point, because the oxytocin treatment prior to the 0.5-hr time point was sufficiently active.. Milk and plasma radioactivity concentrations were measured by —

Results: Drug-related radioactivity was observed in milk samples collected from lactating rats at 0.5 to 25 hr after dosing with levels ranging from 0.012 to 0.353% of the administered dose/gram sample. A C_{max} value of 0.353% of the administered dose/gram sample was observed in the milk sample collected at 1 hr after dosing. Plasma radioactivity concentrations at 0.5 and 1 hr after dosing were 30 and 5.6 times corresponding milk concentrations, respectively; however, at 3 hr after dosing, plasma and milk concentrations were approximately equivalent. At the 6 and 25 hr after dosing, radioactivity concentrations in plasma had declined more rapidly and milk concentrations exceeded those observed in plasma.

Mean concentrations of drug-related radioactivity (% of the radioactive dose per gram sample) in milk and plasma samples obtained from lactating rats that received [^{35}S]-Org 31540/SR90107A by the intravenous route at a dose of 10 mg/kg.

Sample collection time, hr	Milk	Plasma
0.5	0.110	3.301
1	0.353	1.981
3	0.293	0.303
6	0.121	0.046
25	0.012	0.005

Animal Treatment and Determination of Radioactivity in Plasma and in Tissues of Pregnant Rats and Their Fetuses Following Multiple Intravenous Treatments with [^{35}S]-Org 31540/SR90107A (Amendment #027; Report 5014).

Methods: Placental transfer of [^{35}S]-Org 31540/SR90107A was examined in pregnant female Sprague-Dawley rats that received radiolabeled drug by the intravenous route at a dose of 10 mg/kg/day from days 9 to 10 of gestation (Group 1; two doses) or days 9 to 17 (Group 2; nine doses). Pregnant rats were sacrificed at 0.5, 1, 4, 8, and 24 hr after the last dose. One pregnant female rats was sacrificed at each time point. Plasma was obtained from maternal blood samples. For each Group 1 female rat, the maternal uterus and 3 fetuses including placentas (complete contents of amniotic membrane) were excised. For each Group 2 female rat, the amniotic fluid was sampled, and the maternal uterus, 3 fetuses, and placentas were excised. For each fetus collected from Group 2 dams, umbilical blood, the liver, and carcass were excised. For Group 1 female rats, drug-related radioactivity concentrations were measured in maternal plasma, maternal uterus, and fetus (+ placenta). For Group 2 female rats, drug-related concentrations were measured in maternal plasma, maternal uterus, and fetus (i.e., placenta, blood, liver, and carcass).

Results: For Group 1, radioactivity concentrations in the maternal uterus and fetus + placenta were lower than that observed in maternal plasma at 0.5 and 1 hr after dosing; however, at later time points, due to a slower decline of radioactivity concentrations, levels in the maternal uterus and fetus + placenta were higher as compared to the

maternal plasma. Similarly for Group 2, radioactivity concentrations in the maternal uterus and amniotic fluid were lower than observed in maternal plasma at 0.5 and 1 hr after dosing; however, at later time points, due to a slower decline of radioactivity concentrations, levels in the maternal uterus and amniotic fluid were higher as compared to maternal plasma. Radioactivity concentrations in the fetal blood, liver, and carcass were significantly lower than that observed in maternal plasma at 0.5, 1, or 4 hr after dosing. At later time points, the decline in radioactivity concentrations in the fetal blood, liver, and carcass were slower as compared to maternal plasma. Radioactivity concentrations in fetal blood, liver, and carcass samples obtained from Group 2 suggest that placental transfer of drug-related radioactivity (i.e., parent compound and/or metabolites) was low.

Table 7a

The concentration of radioactivity (percentage of the last administered radioactive dose) in the plasma, uterus and fetus including placenta (per gram sample) of pregnant rats which received 2 daily intravenous treatments with 10 $mg \cdot kg^{-1}$ (ca. 0.5 $MBq \cdot kg^{-1}$) [^{35}S]-Org 31540/SR 90107A (mean of a triplicate sample)

Group 1

rat code	sample time (hours)	plasma (% dose per g)	uterus (% dose per g)	fetus (+ placenta) (% dose per g)
8-D6AA	0.49	0.540	0.402	0.303
E-0763	0.99	0.281	0.202	0.166
9-BC46	4.17	0.043	0.055	0.065
1E-A980	8.04	0.013	0.038	0.029
25-49C2	24.22	0.002	0.012	0.009

Table 7b

The concentration of radioactivity (percentage of the last administered radioactive dose) in the plasma, uterus and fetal samples (per gram sample) of pregnant rats which received 9 daily intravenous treatments with 10 $mg \cdot kg^{-1}$ (ca. 0.5 $MBq \cdot kg^{-1}$) [^{35}S]-Org 31540/SR 90107A (mean of a triplicate sample)

Group 2

rat code	time (hours)	radioact. in pregnant rats (% of the last dose)		radioactivity in fetuses (% of the last dose)			
transpdr	post dose	plasma	uterus	placenta	blood	liver	carcass
4E-FCFB	0.53	0.500	0.284	0.233	0.027	0.008	0.016
62-60D7	1.08	0.214	0.148	0.167	0.027	0.007	0.013
F5-5F6D	4.05	0.047	0.066	0.133	0.025	0.007	0.014
49-E481	8.17	0.009	0.037	0.084	0.010	0.006	0.009
49-EC70	24.14	0.003	0.018	0.096	0.004	0.002	0.008

Metabolism

In vitro

In vitro metabolism of [^{35}S]-Org 31540/SR 90107A by rat, rabbit, monkey, and human postmitochondrial liver fractions (SDGRR 4238).

Methods: The objective of this study was to establish whether [^{35}S]-Org 31540/SR 90107A was metabolized by rat, rabbit, monkey, and human postmitochondrial liver fractions through determination of both anti-Xa activity and the radioactive profile at selected time points. Postmitochondrial liver fractions from male rat, female rabbit, male monkey, and human (two males and one female) were incubated with [^{35}S]-Org 31540/SR 90107A (5.9 µg/ml and 0.49 µCi/ml) at 37°C. Incubation samples for determination of anti-Xa activity and the radioactive profile were taken at 0, 5, 15, 30, 45, and 60 min after the start of the incubation. Radioactive profiles were only determined at 0 and 60 min. Anti-Xa activity was measured using the chromogenic substrate ——— and through comparison with a standard curve. The radioactive profile was analyzed by ———————————————

Solvent A was 0.5 N NaCl and Solvent B was 2 M NaCl, and a gradient was used for separation of [^{35}S]-Org 31540/SR 90107A. Incubations without NADP served as a control for non-NADPH-dependent reactions. [^{14}C]-testosterone was used as a positive control.

Results: The concentration of unchanged Org 31540/SR 90107A, as determined on the basis of anti-Xa activity, did not decrease during the 60 min incubation with rat, rabbit, monkey, and human postmitochondrial liver fractions suggesting this compound was not metabolized under the conditions used. In contrast, control incubations with [^{14}C]-testosterone showed 63, 31, 36, 34, 58, and 78% conversion of [^{14}C]-testosterone for rat, rabbit, monkey, and the three human postmitochondrial liver fractions, demonstrating that preparations were enzymatically active. Radioactive profiles at 0 and 60 min showed the presence of only one radioactive peak corresponding to [^{35}S]-Org 31540/SR 90107A either with or without NADP. [^{35}S]-Org 31540/SR 90107A was not metabolized by rat, rabbit, monkey, and human postmitochondrial liver fractions under the conditions used.

In vitro metabolism of [^{35}S]-Org 31540/SR 90107A by rat and human hepatocytes (SDGRR 4255).

Methods: The objective of this study was to establish whether [^{35}S]-Org 31540/SR 90107A was metabolized by rat and human hepatocytes through determination of the radioactivity concentration and anti-Xa activity in cell medium at time points up to 3 hr. Further, the radioactive profile in cell medium and cell extracts at those time points was characterized by HPLC. Rat and human hepatocytes were isolated by collagenase perfusion. Rat hepatocytes (3.1 x 10^6 cells/mL) were incubated with [^{35}S]-Org 31540/SR 90107A (2.3 µCi/mL, equivalent to 9.2 µg/mL). Human hepatocytes (6.4 x 10^6 cells/mL) were incubated with [^{35}S]-Org 31540/SR 90107A (2.8 µCi/mL, equivalent to 23.4 µg/mL). Incubations were performed at 37°C and samples were taken at 0, 30 min, 1 h, 2 h, and 3 h. Cells and medium were separated by centrifugation. The concentration of anti-Xa activity was determined in cell medium. The concentration of radioactivity and the radioactive profiles were determined in methanolic cell extracts and in cell medium.

Results: The concentration of anti-Xa activity in the cell medium did not change during the 3 hr incubation of [^{35}S]-Org 31540/SR 90107A with rat or human hepatocytes. The lack of change in anti-Xa activity suggests that [^{35}S]-Org 31540/SR 90107A was not metabolized under the conditions used. The radioactive profiles were determined by HPLC in cell extract and cell medium. Greater than 99% of the radioactivity was associated with the cell medium. All radioactive profiles obtained for the cell medium were identical with the HPLC profile of [^{35}S]-Org 31540/SR 90107A. The radioactive profile obtained for the 3 hr rat hepatocyte cell extracts contained a single peak with the retention time corresponding to [^{35}S]-Org 31540/SR 90107A. The radioactive profile obtained for 3 hr human hepatocyte cell extracts contained a peak with a retention time of [^{35}S]-Org 31540/SR 90107A and a peak accounted for by the presence of [^{35}S]-SO_4^{2-} ions. There were no indications for the formation of desulfated radiolabeled metabolites. The radioactive profiles confirm that [^{35}S]-Org 31540/SR 90107A was not metabolized during the 3 hr incubation with rat or human hepatocytes.

In situ metabolism of [^{35}S]-Org 31540/SR 90107A in the rat liver perfusion (SDGRR 4243).

Methods: The objective of the study was to establish whether [^{35}S]-Org 31540/SR 90107A was metabolized in a rat liver perfusion setup through (1) determination of the anti-Xa activity in rat liver perfusate and concentration of radioactivity in rat liver perfusate and bile at time points up to 2 hr, and (2)

determination of the radioactivity profiles in rat liver perfusate and bile at those time points. [^{35}S]-Org 31540/SR 90107A was subjected to in situ perfusion in male rat liver. After a single pass pre-perfusion with oxygenated Krebs-Henseleit buffer for approximately 20 min, the liver was subjected to a circulatory perfusion for 2 hr with oxygenated Krebs-Henseleit-BSA perfusion buffer containing 10.2 µg/mL (0.53 µCi/mL) [^{35}S]-Org 31540/SR 90107A. Perfusate samples were taken for determination of anti-Xa activity and the radioactivity profile at 0, 5, 15, 30, 45, 60, 90, and 120 min after the start of the perfusion. Bile was collected throughout the circulatory perfusion period. The concentration of radioactivity was determined in perfusate samples. Radioactivity profiles were analyzed by HPLC in perfusate samples and bile.

Results: Anti-Xa activity decreased slightly at 5 min, as compared with the time 0 sample; however, the anti-Xa activity and radioactivity concentration in the perfusate samples did not show further changes during the 2 hr rat liver perfusion. The slight decrease was mainly explained by the fact that the total volume of perfusion buffer was increased by approximately 8% with the volume of Krebs-Henseleit buffer present in the tubing and the liver when the circulatory perfusion was started. Bile contained less than 0.1% of administered radioactivity. The radioactivity profile was analyzed by HPLC in the perfusate samples and bile. All radioactivity profiles showed the presence of one peak with the retention time of [^{35}S]-Org 31540/SR 90107A, confirming that [^{35}S]-Org 31540/SR 90107A was not metabolized during the 2 hr rat liver perfusion. These measurements indicate that [^{35}S]-Org 31540/SR 90107A was not metabolized in the rat liver perfusion under the conditions used. Less than 0.1% of administered radioactivity was excreted in the bile.

In vivo

Effects of a 2-Week Repeated Intravenous Administration of SR 90107A (0.46, 2.29, and 11.46 mg/kg/day) on Various Liver Enzyme Activities in Sprague Dawley Rats (Amendment #014; Report 693.5.017).

Methods: Hepatic drug metabolizing enzymes were measured in Sprague-Dawley rats that received SR90107A at intravenous doses of 0, 0.4, 2, and 10 mg/kg/day (doses expressed in base form?) for 2 weeks (14-15 days). There were 10 rats/sex/group. Control animals received the vehicle, isotonic NaCl solution. The intravenous dose volume was 2 mL/kg. Samples of liver were obtained at necropsy and microsomes were prepared. Cytochrome P450 content was measured. Monooxygenase activities toward 7-ethoxyresorufin, 7-pentoxyresorufin, aminopyrine, aniline, and erythromycin were

measured to assess induction of CYP1A, CYP2B, CYP2C, CYP2E, and CYP3A, respectively.

Measurement of cytochrome P450 isozymes.

Cytochrome P450 subfamily	Characteristic enzyme activity
Cytochrome P450 1A	7-Ethoxyresorufin O-deethylase
Cytochrome P450 2B	7-Pentoxyresorufin O-dealkylase
Cytochrome P450 2C	Aminopyrine N-demethylase
Cytochrome P450 2E	Aniline hydroxylase
Cytochrome P450 3A	Erythromycin N-demethylase

Results: SR 90107A had no effects on relative liver weight, cytochrome P450 content, or hepatic monooxygenase enzyme activities in rats. 7-ethoxyresorufin O-deethylase (EROD) activities for the male rats at 0.4 and 10 mg/kg/day were decreased to 77.8 and 74.4% of the control (0.203 nmoles/mg microsomal protein/min), respectively; however, there was no evidence of dose response relationship as the activity for male rats at 2 mg/kg/day was not changed. No change in EROD activities was evident for female treatment groups.

Monkeys

Effects of a 2 Week Repeated Intravenous Administration of SR 90107A (0.46, 2.29, and 11.46 mg/kg/day) on Various Liver Enzyme Activities in Macaca Monkeys (Amendment #014 and #040; Reports 693.5.018 and 693.5.020).

Methods: Hepatic drug metabolizing enzyme activities were measured in macaca fascicularis monkeys following treatment with SR90107A at intravenous doses of 0, 0.4, 2, and 10 mg/kg/day (doses expressed in salified form) for 2 weeks (14-19 days). In Report 693.5.018 submitted with Amendment #014, it was incorrectly reported that doses were expressed as the base form. This error was corrected in Report 693.5.020 submitted with Amendment #040. There were 3 monkeys/sex/group. The intravenous dose volume was 1 mL/kg. Samples of liver were removed at necropsy and microsomes were prepared. Cytochrome P450 content was measured. Monooxygenase activities toward 7-ethoxyresorufin, aminopyrine, aniline, and erythromycin were measured to assess induction of CYP1A, CYP2C, CYP2E, and CYP3A, respectively.

Measurement of cytochrome P450 subfamilies.

Cytochrome P450 subfamily	Characteristic enzyme activity
Cytochrome P450 1A	7-Ethoxyresorufin O-deethylase

Cytochrome P450 2C	Aminopyrine N-demethylase
Cytochrome P450 2E	Aniline hydroxylase
Cytochrome P450 3A	Erythromycin N-demethylase

Results: SR 90107A had no effects on relative liver weight, cytochrome P450 content, or hepatic monooxygenase enzyme activities in monkeys.

Investigating the Potential for ORG31540/SR90107A to Inhibit Cytochrome P450 (CYP) Enzymes using Human Liver Microsomes In Vitro (Amendment #096; Report 693.6.040).

Methods: Inhibitory properties of Org 31540/SR90107A toward human liver cytochrome P450 enzymes, CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4, were assessed in vitro using human liver microsomes. Reaction mixtures contained microsomes and Org 31540/SR90107A (200 µM, 346 µg/mL salified). Protein concentration and mixture volume were optimized for each assay. Mixtures were brought to 37°C with an isoform selective substrate with or without Org 31540/SR90107A or selective inhibitor, followed by addition of an NADPH generating system. Each reaction was terminated after a specified period and the product formed was quantified using

Relationships between cytochrome P450 isoforms, specific substrate(s), and inhibitor(s).

Substrate, µM	CYP	Reaction	Inhibitor
Phenacetin, 10 µM	1A2	O-deethylation	Furafylline
Coumarin, 1 µM	2A6	7-hydroxylation	Pilocarpine
Tolbutamide, 200 µM	2C9	Methyl hydroxylation	Sulfaphenazole
Mephenytoin, 75 µM	2C19	4'-hydroxylation	Tranylcypromine
Bufuralol, 20 µM	2D6	1'-hydroxylation	Quinidine
Chlorzoxazone, 75 µM	2E1	6-hydroxylation	Diethyldithiocarbamate
Nifedipine, 30 µM	3A4	oxidation	Ketoconazole

Results: Org 31540/SR90107A at 200 µM (346 µg/mL salified) did not significantly inhibit (defined as >30% inhibition relative to control values by the sponsor) CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4. This concentration was approximately 150-time greater than the highest mean plasma C_{max} concentration (2.25 µg/mL) observed during Phase I studies. Inhibition of coumarin 7-hydroxylation (i.e., CYP2A6) by ORG31540/SR90107A ranged from 72.1 to 82.6% of the control. No significant inhibition was observed for any other reactions (i.e., generally <10% inhibition).

Excretion

An excretion study after a single intravenous or a single subcutaneous dose of [^{35}S]-Org 31540/SR 90107A to male and female Wistar rats (SDGRR 4256).

Methods: The objectives of this study were to determine: 1) the extent of excretion of radioactivity in urine and fecal samples, obtained from male and female Wistar rats treated with either a single intravenous or subcutaneous dose of [^{35}S]-Org 31540/SR 90107A; (2) the anti-Xa activity in urine samples, obtained from male and female rats treated with either a single intravenous or subcutaneous dose of [^{35}S]-Org 31540/SR 90107A; (3) the radioactivity profile of [^{35}S]-Org 31540/SR 90107A in selected urine samples, obtained from male and female rats treated with a single intravenous or subcutaneous dose of [^{35}S]-Org 31540/SR 90107A; (4) the radioactivity profile of [^{35}S]-Org 31540/SR 90107A in selected fecal samples, obtained from male

and female rats treated with either a single intravenous or subcutaneous dose of [^{35}S]-Org 31540/SR 90107A. Rats (3 rats/sex/dose route) received either a single intravenous or subcutaneous dose of [^{35}S]-Org 31540/SR 90107A (equivalent to 410 µg/kg). Following administration of the radioactive doses, urine and fecal samples were collected in 24-hr fractions up to 168 hr post dosing.

Results:

Table 16. Excretion of anti-Xa activity in urine following either a single intravenous or subcutaneous dose of [^{35}S]-Org 31540/SR 90107A to male and female rats (Adapted from sponsor's table in SDGRR 4256).

	Anti-Xa Activity			
	Intravenous Route		Subcutaneous Route	
Time (hr)	Male, n=3	Female, n=2	Male, n=3	Female, n=2
0-24	76.1 ± 4.9	76.4	77.0 ± 2.3	65.0
24-48	0.7 ± 0.1	0.6	0.3	

Table 17. Excretion of radioactivity in urine and feces following a single intravenous or subcutaneous dose of [^{35}S]-Org 31540/SR 90107A to male and female rats (Adapted from sponsor's table in SDGRR 4256).

	Radioactivity							
	Intravenous				Subcutaneous			
Time	Male		Female		Male		Female	
(hr)	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces
0-24	80.8 ± 4.0	5.1 ± 3.2	89.0	2.7	84.6 ± 1.1	5.0 ± 4.6	76.8	4.5
24-48	1.4 ± 0.2	0.8 ± 0.2	1.4	0.8	1.4 ± 0.5	1.0 ± 0.3	1.4	1.2

Following either a single intravenous or subcutaneous dose of [^{35}S]-Org 31540/SR 90107A to male and female rats, 60.3 to 83.4% of administered anti-Xa activity was recovered in urine and 85.2 to 100.5% of administered radioactivity was recovered in urine and feces within 7 days post administration. The majority of the administered dose was excreted into the urine during the first 24

hr. The radioactivity present in urine was predominantly composed of the unchanged pentasaccharide. In addition, some of the radioactivity was recovered in the form of $[^{35}S]$-SO_4^{2-} ions. No desulfated radiolabeled metabolites were observed.

Excretion balance following a single intravenous or subcutaneous administration (0.4 mg/kg) of $[^{35}S]$-SR 90107A (Org 31540) to male macaca fascicularis monkeys (Report 693.5.007).

Methods: This study examined the excretion balance following a single intravenous or subcutaneous administration (0.4 mg/kg) of $[^{35}S]$-SR 90107A (Org 31540) to male macaca fascicularis monkeys. Animals weighed 2.92 to 3.88 kg. A solution of SR 90107A and $[^{35}S]$-SR 90107A was prepared immediately prior to usage (approximately 3.2 mg/ml with a calculated specific activity of 10.7 µCi/mg). On Day 1, animals received the test compound either intravenously in the femoral vein (bolus at 0.6 ml/kg) or subcutaneously (0.13 ml/kg). SR 90107 was given as an isotopic dilution at a dose of 0.4 mg/kg, which corresponded to approximately 4 µCi/kg. On Day 15, this schedule was reversed, animals that initially received the intravenous dose, now received the subcutaneous administration. Animals that initially received the subcutaneous dose, now received the intravenous administration. Urine samples were collected at the following time intervals: 0-6, 6-24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hr. Fecal and cage wash samples were collected by 24 hr-fractions over a 168 hr-period. One blood sample was collected from each animal, 2 hr after intravenous or subcutaneous administration.

Results: Following either a single intravenous or subcutaneous administration of 0.4 mg/kg $[^{35}S]$-SR 90107A, the plasma concentrations were for 1.98 ± 0.16 mg Eq/L and 2.36 ± 0.24 mg Eq/L, respectively, and the blood concentrations accounted for 1.19 ± 0.07 mg Eq/kg and 1.40 ± 0.07 mg Eq/kg. At the 2 hr-sampling time, the radioactivity was almost completely distributed in the plasma, regardless of the route of administration (88 to 100%). Following either intravenous or subcutaneous administration of $[^{35}S]$-SR 90107A, the drug was primarily eliminated in the urine during the first 24 hr period.

Table 18. Mean cumulative excretion following a single intravenous administration of 0.4 mg/kg [^{35}S]-SR 90107A to male monkeys (values expressed as a percentage of the administered dose) (Adapted from Sponsor's table in Report 693.5.007).

Time (hr)	Urine	Feces	Washes	Total
0-24	65.87 ± 17.88	0.03 ± 0.03	7.40 ± 4.41	73.30 ± 14.27
24-48	70.17 ± 17.74	0.15 ± 0.10	8.63 ± 5.19	78.95 ± 13.61
0-168	71.95 ± 17.79	0.21 ± 0.20	9.23 ± 5.67	81.39 ± 13.16

Table 19. Mean cumulative excretion following a single subcutaneous administration of 0.4 mg/kg [^{35}S]-SR 90107A to male monkeys (values expressed as a percentage of the administered dose) (Adapted from Sponsor's table in Report 693.5.007).

Time (hr)	Urine	Feces	Washes	Total
0-24	66.03 ± 3.24	0.24 ± 0.12	7.66 ± 1.73	73.93 ± 2.77
24-48	69.96 ± 2.98	0.34 ± 0.25	8.74 ± 1.75	79.04 ± 2.07
0-168	71.97 ± 3.01	0.37 ± 0.25	9.52 ± 2.07	81.86 ± 2.47

Radioactivity profiles of [^{35}S]-Org 31540/SR 90107A in plasma and urine from male Cynomolgus monkeys following a single intravenous or a single subcutaneous dose (SDGRR 4240).

Methods: The objectives of this study was to determine the radioactivity profiles of [^{35}S]-Org 31540/SR 90107A in selected plasma and urine samples, obtained from male Cynomolgus monkeys treated with either a single intravenous or subcutaneous dose of [^{35}S]-Org 31540/SR 90107A. On Day 1, animals received either intravenously or subcutaneously, an isotopic dilution of SR 90107 at a dose of 0.4 mg/kg. On Day 15, this scheduled was reversed, animals that initially received the intravenous dose, now received the subcutaneous administration. Animals that initially received the subcutaneous dose, now received the intravenous administration.

Results: Following either a single intravenous or subcutaneous dose of [^{35}S]-Org 31540/SR 90107A to male Cynomolgus monkeys, radioactivity profiles of plasma samples obtained at 2 hr post dosing contained one peak of radioactivity with a retention time corresponding to the unchanged pentasaccharide. Following either intravenous or subcutaneous administration, the major part of the radioactivity present in urine was also accounted for by the presence of the unchanged pentasaccharide. In addition, some of the radioactivity in the urine was recovered in the form of [^{35}S]-SO_4^{2-} ions. No desulfated radiolabeled pentasaccharide metabolites were observed.

The pharmacokinetic studies with Org31540/SR90107A were conducted in rats, rabbits, and monkeys. In single dose IV/SC study in rats (0.17 and 0.35 mg/kg for IV and SC, respectively), the Cmax and AUC increased dose proportionally. The elimination half-life was approximately 1 h, the clearance was ——— ml/h/kg and the volume of distribution was ——— ml/kg. The absolute bioavailability after SC administration was 115% at 0.35 mg/kg dose level. Bioavailability value greater than 100% were apparently due to variability between samples and experimental errors that were inherent in the procedures used. In a single dose study in rabbits at 0.17 mg/kg, IV, the $AUC_{0-\alpha}$ was 7.04 μg.h/ml with a clearance value of 25 ml/h/kg and a volume of distribution of 63 ml/kg. In cynomolgus monkeys, Org31540/SR90107A was administered as a single IV dose of 8.8 mg/kg followed by 10 mg/kg, SC for 8 to 10 days. In this study, the terminal half-life was 5 to 6 hours and the bioavailability after SC administration was 71 to 77%. After a single IV dose of 10 mg/kg in cynomolgus monkeys, the terminal half-life was found to be 2.7 h, the clearance was 0.11 L/h/kg and the volume of distribution was 440 ml/kg. In single IV and SC dose (100, 250, and 500 μg/kg) studies in macaques, the terminal half-life was 6 hours and the mean plasma clearance was 13 to 15 ml/h/kg. In a single dose (0.17 mg/kg, IV and SC) in baboons, the Cmax of 0.8 μg/ml was reached at 1.7 h with an elimination half-life of 5.4 hours. In a second single dose study in baboons (0.27 mg/kg, SC), Cmax of 2.32 μg/ml was reached at Tmax of 2.5 h with a terminal half-life of 4.4 hours. *In vitro* binding studies using rat, monkey and human blood, Org31540/SR90107A was exclusively found in plasma (96 to 101%) in all species. A very low fraction was associated with blood cells in all species. The *in vitro* protein binding studies were conducted using rat, monkey and human plasma. About 90, 88 and 92% protein binding was observed in rat, monkey and human, respectively. There was no species difference in plasma protein binding and at clinically relevant concentration (1 to 2 μg/ml), plasma protein binding for Org31540/SR90107A was approximately 90%. The tissue distribution was studied in male rats after a SC dose of 0.4 mg/kg using ——— ——— . Maximum radioactivity was observed at 0.25 h after treatment and was observed in bladder content (urine) and in tissues involved in metabolism and excretion (blood, lung, kidney and liver). No retention or accumulation or target organ was noted. The placental transfer of radioactivity was examined in SD rats. On gestation days 10 and 17, relatively high levels of radioactivity were seen in the uterus (0.402% dose per g) and placenta (0.303% dose per g) with a tissue plasma ratio of 0.5 at 0.5 h post-treatment. Low levels of radioactivity were also found in the fetuses (0.016 to 0.233% of last dose), which suggested low level of placental transfer of Org31540/SR90107A. The excretion of radioactivity into milk was studied in lactating SD rats after a single IV dose of 10 mg/kg of Org31540/SR90107A. The excretion in

milk was found to be 0.35% of the administered dose per gram of milk at 1 h post-dose, which had decreased to 0.012% per gram of milk at 25 h post-dose. The metabolism of Org31540/SR90107A was studied in *in vitro* models of hepatic metabolism: post-mitochondrial liver fractions from rat, rabbit, macaque (*M. fascularis*) and human; rat and human hepatocytes; and an *in situ* perfused rat liver preparation. There was no evidence of biotransformation of Org31540/SR90107A in all these preparations. The metabolism of Org31540/SR90107A was studied *in vivo* in rats and macaques after a single IV or SC dose of 0.4 mg/kg. Metabolite profiles in the urine and feces of rat and macaque indicated that Org31540/SR90107A did not undergo metabolism. The effect of Org31540/SR90107A on the activity of hepatic drug metabolizing enzymes was examined in liver samples of rat and macaque, obtained after 2 weeks of IV dosing at 0.4, 2, and 10 mg/kg/day. Org31540/SR90107A has no effect on total cytochrome P450 (CYP), 7-pentoxyresorufin N-dealkylase (CYP2B), aminopyrine N-demethylase (CYP2C), aniline hydroxylase (CYP2E) and erythromycin N-demethylase (CYP3A). The excretion of Org31540/SR90107A was examined in rat and macaque after single IV or SC dose of Org31540/SR90107A at 0.4 mg/kg. Majority of the radioactivity was recovered in the urine (80 to 90%) as unchanged pentasaccharide. In macaque, the majority of the radioactivity (72%) excreted in the urine as unchanged drug within 24 h of dosing and less than 0.5% was excreted through feces and the remaining was recovered in the cage washing. The pharmacokinetic parameters of Org31540/SR90107A after single doses in animals and humans are summarized in the following table (from sponsor's submission). It is to be mentioned here that the pharmacokinetic parameters after s.c. and i.v. administration are comparable in rats.

Table (5.3.2) 1 - Plasma Pharmacokinetic Parameters in Male Animals and Humans Following a Single Administration of Org31540/SR90107A (Definitive Studies) [Mean]

		C_{max} (µg/mL)	t_{max} (h)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (µg·h/mL)	CL (mL/h/kg)	Vd (mL/kg)
Rat IV	0.4 mg/kg	na	na	1.1	4.0	90	100
	2 mg/kg	na	na	1.2	9.4	190	190
	10 mg/kg	na	na	1.1	27.8	310	210
Macaque SC	10 mg/kg	11.3	1-2	5.7	114	80	662
Human SC	mg (0.035 mg/kg)[a]	0.34	1.7	17.2	6.65	4.82[b]	117[c]

na = not applicable

[a] Based on average human bodyweight of 70 kg.

[b] Converted from units of mL/min in the original report.

[c] Converted from units of L in the original report.

Ref: Rat [SDGRR4528], Macaque [ABS0300], Human [BDR3780]

TOXICOLOGY:

Acute

ACUTE TOXICITY IN MICE, RATS, AND MONKEYS:

Single-dose intravenous or subcutaneous toxicity studies in mice, rats, and monkeys.

Methods: The sponsor conducted several studies to examine the single dose toxicity of Org 31540/SR 90107A in mice, rats, and monkeys when administered by the subcutaneous or intravenous routes. OF1 Swiss mice or Sprague Dawley rats (5 animals/sex/group) received Org 31540/SR 90107A at dose levels of 0 and 40 mg/kg by either the intravenous or subcutaneous route. Cynomolgus monkeys (Macaca Fascicularis) (2 males and 2 females) received a single subcutaneous administration of Org 31540/SR 90107A at a dose level of 40 mg/kg. For all studies, mortality and changes of body weight were observed for 14 to 15 days following administration. A necropsy was performed on each animal sacrificed at the end of the study and the injection site was submitted to a microscopic examination.

Results: For mouse, rat, and monkey studies, there was no mortality following a single dose of Org 31540/SR 90107A at a dose level of 40 mg/kg administered by either the intravenous or subcutaneous route. For mouse studies, there were no macroscopic lesions following intravenous administration; however, following subcutaneous treatment, one male and two females were observed with slight hematomas at the injection point site. For the intravenous toxicity study in rat, females were found to have a 10.6% loss of body weight as compared with a 14% increase for control female group. The sponsors have attributed this weight loss to a drinking anomaly unrelated to the test article. Similarly for the subcutaneous rat study, mean body weight for the female treatment group at day 15 was 85% of that observed for the control; however, weight loss appeared to be related to decreased food consumption, which was 56% of the control between days 8 and 15. For the subcutaneous toxicity study in monkeys, slight decreases in food consumption were observed on day 1 in 2 males (50% of food consumed) and one female (25% of food consumed) as compared to pretreatment values which ranged from 75 to 100%. A subcutaneous hematoma was found at the injection site in one female.

Incidence of mortality in mice, rats, and monkeys following a single dose of Org 31540/SR 90107A at 0 or 40 mg/kg administered by either the subcutaneous or intravenous route.

Dose	Mice		Rats		Monkeys
mg/kg	SC	IV	SC	IV	SC
0	0	0	0	0	0
40	0	0	0	0	0

Org 31540/SR 90107A at 40 mg/kg by either the intravenous or subcutaneous route did not cause mortality in mice or rats. For monkeys given a 40 mg/kg dose by the subcutaneous route, there was no mortality. Hematomas were observed at the injection sites for studies with mice and monkey, in which Org 31540/SR 90107A was administered subcutaneously.

SUBACUTE TOXICOLOGY

RATS

A 2 week intravenous toxicity study in the rat (Report 693.3.031).

Testing Laboratory: Sanofi Recherche
371 rue du Professeur J. Blayac
34184 Montpellier CEDEX 04
France

Study Started: October 24, 1994

Study completed: March 19, 1996

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

Animals: Sprague Dawley rats. At the beginning of the treatment period, animals were approximately 7 weeks old and had a mean body weight of 270 g for males and 200 g for females.

Drug Batch: SR 90107A Batch 92N037.

Methods: Three groups of Sprague Dawley rats received Org 31540 by the intravenous (caudal vein) route at dose levels of 0.4, 2, or 10 mg/kg daily for 14 days. The control group received the vehicle. Each group consisted of 10 males and 10 females. The animals were observed at least twice daily throughout the study

for clinical signs. Electrocardiography was performed on days -4 and +10 (2 to 4 hr after test compound administration). Ophthalmological examinations were performed on days -3 and +11. Animal body weights and food consumption were measured weekly during the treatment period. Blood samples for hematology and plasma biochemistry were collected from the retro-orbital sinus of all animals fasted overnight. Hematology samples were collected on days -10 and +12. Samples for plasma biochemistry were collected on days -7 and +15. Urinalysis was performed on the first five animals/sex/group on day +12, on overnight urine samples collected following oral administration on the previous day of 10 ml water. Necropsy was performed on days 16 to 18.

Results:

1. **Observed Effects:** Most observations were focused toward the tail where the test compound was intravenously injected. Local tolerance of the tail was assessed in control and treatment groups. Redness was noted in the distal and then in the median parts. The redness, observed in treated females only, appeared at day 4 (0.4 mg/kg/day, 5 of 10 animals), day 8 (2 mg/kg/day, 1 of 10 animals) and day 3 (10 mg/kg/day, 5 of 10 animals) and was attributed to localized hemorrhages caused by venous puncture trauma (veins of females are smaller than those of males) and the pharmacological activity of the test compound. One female in the high dose group was found to have black discoloration of the tail tip (distal part) from day 11 onwards due to dry gangrene.

2. **Mortality:** None.

3. **Body Weight and Food Consumption:** Body weights for male controls on days 1 and 15 were 270 and 318 g; respectively. Body weight gain for the male low, mid, and high dose groups were 85.4, 89.6, and 100% of the control, respectively. Body weights for female controls on days 1 and 15 were 199 and 198 g, respectively. There was no weight change for the female low and mid dose groups, and the female high dose group declined by 2 g. Food consumption was not significantly different between control and treatment groups; however, for all groups, consumption during week 2 was approximately 75% of that observed during 1.

4. **Hematology, Coagulation, and Bone marrow:** Slight changes in various erythrocyte parameter, generally with a magnitude of no more than 5%, were observed in male and female treatment groups; however, these changes may be indicative of small hemorrhagic phenomena occurring in response to test compound administration. There were no significant changes in platelet counts between control and treatment groups.

Erythrocyte counts for the male mid dose group on day 12 were slightly decreased to 96% of the control (7.66 x $10^6/mm^3$), while similar decreases for the low and high dose groups were not significant. For the male high dose group on day 12, mean corpuscular volume (MCV) was increased to 104.9% of the control (65.4 μm^3), while in contrast, mean corpuscular hemoglobin content (MCHC) was decreased to 97% of the control (30.7%).

Hemoglobin levels for the female low and high dose groups on day 12 were both found to have slightly decreased to 96% of the control (158 g/L) without changes of erythrocyte counts, hematocrit, or reticulocytosis. MCHC for the female high dose group was decreased to 96.8% of the control (31.7%). For female mid and high dose groups on day 12, monocyte percentages were decreased to 68.4 and 73.7% of the control value (3.8%), respectively. Monocyte counts for the female mid and high dose groups were 69.6 and 73.9% of the control (0.23 x $10^3/mm^3$).

Femoral bone marrow smears were not evaluated. The sponsor stated that no treatment related abnormalities were reported. Analysis was not performed due to the slight degree of hematological variation observed, of their extramedullary origin and because there was no modification of bone marrow cellularity and no histological changes. For bone marrow cellularity, no changes of the number of cells/mm^3, number of cells/femoral diaphysis, femoral diaphysis weight, and number of cells/gram of femoral diaphysis were observed between control and treatment groups.

5. Blood Chemistry and Urinalysis:

Blood chemistry: Slight changes in a number of blood chemistry parameters (i.e., total protein, globulins, Na^+, Cl^-, Ca^{2+}) were observed for male and female treatment groups; however, these changes were less than 5% and are not believed to have any biological significance.

Urinalysis: Urobilinogen levels in urine, measured with multireagent strips, were significantly increased in the both the male and female high dose groups on day 12, which may be indicative of hemorrhagic or hemolytic processes. Levels for both the male and female high dose groups, were increased to 350% of the control (0.2 EU/dL), respectively. Levels for the male and female mid dose groups were increased to 250% of the control; although, this difference was not statistically significant. The excreted quantity of creatinine for the female high dose treatment group on day 12 was increased to 143.5% of the control (46 μmoles); however, small increases for the low (108.7%) and mid (117.4%) dose groups were not significant.

6. Vital Signs, Physical Examination, and Ophthalmic Examination:

Electrocardiogram: The QTc interval in group 1 (0.4 mg/kg/day) males was lengthened to a 128% of the control duration (2.69); however, no changes were observed for the mid and high dose treatment groups.

Ophthalmic examination: No treatment related effects were observed with ophthalmologic examinations.

7. Organ Weights: No treatment related effects were observed for absolute and relative organ weights.

8. Gross Pathology: A number of minor pathological changes were observed in response to treatment with Org 31540/SR 90107A; however, these changes were not test article-specific and there was not a clear dose-response relationship. For the thymus, congestion and superficial petechiae were observed for both control and treatment groups with a similar incidence. For the lung, gray areas of the surface were noted for the low dose (1/10 males, 2/10 females) and high dose (2/10 males and 1/10 females) treatment groups with no comparable incidence in controls. For the mesenteric lymph nodes, slight or moderate increases of volume were observed for the low (2/10 males and 3/10 females), mid (4/10 males and 1/10 females), and high (1/10 males and 1/10 females) dose treatment groups compared to a lower incidence for the control (0/10 males and 1/10 females). For the Harderian gland, moderate to large-sized hematomas were noted in the low (1/10 males and 1/10 females), mid (1/10 males and 1/10 females), and high (1/10 males) dose treatment groups with no comparable incidence in the control; although, discoloration was noted for 1 male control (n= 10) and 1 male and 1 female in the mid dose group (n = 10/sex).

9. Histopathology: Very slight to marked serofibrino-hemorrhagic, perivascular or hypodermic infiltration was reported for injection sites in the tail. These appear to be directly related to the injection; however, treatment with the test compound increased the incidence and severity of reported changes. For the popliteal lymph nodes, hemorrhagic infiltration was noted for the low (2/10 females, slight), mid (2/10 males, slight and 1/10 females, marked), and high (2/10 females, marked) compared to 1 female control with slight infiltration. Siderophage infiltration in the popliteal lymph nodes was noted in the low (2/10 females), mid (3/10 females), and high (2/10 males and 1/10 females) dose treatment groups with no comparable incidence in the control.

Table 20. Light microscopic examination of injection site (Adapted from Sponsor's table in Report 693.3.031, page 37 [38, stamped page number]).

Dose	0		0.4 mg/kg/day		2 mg/kg/day		10 mg/kg/day	
Sex	M	F	M	F	M	F	M	F
N	10	10	10	10	10	10	10	10
Injection sites: serofibrino-hemorrhagic infiltration								
Perivas-cular	3	1	3	2	1	1	1	
Very slight	4	2	5	4	5	5	5	4
Slight		1		2		2		4
Moderate				1		1		2
Marked								
Hypodermic								
Very slight		1	1	1		2	6	3
Slight		1		3			3	2
Moderate						1		2
Marked				1		1		3
Popliteal lymph nodes: hemorrhagic or siderophage infiltration								
Slight		1		3	2	3	2	
Moderate-marked						1	1	4

A number of minor histopathological changes were observed in response to treatment with Org 31540/SR 90107A; however, these changes were not test article-specific and there was not a clear dose-response relationship. For the eyes, focal or segmental retinal atrophy was noted for the low (4/10 males and 2/10 females), mid (2/10 males and 2/10 females), and high (1/10 males and 4/10 females) dose treatment groups compared with a relatively low incidence in the control of only 1 in 10 females. Lesions due to blood sampling in the retro-orbital sinus were of similar incidence in the control and treatment groups. For the retromandibular lymph nodes, macrophages with black pigment were noted in high dose treatment group (3/10 males and 2/10 females) with no comparable incidence in controls; this observation was attributed to tattoo markings.

A number of changes were noted in the liver for both control and treatment groups. There was no evidence of a dose response relationship or a test article-specific effect. Foci of liver necrosis were noted in 1 animal from the low dose group and 5 animals from the high dose group. Also in the liver, there was slight to marked centrilobular, periportal, or panlobular hepatocyte margination with similar frequency in both the control and treatment groups. Further, there were single to several chronic inflammatory cell foci in both control and treatment groups with similar frequency.

Changes were noted for the adrenal glands, and Harderian glands that occurred with a high frequency in all groups; however, the percentage incidence was not different between control and treatment groups. For the adrenals, there was slight to moderate vacuolation of cortical cells in the control and high dose treatment groups with similar frequency. For the thymus, hemorrhagic infiltration was noted for both the control and high dose treatment groups with similar frequency. For the Harderian glands, inflammatory cell infiltration of various grades was noted for the control and high dose treatment groups with similar frequency.

Rats received Org 31540 by the intravenous route at dose levels of 0.4, 2, or 10 mg/kg day for 14 days. The no effect dose was 2 mg/kg/day. Urobilinogen levels in urine were significantly increased in the both the male and female 10 mg/kg/day, which may be indicative of hemorrhagic or hemolytic processes. Foci of liver necrosis were observed with an increased frequency in the 10 mg/kg/day group; although, a dose response relationship was not observed for this effect. Significant pathological lesions were observed at injection sites, which were attributed to injection trauma aggravated by the pharmacological activity of Org 31540.

Toxicity study for 4 weeks by subcutaneous administration to rats with a 16 day recovery period (SDGRR 2998).

Testing Laboratory: [ ]

Study Started: April 4, 1990

Study completed: July 20, 1991

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

Animals: Sprague Dawley rats. At the beginning of the treatment period, animals were approximately 6 weeks old and had a mean body weight of 213 g for males and 158 g for females.

Drug Batch: ——— 89032/1 or 2 Org 31540 10 mg/ml Test no. ZA-015.

Methods: Three groups of Sprague-Dawley rats received Org 31540 by subcutaneous injection at dose levels of 0.4, 2, or 10 mg/kg daily for 4 weeks. Animals were allocated to principal and satellite groups. The 10 mg/kg/day principal group was composed of 12 males and 12 females. The 0.4 and 2 mg/kg/day principal groups were composed of 6 males and 6 females each. The control principal group, consisting of 12 males and 12 females, received the vehicle alone. At the end of the treatment period, the first 6 surviving animals of the control and high dose level principal groups were kept for a 16-day recovery period. Satellite groups were used for toxicokinetics. Each satellite group was composed of 6 males. Clinical signs were recorded once a day. Mortality was checked twice a day. Body weight and food consumption were recorded once a week. Ophthalmologic examinations were performed before treatment and on week 4. Hematology, blood biochemistry, and urinalysis were performed on weeks 4 and 6. Toxicokinetics were performed on days 1, 14, and 29. At the end of the treatment and recovery periods, the animals were sacrificed, all tissues were examined before and after dissection, the weight of the main organs were recorded and a microscopic examination was performed.

Results:

1. **Observed Effects**: No significant clinical signs could be related to the treatment with Org 31540 at any dose level.

2. **Mortality**: One female of the 0.4 mg/kg/day group was found dead during week 1. Microscopic examination revealed a hematoma at the injection site, meningeal hemorrhage in the brain and spinal cord, and hemorrhage in the pituitary gland, and mandibular lymph node. The cause of death was attributed to a direct mechanical injury related to handling of the animal and the anticoagulant properties of Org 31540.

3. **Body Weight and Food Consumption**: No treatment related effects were observed for body weight and food consumption. Body weights for the male control group at weeks 1 and 5 were 215 and 375 grams, respectively. Weight gain for the male low, mid, and high dose groups were 98.1, 105.6, and 111.2% of the control, respectively. Body weights for the female control group at weeks 1 and 5 were 158 and 241 grams, respectively. Weight gain for the female low, mid, and high dose groups were 108.4, 114.4, and 100% of the control, respectively.

4. **Hematology and Bone Marrow:**

Hematology: A number of small changes were observed with erythrocytes, eosinophils, and polychromatic normoblasts. There were no significant changes in platelet counts between control and treatment groups. For the female high dose group at 4 weeks, there were slight increases in the hemoglobin level, PCV, and MCV of less than 10%; however, the red blood cell count was unchanged. The percentage eosinophils for the male high dose group at the end of the 4 week treatment period was increased to 2% compared with 1% for the controls.

Bone Marrow: Bone marrow analysis was performed for the control and high dose treatment groups. At week 5, the percentage of eosinophil myelocytes for the high dose male group was 0% as compared with 1% for the control group; however, there was no difference at the end of the recovery period. Polychromatic normoblasts were increased in the male high dose group at the end of the recovery period from 8.4% in the control to 11.4%.

5. **Blood Chemistry and Urinalysis:**

Blood chemistry: At week 4, a decrease in blood glucose levels was observed for all male and female treatment groups. For male low, mid, and high dose treatments groups, glucose levels were decreased to 83, 88, and 90.3% of the control (8.08 mmol/L), respectively. For female low, mid, and high dose treatment groups, glucose levels were decreased to 79, 80, and 78.5% of the control (8.28 mmol/L), respectively. Blood triglyceride levels for female low, mid, and high dose treatment groups at 4 weeks were also decreased to 71, 58.5, and 66% of the control (0.41 mmol/L), respectively. These changes in blood glucose and triglyceride levels were not observed for the male or female treatment groups at the end of the recovery period. There were slight changes in a number of other blood chemistry parameters (i.e., Na^+, Cl^-, urea, A2-Globulin, aspartate aminotransferase) for male and female treatment groups, which were statistically significant; however, the percentage change was generally less than 5% and considered to have little biological significance.

Urinalysis: The urine volume for the male low dose treatment was decreased to 52% of the control (23 mL); however, decreases for the mid and high dose groups were not statistically significant. No changes were noted for the red blood cells, bilirubin, or urobilinogen, which might be indicative of hemorrhage.

6. **Ophthalmic Examination**: No treatment related effects were found with ophthalmic examinations.

7. **Organ Weights**: Body weights listed in the Relative Organ Weight tables for the end of the 4 week treatment period are lower than those listed in the body weight tables under week 5. Weight loss appears to have approached 10%. For the male high dose group, there was a slight increase of the absolute and relative kidney weight to approximately 114% of the control (3.109 g and 0.867%); however, this change was not apparent at the end of the recovery period. For the female high dose group at the end of the recovery period, the absolute and relative liver weight was decreased to approximately 86% of the control (7.810 g and 3.265%).

8. **Gross Pathology**: For the female high dose group at the end of the 4 week treatment period, one animal had a hematoma at the injection site.

9. **Histopathology**: Slight hemorrhages were found in the thymus, lungs, and Harderian glands, with comparable incidence and severity, among the control and treatment groups. For the female high dose group at the end of the 4 week treatment, mucosal edema was observed in the forestomach for 2 of 6 animals with no comparable finding in controls. For the male high dose group after the 4 week treatment period, 2 of 6 animals were observed with mononuclear cell infiltration in the subcutaneous tissue around the injection site, with no comparable incidence in the control or other treatment groups.

10. **Drug Plasma Levels**: Toxicokinetic data was not reported.

Rats received Org 31540 by subcutaneous injection at dose levels of 0.4, 2, or 10 mg/kg daily for 4 weeks. The no effect level was 2 mg/kg/day. No treatment-related mortality occurred in any group. There was no target organ of toxicity. For the 10 mg/kg/day treatment group at 4 weeks, 2 of 6 females were observed with mucosal edema in the forestomach. Effects observed with the 10 mg/kg/day dosage appeared to be reversible.

A 13 Week Intravenous Toxicity Study Including Toxicokinetics with Org 31540/SR 90107A in Wistar Rats (Report No. 4528).

Testing Laboratory: Scientific Development Group
N.V. Organon
Oss
The Netherlands

Study Started: October 18, 1995

Study Completed: October 29, 1996

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

Animals: Male and female Wistar rats (Hsd/Cpb: WU (Spf)) were used in this study. At the initiation of treatment, males weighed 233-274 grams and females weighed 148-208 grams. The age range of the animals was not reported.

Drug Batch: Org 31540/SR 90107A, Batch RML 18. The test article was manufactured by Sanofi Recherche, Montpellier Cedex, France.

Methods: This study examined the toxicity and toxicokinetics of Org 31540/SR 90107A when administered by the intravenous route to rats at dose levels of 0, 0.4, 2, and 10 mg/kg/day (i.e., 260, 1300, and 6500 anti-Xa units/kg/day, respectively) for 13 weeks. These doses were identical to those used for the two week intravenous toxicity study. Each group consisted of 10 rats/sex. The test article was administered as a sterile, 0.9% NaCl, pyrogen-free solution of either 0.2, 1, or 5 mg/mL. Controls received a 0.9% NaCl solution. The dosing volume was 2 mL/kg. Animals were observed at least once daily at various times for clinical signs (i.e., behavioral and physical abnormalities). Ophthalmic examinations were performed in all groups on one-half the number of animals/sex prior to the initiation of the treatment and at the end of the 13 week treatment period. Body weight was determined twice weekly during the first 4 weeks of treatment and once weekly thereafter. Food consumption was measured once weekly. Hematology, blood clotting, blood biochemistry, and urinalysis were performed at the end of the treatment period on all animals after fasting overnight. Toxicokinetic analysis was performed after the first dosage on day 1 and after 12 weeks of daily intravenous dosing. Blood samples were taken from 2 rats per group per time point at the following time points: 0, 5, 10, 20, and 40 min and 1, 2, 4, 6, and 24 hr post dosing. The concentration of Org 31540/ SR 90107A in plasma was determined by the measurement of anti-Xa activity allowing for calculation of toxicokinetic parameters. All animals were sacrificed after the 13 week treatment period. Gross pathological and organ weight analyses were performed for the control and all treatment groups. Histopathological analysis was performed for the control and 10 mg/kg/day treatment groups.

Results:

1. **Observed Effects:** Signs related to intravenous treatment (i.e., tail lesions, minor hemorrhage, hematoma) were observed for the control and treatment groups. In concordance with the sponsor, these effects appear to be the results from the mechanical insult of heating, distention, and puncture of the vein. One male in the 10 mg/kg/day group broke its teeth during week 13; the food for this animal was moistened to allow it to eat. Signs of eye irritation were noted in the control and 0.4 mg/kg/day groups.

2. **Mortality:** None.

3. **Body Weight and Food Consumption:** There were no significant effects on body weight gain or food consumption for the 0.4 and 2 mg/kg/day groups. Body weight gain for the male 10 mg/kg/day group was impaired by >10%, while there was no effect on the female 10 mg/kg/day group. Body weights for the male control group at the start and end of the 13 week treatment period were 250 and 395 grams, respectively. Weight gains for the male 0.4, 2, and 10 mg/kg/day groups were 98.6, 97.9, and 89.0% of the control,

respectively. Body weights for the female control group at the start and end of the 13 week treatment period were 178 and 243 grams, respectively. Weight gains for the female 0.4, 2, and 10 mg/kg/day groups were 100, 89.2, and 100% of the control, respectively. There were no treatment-related changes in food consumption.

4. Hematology, Coagulation, and Bone Marrow:

A. Hematology: The platelet count for the male 10 mg/kg/day group was significantly elevated to 109.3% of the control (1132 x 10^9/L). The platelet large cell ratio for the male 10 mg/kg/day group was reduced to 7.9% as compared to 9.6% for the control. The neutrophil counts for the male 2 and 10 mg/kg/day groups were reduced to 83.1 and 69.2% of the control (1.3 x 10^9/L); although, these changes were not significant.

The were no significant changes in platelet counts for the female treatment groups.

B. Coagulation: There were no treatment-related changes in prothrombin time or activated partial thromboplastin time.

5. Blood Chemistry and Urinalysis:

A. Blood Chemistry: A number of small changes in blood chemistry parameters were observed; although, there was little if any evidence of a dose response relationship. Plasma hemoglobin levels for the male 0.4, 2, and 10 mg/kg/day groups were reduced to 84.8, 84.8, and 69.7% of the control (3.3 µmole/L), respectively; although, only the change for the high dose group was significant. Triglyceride levels for the male 0.4, 2, and 10 mg/kg/day groups were reduced to 87.5, 73.4, and 68.7% of the control (0.64 mmole/L), respectively; although, these changes were not significant.

B. Urinalysis: No treatment-related findings.

6. Ophthalmic Examination: There were no significant treatment-related ophthalmic effects. One control female was found with a small ectasia on a retinal vessel at the end of the treatment period. For the 0.4 mg/kg/day group, 1 male was found with red discoloration of the medial canthus bilaterally at the end of the treatment period. For the 10 mg/kg/day group, 1 male was found with a dark papil unilaterally during predosing. At the end of the treatment period, this male was found with redness around the papil bilaterally.

7. Organ Weights: Organ weight changes were principally confined to the male 10 mg/kg/day group and there were no histopathological correlations. Absolute and relative thymus weight for the male 10 mg/kg/day group were increased to 113 and 116.7% of the control

respectively. Body weights for the female control group at the start and end of the 13 week treatment period were 178 and 243 grams, respectively. Weight gains for the female 0.4, 2, and 10 mg/kg/day groups were 100, 89.2, and 100% of the control, respectively. There were no treatment-related changes in food consumption.

4. **Hematology, Coagulation, and Bone Marrow**:

A. Hematology: The platelet count for the male 10 mg/kg/day group was significantly elevated to 109.3% of the control (1132 x 10^9/L). The platelet large cell ratio for the male 10 mg/kg/day group was reduced to 7.9% as compared to 9.6% for the control. The neutrophil counts for the male 2 and 10 mg/kg/day groups were reduced to 83.1 and 69.2% of the control (1.3 x 10^9/L); although, these changes were not significant.

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B. Urinalysis: No treatment-related findings.

6. **Ophthalmic Examination**: There were no significant treatment-related ophthalmic effects. One control female was found with a small ectasia on a retinal vessel at the end of the treatment period. For the 0.4 mg/kg/day group, 1 male was found with red discoloration of the medial canthus bilaterally at the end of the treatment period. For the 10 mg/kg/day group, 1 male was found with a dark papil unilaterally during predosing. At the end of the treatment period, this male was found with redness around the papil bilaterally.

7. **Organ Weights**: Organ weight changes were principally confined to the male 10 mg/kg/day group and there were no histopathological correlations. Absolute and relative thymus weight for the male 10 mg/kg/day group were increased to 113 and 116.7% of the control

9. **Histopathology**: Histopathological analysis and evaluation of the treatment site were only performed for the control and 10 mg/kg/day groups.

A. Histopathological Analysis: There were no significant treatment-related lesions; however, for the adrenal glands of 2 (20%) males in the 10 mg/kg/day group, cortical cell vacuolation in the zona fasciculata was found.

Table 2. Incidence of major histopathological findings for rats treated with 0 or 10 mg/kg/day pentasaccharide for 13 weeks.

Histopathology n = 10/group	Pentasaccharide, mg/kg/day							
	0		0.4		2		10	
	M	F	M	F	M	F	M	F
Adrenal gland -cortical cell vacuolation in zona fasciculata							2	
Stomach -serosal hemorrhage and fibrin deposits surrounded with a mixed inflammatory cell infiltrate								1
Duodenum -mucosal mononuclear cell aggregation							1	
Testes -foci of degeneration of germinal epithelium							1	
Urinary bladder -proteinous conglomerate							1	
Thyroid gland -follicular cyst							1	

B. Analysis of The Site of Treatment: Histopathological lesions at the site of treatment appear to be primarily related to injection trauma. The same types of lesions are reported for the control and 10 mg/kg/day groups; although, treatment with the test article increased the incidence for a few lesions (i.e., crusting and foreign body granulomas).

10. **Drug Plasma Levels**: Following either a single intravenous dose or 12 weeks of intravenous dosing at 0.4, 2, or 10 mg/kg/day pentasaccharide to rats, the respective AUC values were not proportional to dose. Following a single dose, AUC values for 2 and 10 mg/kg/day doses were only 2.5 and 7 times the AUC observed for the 0.4 mg/kg/day dose, respectively. For multiple dosing over a 12 week period, AUC values for 2 and 10 mg/kg/day doses were only 2.8 and 7.1 times the AUC observed for the 0.4 mg/kg/day dose, respectively. Previous studies have suggested that doses >1 mg/kg saturate circulating AT-III levels. Comparison of a single intravenous dose versus multiple doses over a 12 week period found that AUC values obtained with multiple doses were higher. Following a single intravenous dose, AUC values between males and females were not different. However, with multiple dosing, AUC values for males appeared to be higher than those observed for females. The halflife of elimination was approximately 1 hr following either a single dose or multiple dosing. The volume of distribution following a single dose of 0.4 mg/kg/day or multiple dosing with 0.4 or 2.0 mg/kg/day was consistent with the blood volume. However, with a single dose of 2 or 10 mg/kg/day or multiple dosing with 10 mg/kg/day, the volume of distribution exceeded the blood volume. Following both single intravenous dosing and multiple intravenous dosing, clearance increased as the dose was elevated; although, it was not proportional to dose. Previous excretion studies have found that the pentasaccharide was primarily eliminated in the urine, and clearance values, listed below, were close to the published value for glomerular filtration rate of 0.31 L/h/kg found with rats.

Table 3. Toxicokinetic parameters following either a single dose or 12 weeks of multiple dosing of 0.4, 2, or 10 mg/kg/day pentasaccharide to rats (Adapted from Sponsor's table in Addendum II of Report No. 4528).

Dose mg/kg	Sex	$AUC_{0-\infty}$ μg-hr-mL^{-1}		AUC_{0-} μg-hr-mL^{-1}		t½ hr		V_d L/kg		CL L-h^{-1}-kg^{-1}	
		SD	MD	SD	MD	SD	MD	SD	MD	SD	MD
0.4	M	4.3	6.0	4.6	6.3	1.05	0.95	0.10	0.07	0.09	0.06
	F	4.2	5.0	4.7	5.2	1.33	0.89	0.08	0.07	0.09	0.08
2	M	10.5	16.8	10.8	17.2	1.24	1.18	0.19	0.05	0.19	0.12
	F	10.9	13.8	11.5	14.1	1.02	1.05	0.20	0.11	0.17	0.14
10	M	32.0	46.7	32.4	47.5	1.06	1.19	0.21	0.11	0.31	0.21
	F	27.7	31.6	27.9	31.9	1.02	0.99	0.15	0.13	0.36	0.31

SD = Single Dose
MD = Multiple Dosing

Rats received Org 31540/SR 90107A (pentasaccharide) by the intravenous route at doses of 0, 0.4, 2, and 10 mg/kg/day for 13 weeks. The no effect level was 2 mg/kg/day. There was no mortality for any treatment groups. For the male 10 mg/kg/day group, weight gain was impaired by 11%. For two males of the 10 mg/kg/day group, cortical cell vacuolation in the zona fasciculata of the adrenal gland was observed. Toxicokinetics of Org 31540/SR 90107A were evaluated following either a single intravenous dose or multiple intravenous dosing for 12 weeks at 0.4, 2, and 10 mg/kg/day. AUC values were not proportional to dose. Previous studies have suggested that doses >1 mg/kg saturate circulating AT-III levels. AUC values for multiple dosing were higher than those found for single doses. Following a single intravenous dose, AUC values between males and females were not different; however, with multiple dosing, AUC values for males were higher. The halflife of elimination was approximately 1 hr following either a single dose or multiple dosing.

MONKEYS

A 2 week intravenous toxicity study in the macaque (Report 693.3.030).

Testing Laboratory: Sanofi Recherche
371 rue du Professeur J. Blayac
34184 Montpellier CEDEX 04
France

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Study Started: November 2, 1994

Study Completed: March 19, 1996

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

Animals: Cynomolgus monkey (Macaca fascicularis). Precise ages were not known because animals were collected from the wild. Initial body weight range was 2.3 to 3.6 kg for males and females.

Drug Batch: SR 90107A, Batch 92N037.

Methods: The aim of this study was to assess the subacute toxicity of SR 90107A to the cynomolgus monkey following repeated administration by the intravenous route during at least 15 days. The test compound (SR 90107 A) was administered intravenously at dose levels of 0.4, 2, or 10 mg/kg/day in the left or right hindlimb. Control animals received the vehicle. Each group consisted of 3 males and 3 females. Animals were observed twice daily throughout the study for clinical signs and mortality. Electrocardiography was performed on days -6 and +14 (2 to 4 hr after test compound administration). An ophthalmological examination was performed on days -5 and +14 on all animals. Body weights were measured weekly. Food consumption was estimated daily, from which mean individual daily values were calculated on a weekly basis. Blood samples for hematology and plasma biochemistry were collected on days -12, +13, and +15 (hematology only). Urinalysis was performed on all animals on days -7 and +14 on overnight urine samples collected following oral administration (gavage) on the previous day of 20 ml/kg water. Necropsy was performed from day 16 to day 20 on all surviving animals.

Results:

1. **Observed Effects**: For both the control and treatment groups, redness, due to serohemorrhagic infiltration from injection trauma, was observed at injection sites on both the right and left hindlimbs. Large hematomas were noted on the left elbow or forelimb in two females from the high dose group from days 7 and 3 onwards, respectively. These hematomas were first noted as redness and then as tumefaction. The sponsor attributed them to handling trauma, probably aggravated by the pharmacological effects of SR 90107A.

2. **Mortality**: There were no deaths in the present study; however, one male macaque, in the intravenous toxicokinetics study (Report 693.5.009), treated with a single dose of 10 mg/kg, was found dead, 24 hr after dosing due to hemorrhage at the blood sampling site.

3. **Body Weight and Food Consumption**: There were no treatment related effects on body weight gain or food consumption. Initial body weights for the male 0, 0.4, 2, and 10 mg/kg/day groups were 2683, 2987, 2850, and 2963 g, respectively. At day 14, the male 0, 0.4, 2, and 10 mg/kg/day treatment groups had lost 8, 12.2, 13.8, and 5.6% of their body weight at day 1. At week 1, food consumption for the male low, mid, and high dose groups was 126, 171, and 136.7% of the control, respectively; although, only the mid dose group was significantly different. At week 2, food consumption for the male low, mid, and high dose groups was 119, 123.6, and 109.5% of the control; although, these differences were not significant. Initial body weights for the female 0, 0.4, 2, and 10 mg/kg/day treatment groups were 2573, 2700, 2840, and 2517 g, respectively. At day 14, the female 0, 0.4, 2, and 10 mg/kg/day treatment groups had lost 10.5, 16.4, 11.9, and 13.5% of their body weight at day 1. There were no differences in food consumption between the female control and treatment groups.

4. **Hematology, Coagulation, and Bone marrow**:

Hematology: There were no significant differences in platelet counts between control and treatment groups. For the female high dose group, erythrocyte counts on day 13 were decreased to 73% of the control ($5.81 \times 10^6/mm^3$); although, this value was not significantly different. However, the PCV for the female high dose group on day 13 was significantly decreased to 35.9% as compared with a control value of 45.5%. Also, for the female high dose treatment group, the reticulocyte percentage was increased to 375% of the control (1.76%); although, this value was not statistically significant. For the male low, mid, and high dose treatment groups, fibrinogen levels on day 13 were increased to 107, 121, and 135% of the control (1.32 g/L). For the female mid and high dose groups, fibrinogen levels on day 13 were increased to 114 and 141.7% of the control (1.97 g/L).

Coagulation: Prothrombin time (PT) was increased in both male and female treatment groups on day 15. For the male low, mid, and high dose treatment groups, PT was increased to 110.7, 113.4, and 119.6% of the control value (11.2 sec); however, these increases were not statistically significant. PT for the female low, mid, and high dose treatment groups were significantly increased to 109, 116, and 120.7% of the control (11.1 sec). Activated partial thromboplastin time (APTT) was increased in

both male and female treatment groups on day 15. For the male low, mid, and high dose treatment groups, APTT was increased to 116.5, 119, and 143% of the control value (22.9 sec); although, only the high dose value was statistically significant. APTT for the female low, mid, and high dose treatment groups were increased to 117.4, 119, and 153% of the control (23.0 sec); although, only the mid and high dose values were statistically significant.

Bone Marrow: An examination of femoral and radial bone marrow suggested increased erythropoiesis. For the female high dose treatment group, the % reticulocytes was increased to 375% of the control; although, this value was not statistically significant.

5. **Blood Chemistry and Urinalysis**:

Blood chemistry: For male low, mid, and high dose treatment groups, plasma glucose levels on day 13 were significantly increased to 139.5, 129.1, and 128.9% of the control (4.18 mM). No comparable effect was observed for female treatment groups. For the female mid and high dose treatment groups, plasma bilirubin levels on day 13 were increased to 188 and 253% of the control (1.7 μM), respectively; although, only the mid dose group was statistically significant. For the female mid and high dose groups, plasma lactate dehydrogenase activity on day 13 was increased to 138.6 and 168.4% of the control (407 IU/L), respectively; although, these values were not statistically significant.

Urinalysis: For male low, mid, and high dose treatment groups, excreted quantities of chloride in the urine were increased to 402.8, 158, and 222% of the control (615 μmoles), respectively; although, only the high dose group was statistically different. For the male low and high dose treatment groups, erythrocytes were detected in the urine on day 14 at values of 1.3 ± 0.67 and 1.0 ± 0.0, respectively as compared with a control value of 0 ± 0; although, only the high dose group was significantly different. For the female low, mid, and high dose groups, erythrocytes were detected in the urine on day 14 at values of 1.0 ± 0.0, 1.3 ± 0.33, and 1.0 ± 0.58, respectively, compared with a control value of 0.0 ± 0.0.

6. **Electrocardiographic and Ophthalmic Examinations**: No treatment related effects were found during either electrocardiographic or ophthalmologic examinations.

7. Organ Weights: The relative thymus weight for male low, mid, and high dose treatment groups was decreased to 56.4, 51.2, and 55.8% of the control (1684 mg/kg); statistical analysis revealed that the low and mid dose groups were significantly different from the control. Changes in absolute thymus weights for the male low, mid, and high dose groups paralleled relative weights. Relative thymus weights for the female mid and high dose groups were decreased to 83.9 and 68.0% of the control (945 mg/kg); however, these values were not statistically significant. Relative thyroid weights for male low, mid, and high dose treatment groups were increased to 148, 160, and 152% of the control (216 mg/kg); however, these values were not statistically significant. Absolute and relative testes, prostate, and seminal vesicle weights for the male low and high dose treatment groups were increased over corresponding control values; however, no changes were evident for the mid dose group.

8. Gross Pathology: Treatment related lesions were observed at the injection sites: hematomas, thickening of the vein wall, cutaneous swelling and redness as well as edema and desquamation, and dark discoloration of the popliteal lymph nodes (draining nodes). Hematomas and thickening of the vein wall, observed in all groups, were mainly due to injection trauma. Hematomas were more extended in animals receiving SR 90107 A, particularly at the high dose level.

Table 21. Incidence of hematomas at the injection site (Adapted from Sponsor's table in Report 693.3.030).

Dose	0		0.4 mg/kg/d		2 mg/kg/d		10 mg/kg/d	
N	6		6		6		6	
	R	L	R	L	R	L	R	L
Injection Sites Subcutaneous Hematomas								
Small	3	3	2	1	5	1	1	3
Medium to large	1	2	2	3	-	3	4	3

A hematoma was also noted on the left eye brow in a male from the mid dose group.

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Hematomas were observed on the whole left arm or left forearm (up to the elbow) in two females of the high dose treatment group. These large hematomas were reported during clinical examination and induced marked anemia. They were associated with health deterioration characterized by marked decreased thymus volume on macroscopic examination. These hematomas were probably the result of trauma and the pharmacological properties of SR 90107A; the pale discoloration noted in the lungs of this animal could be related to anemia.

For the thymus, superficial petechiae were observed for 1 male in the low dose group and one male and one female in the mid dose group with no comparable incidence in the control. For the lungs, grey areas were noted on the surface for 1 male control, 1 female in the low dose group, 1 male and 2 females in the mid dose group, and 1 male in the high dose group. For the popliteal lymph nodes, marked discoloration of one or both nodes was observed for 2 females of the low dose group and one female of the high dose group.

9. **Histopathology**: At the injection sites, hemorrhagic or sero-hemorrhagic infiltration (edematous) and hematomas were present. Inflammatory cell infiltration into these sites occurred. This ranged from acute in 1 case to subacute in a few cases to fibrous in most cases. These lesions were attributed to injection trauma, noted in the perivascular and hypodermic tissues, and occurred with similar frequency between control and treatment groups. They were associated with various lesions also related to injection trauma: veins, narrow vascular rupture, fibrosis of media; dermis, hemorrhagic or sero-hemorrhagic infiltration and subacute, granulomatous, chronic or fibrous inflammatory cell infiltration; epidermis, hyperplasia; and draining lymph nodes (popliteal and iliac lymph nodes), hemorrhagic and erythro-sidero-macrophagic infiltration, and sometimes fibrosis.

Table 22. Histopathological lesions at the injection site (Adapted from Sponsor's table in Report 693.3.030).

Dose	0		0.4 mg/kg/d		2 mg/kg/d		10 mg/kg/d	
N	6		6		6		6	
Side	R	L	R	L	R	L	R	L
Injection Sites: perivascular tissues and hypodermis								
Hemorrhagic or sero-hemorrhagic infiltration/hematoma								
Slight/ small	3	3	2	2	3	2	5	2
Moderate/ medium	2	1	2	4	1	3	1	2
Marked/ large		1						1
Inflammatory cell infiltration								
Slight	3	4	4	1	2	2	3	2
Moderate	2	2	1	5	2	3	2	3
Bone Marrow								
Increased Erythropoiesis								
Slight					1		1	
Moderate/ marked							3	

Changes were noted for the kidney, thymus, popliteal lymph nodes, stomach, and mesenteric lymph nodes where the percentage incidence for treatment groups was slightly higher than the control group. These were not test article specific effects and a dose response relationship was not evident. For the kidney, chronic cortical inflammatory cell infiltration or tubulo-interstitial nephritis (slight focal or multifocal)was noted for two males in each of the low, mid, and high dose groups with no incidence in the control group. For the thymus, involution (slight physiological to chronic) was noted for 1 female control, 2 males of the low dose group, 1 male and 1 female of the mid dose group, and 2 males and 2 females of the high dose group. For the popliteal lymph nodes, macrophages, erythrophage and/or siderophage infiltration (ranging from slight to moderate, uni or bilateral) was observed for 1 female control, 3 males and 3 females of the low dose group, 3 males and 1 female of the mid dose group, and 2 males and 2 females of the high dose group. For the stomach, multifocal or zonal fibrosis with atrophy in the inner layer or both layers of the muscularis was observed for 1

female control, 1 female of the low dose group, 2 males and 1 female of the mid dose group, and 1 male and 1 female of the high dose group. For the mesenteric lymph node, slight macrophage or siderophage infiltration was observed for 1 female control, 1 male and 2 females of the low dose group, 1 female of the mid dose group, and 2 males and 1 female of the high dose group.

For the liver, centrilobular or panlobular hepatocyte cytoplasmic margination was observed for all animals.

10. Drug Plasma Levels: Anti-Xa activity was significantly enhanced on day 15, one hr after drug administration in both male and female treatment groups. Anti-Xa activity for male low, mid, and high dose animals was 1.49, 4.17, and 15 IU/ml, respectively. Anti-Xa activity for female low, mid, and high dose animals was 1.70, 4.27, and 14.67 IU/ml, respectively. On day 15, prior to drug administration, there was no appreciable anti-Xa activity.

Cynomolgus monkeys received SR 90107A by the intravenous route at dose levels of 0.4, 2, or 10 mg/kg/day for 15 days. The no effect level was 2 mg/kg/day. One male treated with a single dose of 10 mg/kg in the toxicokinetics study died as a result of hemorrhage. There was no target organ of toxicity. Significant pathological lesions were associated with the injection site, and attributed to injection trauma aggravated by the pharmacological activity of SR 90107A, particularly in the high dose treatment group. For the high dose treatment group, there was evidence of hematomas at the injection site as well as increased erythropoiesis in the bone marrow as a compensatory response to hemorrhage.

Org 31540/CY 234 4-week subchronic toxicity study by repeated subcutaneous injection followed by a 2 week recovery period in cynomolgus monkeys (SDGRR 2861).

Testing Laboratory: [ ]

Study Started: January 10, 1990

Study completed: February 25, 1991

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

Animals: Cynomolgus monkeys. Precise ages were not known, because these animals were wild-caught. They had a mean body weight of 3.5 ± 0.3 kg for males and 2.4 ± 0.2 kg for females on day 1.

Drug Batch: ——— 89032/1 or 2 Org 31540 10 mg/ml Test #ZA-015.

Methods: Twenty-eight cynomolgus monkeys (14 males and 14 females) were used in this study. Control and high dose treatment groups consisted of 4 males and 4 females each. The low and middle dose treatment groups consisted of 3 males and 3 females each. The test substance was administered daily by subcutaneous injection at dose levels of 0.4, 2, or 10 mg/kg/day for 28 days. Clinical examinations, monitoring for any mortality, and estimation of food consumption were performed daily. Body weight was recorded once before the end of treatment and at weekly intervals until the end of the study. Hematological examination, blood biochemical analysis, and urinalysis were performed during the predosing period and on week 4. Electrocardiographic examinations were performed before the beginning of treatment, on weeks 2 and 4 (2 to 4 hr after treatment), and at the end of the recovery period. Ophthalmologic examinations were performed before the beginning of treatment and on week 4 (2 to 4 hr after treatment). After 28 days of treatment, one male and one female from the control and high dose treatment groups were chosen for a recovery period of 2 weeks. At the end of the treatment and recovery periods, the animals were sacrificed. All tissues were examined macroscopically, the weights of the main organs were recorded and histological examination was performed.

Results:

1. **Observed Effects**: A swelling at the injection site was observed in 2 males and one female of the 10 mg/kg/day group. For one male, the swelling was noted after 7 days of treatment and disappeared 8 days later. For the other male, it was noted after 21 days of treatment and disappeared 3 days later. In the female, the swelling was only noted on the last day of treatment. For a female of the 0.4 mg/kg/day group, a swelling of the right thigh was observed on the last day of treatment, which was probably caused by injection of the anesthetic agent.

One male of the 0.4 mg/kg/day group showed an incomplete paralysis of the right arm from day 14 until the end of treatment. The absence of a similar change in the leg of the same side, probably excluded a possible central effect. The paralysis could be due to the presence of a small hematoma on a nerve. A wound at the tip of the tail was noted in this animal from day 10 until the end of the treatment. The wound was disinfected daily from day 14 with Betadine (an iodine solution).

2. **Mortality**: None.

3. **Body Weight and Food Consumption**: No treatment related effects were observed for body weight or food consumption. Body weights for the male 0, 0.4, 2, and 10 mg/kg/day treatment groups at week 1 were 3.4, 3.6, 3.6, and 3.4 kg, respectively. Body weight gain for the male 0, 0.4, 2, and 10 mg/kg/day groups were 0.2, 0.1, 0.2, and 0 kg, respectively. Body weights for the female 0, 0.4, 2, and 10 mg/kg/day treatment groups at week 1 were 2.4, 2.5, 2.5, and 2.3 kg, respectively. Body weight gain for the male 0, 0.4, 2, and 10 mg/kg/day groups were 0.1, 0, 0, and 0 kg, respectively.

4. **Hematology, Coagulation, and Bone Marrow**:

Hematology: For one male of the 10 mg/kg/day at week 4, decreases of the erythrocyte count, hemoglobin content, and PCV to approximately 50% of the control value (——————— ——— were observed. These changes were not observed after the recovery period. There were no changes of either bilirubin levels or the maturation index of the bone marrow erythroid elements. There were no significant changes in platelet counts between control and treatment groups. White blood cells for female low, mid, and high dose treatment groups at 4 weeks were decreased to 80.4, 61.6, and 77.4% of the control (13.3 G/l).

Coagulation: An increase in the activated partial thromboplastin time (APTT) was observed for both male and female low, mid, and high treatment groups, when measured 2 hr after test compound administration. For male low, mid, and high dose groups, the APTT was increased to 124, 145.6, and 162.4% of the control value (23.7 sec). For female low, mid, and high dose groups, the APTT was increased to 108, 122.4, and 145.9% of the control value (25.9 sec). Prior to the injection on day 28, APTT for both male and female treatment groups were not different from the control values. In contrast to the increase of APTT, no treatment-related effects on thrombin time were observed; although, a slight increase was found for 1 female in the high dose group.

Bone Marrow: After either the 4 week study period or the recovery period, no changes were observed in either the maturation index or the in the myeloid/erythroid ratio from quantitative analysis of bone marrow smears. There were no treatment related effects on the distribution of megakarocytes.

5. **Blood Chemistry and Urinalysis:**

Blood Chemistry: Blood glucose levels for the female high dose treatment group at 4 weeks were increased to 172% of the control (4.75 mmol/L); although, no changes were observed for the low and mid dose groups. The creatinine value for one female of the high dose group was increased to 135% of the control value (; however, for the high dose group as a whole, there was no significant difference from the control.

Urinalysis: No treatment related effects were noted during urinalysis.

6. **Electrocardiographic and Ophthalmic Examination:** No treatment- related effects were observed on heart rate or electrocardiographic parameters (PQ, QRS, and QT intervals). No treatment related effects were found with ophthalmic examinations.

7. **Organ Weights:** Significant variations were noted in absolute and relative weights for several organs (i.e., spleen, adrenal glands, thyroid gland, pituitary gland, testes, prostate, ovaries, and thymus) between control and treatment groups. These findings are somewhat complicated by the consideration that these animals were caught in the wild and vary in age and sexual maturity. Many of these changes were not found in the recovery groups and in some cases were even reversed; however, each recovery group was only composed of one animal and it is difficult to interpret the significance of these changes in comparison with 4 week control and treatment groups. In general, macroscopic and microscopic pathological changes do not correlate with altered absolute and relative organ weights, and there was no clear dose response relationship.

Spleen: Absolute spleen weights for male low, mid, and high dose groups were increased to 148, 192, and 155% of the control (4.864 g), respectively; although, only the change for high dose group was significant. Relative spleen weights for male low, mid, and high dose groups were increased to 135, 173, and 155% of the control group (1.446 g/kg). Absolute spleen weights for female low and high dose groups were decreased to 72.9 and 59% of the control (8.156 g); although, the mean for the mid dose group was slightly higher than the control.

Liver: Relative liver weights for male low, mid, and high dose groups were increased to 101, 103, and 123% of the control (17.884 g/kg).

Testes: Absolute testes weights for male low, mid, and high dose groups were decreased to 67.8, 81.8, and 26.1% of the control (15.555 g); although, there was significant variation within groups and statistical significance was not reached. Relative testes weights for male low, mid, and high dose groups were decreased to 59.6, 70.5, and 26.2% of the control (4.735 g/kg); however, only the value for the high dose group was significant.

Prostate: Absolute prostate values for male low, mid, and high dose groups were decreased to 63.6, 69.9, and 27.6% of the control (2.154 g); although, only the high dose value was significant. Relative prostate values for male low, mid, and high dose groups were decreased to 56.7, 61, and 26.7% of the control (0.657 g/kg); and appear to closely parallel values observed for testes.

Thyroid: Absolute thyroid weights for male low, mid, and high dose groups were increased to 155, 157.6, and 119.5% of the control (0.297 g); although, only the mid dose was significantly higher and relative values were not different from the control.

Thymus: Absolute thymus weights for male low, mid, and high dose groups were increased to 122.6, 151.8, and 180.6% of the control (2.619 g); however, there was extremely high variation within groups and these groups were not significantly different. Relative thymus weights for male low, mid, and high dose groups were increased to 116.8, 146, and 172.4% of the control (0.772 g/kg); although, variation within groups was extremely high.

Ovaries: Absolute ovary weights for the female mid and high dose groups were both increased to 150% of the control (0.201 g). Relative ovary weights were similarly increased.

Kidney: The absolute kidney weight for the female high dose group was decreased to 80% of the control (13.203 g); although, this value was not statistically significant.

8. **Gross Pathology:** At the end of the treatment period, hematomas were found at the injection site for 1 female in the 0.4 mg/kg/day and for 1 male and 1 female in the 10 mg/kg/day group. Also, a hematoma was found on the right leg for 1 female in the 0.4 mg/kg/day group.

Changes were noted for the caecum, liver, and lung in both control and treatment groups that occurred with a high frequency within each group; although, the percentage incidence was not different between groups.

9. **Histopathology:** For the subcutaneous tissue at the injection site, there was hemorrhage for two females in the low dose group and 1 male in the high dose group.

For animals in the control and treatment groups, there was evidence of mononuclear cell aggregates in the liver, skin, brain, parotid glands, mandibular glands, tongue, skeletal muscle, heart, adrenal glands, pancreas, and gall bladder. Further, evidence of parasitic infection for these animals in the 4 week treatment groups was found in the caecum, colon, rectum, stomach, and tongue skeletal muscle. Almost all animals in the control and treatment groups during the treatment and recovery periods had evidence of chronic interstitial pneumonia.

For the prostate, there were mononuclear cell aggregates for only 1 male control as compared to all male low, mid, and high dose animals. There were small tubuloalveolar units for one male in each of the low and mid dose groups and all three males in the high dose group. There was increased fibromuscular stroma for one male in each of the low and mid dose groups and all three males in the high dose group. These differences were also noted in the recovery group male.

For the testes, there was a small seminiferous tubule for one male in each of the low and mid dose groups and two males in the high dose group. There was reduced spermiogen for one male in each of the low and mid dose groups and all three males in the high dose group. These differences were also noted in the recovery group male.

For the epididymis, there was interstitial mononuclear cell aggregates for one male in each of the low and high dose groups. There was oligospermia for one male in each of the low and mid dose groups and all three males in the high dose group. These differences were also noted in the recovery group male.

Cynomolgus monkeys received Org 31540/SR 90107A by subcutaneous injection at dose levels of 0.4, 2, or 10 mg/kg/day for 26 days. The no effect level was 2 mg/kg/day. There was no treatment-related mortality in any group. There was no target organ of toxicity. For one male of the 10 mg/kg/day group at week 4, decreases of the erythrocyte count, hemoglobin content, and PCV to approximately 50% of the control value were observed. Blood glucose levels for the female high dose treatment group at 4 weeks were increased to 172% of the control; although, no changes were observed for the low and mid dose groups. These animals were caught in the wild and vary in age and sexual maturity; however, the incidence of histopathological changes in the testes, prostate, and epididymis were higher in the male 10 mg/kg/day treatment group.

Three Months Intravenous Toxicity Study in the Macaque (Amendment #034; Report 693.3.037).

Testing Laboratory: Department of Toxicology and General Pharmacology
Sanofi Recherche
371 rue du Professeur J. Blayac
34184 Montpellier Cedex 04
France

Study Started: December 11, 1985

Study Completed: April 3, 1997

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

Animals: Cynomolgus monkeys (Macaca fascicularis) supplied from [redacted] The monkeys were classified as young animals (i.e., animals were caught in the wild and ages were unknown) and body weight ranges were 2.7 to 5 kg for male monkeys and 2.6 to 3.1 kg for female monkeys.

Drug Batch: SR 90107A, Batch M 279T. The test compound was supplied in a salified form as a 10 mg/mL solution in 1 mL syringes.

Methods: In a three-month intravenous toxicology study, cynomolgus monkeys received SR90107A at doses of 0, 0.4, 2, and 10 mg/kg/day (doses expressed as salified form). Control animals received the vehicle, isotonic NaCl. There were 4 monkeys/sex/group. SR90107A was administered by the intravenous route using a dosage volume of 1 mL/kg to hindlimb veins and secondarily to forelimb veins. Animals were observed at least two times per day for clinical signs of toxicity and moribundity/mortality. Tolerability at the injection site was evaluated daily. Electrocardiograms were recorded on day -5 and on days 31 and 95, prior to dosing

and consisted of three standard limb leads I, II, and III and three augmented limb leads, aVR, aVL, and aVL. The following parameters were measured: heart rate, RR duration, rhythm, PR duration, P duration, QRS duration, QT duration, $QT_{corrected}$, R amplitude (lead II), T amplitude (lead II), and T configuration (lead II). Ophthalmic examinations were conducted on all animals on days -3, 33, and 93. Body weights were measured on a weekly basis throughout the treatment period. Food consumption was estimated on a daily basis. Blood for determination of hematology and plasma biochemistry parameters was collected on day -11 and days 30 and 92, prior to dosing. Blood for determination of coagulation parameters (i.e., prothrombin time, activated partial thromboplastin time, thrombin clotting time, prothrombin time, fibrinogen levels, and plasma levels of anti-Factor Xa activity) was collected on days -21 and -11, prior to the start of treatment, and on days 32 and 94, prior to and 1 hr after dosing. Blood for determination of lymphocyte subpopulations, thyroxin (T_4), and triiodothyronine (T_3) was collected on day -11 and day 92, prior to dosing. Blood for conducting functional tests with circulating lymphocytes (i.e., lymphoblast transformation tests using the following mitogens, concanavillin A, phytohemagglutinin, and pokeweed mitogen, one way mixed lymphocyte culture, and NK cytotoxicity) was collected on day -4 and day 96, prior to dosing. Urine for determination of urinalysis parameters was collected overnight on days -6, 26, and 86 following oral administration of water (20 mL/kg) on the previous day. Terminal sacrifice of all surviving animals occurred from days 99 to 102. All animals were subjected to a complete necropsy. Absolute and relative organ weights were determined for the liver + gallbladder, spleen, kidneys, adrenal glands, thymus, heart, lungs, ovaries, uterus, testes, seminal vesicles, and prostate. Tissues and organs were collected, preserved, processed using standard techniques, and submitted to microscopic examination as follows: injection sites (i.e., right and left hindlimb veins), skin and subcutaneous tissues, mammary tissue, liver, gallbladder, spleen, kidneys, adrenal glands, thymus, heart, lungs, tracheobronchial lymph nodes, urinary bladder, ovaries, fallopian tubes, corpus uteri, uterine cervix, vagina, testes, epididymides, seminal vesicles, prostate, aorta, sciatic nerve, popliteal lymph nodes, crural muscle, pancreas, esophagus, stomach contents, pancreas, esophagus, stomach (cardia, fundus, and pyloric area), duodenum, jejunum, ileum, cecum, colon, rectum, mesenteric lymph nodes, parotid glands, submandibular glands, thyroid glands, larynx, trachea, tongue, eyes, optic nerve, brain, pituitary gland, spinal cord, femoral bone marrow, and proximal radial epiphysis. Sections of liver and kidneys were collected for electron microscopic examinations; however, these examinations were not performed.

Results:

1. **Observed Effects:** For the control and treatment groups, localized to extensive redness and slight to marked induration were observed at most injection sites (i.e., hindlimb veins in all animals and forelimb veins in a few animals). These lesions were principally attributed to trauma related to injection and handling. However, the incidence and severity of these changes were increased in SR90107A-treated groups, which was considered to be due to the pharmacological activity of the test compound. These changes displayed no dose response relationships. Hemorrhage and hematoma formations were also observed at injection sites for all treatment groups as well as the

control (see Histopathology); however, the incidence was higher for SR90107A treatment groups at 2 and 10 mg/kg/day. Concomitant changes related to hemorrhage and hematoma formation for a few animals (i.e., 1 or 2) at 2 and 10 mg/kg/day included pallor of mucous membranes, hypothermia, decreased activity, and weakness or prostration.

Local Tolerance (Observations at all injection sites)

Lesions at injection sites	0 mg/kg/day		0.4 mg/kg/day		2 mg/kg/day		10 mg/kg/day	
	Male	Female	Male	Female	Male	Female	Male	Female
Local Redness								
-# animals	3	3	4	4	4	3	4	4
-total occurrence	13	35	50	19	48	22	45	49
Extensive Redness								
-# animals	0	0	0	2	1	2	2	3
-total occurrence				4	4	7	6	23
Slight or marked induration								
-# animals	1	1	0	3	3	2	2	4
-total occurrence	2	5		13	4	8	11	22

2. **Mortality:** Mortality occurred for 1 male monkey in each the of the 0.4 and 2 mg/kg/day groups; however, these deaths appeared to be unrelated to treatment as no mortality occurred in the 10 mg/kg/day group. During the treatment period, 1 male monkey (#9) at 0.4 mg/kg/day was found dead on day 73 and 1 male monkey (#20) at 2 mg/kg/day was found dead on day 64. Death for the male monkey at 2 mg/kg/day was attributed to a large recurrent hematoma in the left armpit with consequent anemia (i.e., discoloration of mucous membranes and several organs as well as increased bone marrow erythropoiesis) and deterioration of general health. The hematoma was believed to be caused by handling trauma and the pharmacological activity of SR90107A. The death of the male monkey at 0.4 mg/kg/day was attributed to acute gastric dilatation (i.e., spontaneous pathology according to the sponsor). Macroscopic examination found severe abdominal distention. Microscopic examination of the stomach found gram positive bacilli in the lumen, stretching of muscle cells, and edema in the submucosa. This was accompanied by lung atelectasis (i.e., incomplete expansion of a lung or a portion of a lung). Passive congestion, edema, and hemorrhage were observed in several organs.

3. **Body Weight and Food Consumption:** Body weight gain and food consumption for female monkeys at 10 mg/kg/day were impaired. Body weights of male controls on day's -3 and 99 were 4605 and 4738 g, respectively, yielding a 2.9% increase of initial body weight body. The initial mean body weight of male controls was higher than that of other male treatment groups (i.e., 3703, 3718, and 3780 g for male monkeys on day -3 at 0.4, 2, and 10 mg/kg/day, respectively). Body weight gains of male monkeys at 0.4, 2, and 10 mg/kg/day, expressed as percentage of initial mean body weights on day -3, were 18.3, 8.2, and 10.1%, respectively. Body weights of female controls on days -3 and 99 were 2888 and 2893 g, respectively, yielding a 0.2% increase of initial body weight. Body weight gains of female monkeys at 0.4, 2, and 10 mg/kg/day, expressed as percentage of initial mean body weights on day -3, were 10.3, 11.3, and -0.8%,

respectively. Mean food consumption for male monkeys at 0.4, 2, and 10 mg/kg/day over the 14-week treatment period was 96.9, 92.5, and 93.5% of the control (183.5 g/24 hr), respectively. Mean food consumption for female monkeys at 0.4, 2, and 10 mg/kg/day over the 14-week treatment period was 101.7, 104.7, and 84.2% of the control (169 g/24 hr), respectively.

4. **Hematology and Blood Coagulation**: Primarily at the high dose of 10 mg/kg/day but also at the mid dose of 2 mg/kg/day, decreases in red blood cell counts, hemoglobin levels, and hematocrit were observed on days 30 and 92. Compensatory increases of the reticulocyte percentage were evident. Prothrombin time and activated partial thromboplastin time were increased for all treatment groups on days 32 and 94 at 1 hr after dosing.

Hematology: For female monkeys at 10 mg/kg/day on day 30, red blood cell (RBC) counts, hemoglobin levels, and hematocrit were decreased to 94.1, 93.4, and 94.6% of control values (6.15 x 10^6/mm^3, 122 g/L, and 46.4%), respectively. For male monkeys at 10 mg/kg/day on day 92, RBC counts, hemoglobin levels, and hematocrit were decreased to 80, 80, and 79.2% of control values (6.44 x 10^6/mm^3, 130 g/L, and 47.6%), respectively. For female monkeys at 2 mg/kg/day on day 92, red blood cell (RBC) counts, hemoglobin levels, and hematocrit were decreased to 88.7, 91.9, and 92.4% of control values (6.20 x 10^6/mm^3, 123 g/L, and 44.7%), respectively. For female monkeys at 10 mg/kg/day on day 92, red blood cell (RBC) counts, hemoglobin levels, and hematocrit were decreased to 87.4, 88.6, and 92.2% of control values, respectively. For male monkeys at 2 and 10 mg/kg/day on day 30, reticulocyte percentages were increased to 514.6 and 173.2% of the control (0.41%), respectively. For male monkeys at 10 mg/kg/day on day 92, the reticulocyte percentage was increased to 185.1% of the control (0.49%). For female monkeys at 10 mg/kg/day on day 30, the reticulocyte percentage was increased to 152% of the control (0.60%). For female monkeys at 2 and 10 mg/kg/day on day 92, the reticulocyte percentages were increased to 167.7 and 614.5% of the control (0.62%), respectively.

For female monkeys at 2 and 10 mg/kg/day on day 30, neutrophil percentages were increased to 130 and 198% of the control (32.1%), respectively. For female monkeys at 2 and 10 mg/kg/day on day 30, neutrophil counts were increased to 146.3 and 305% of the control (3.61 x 10^3/mm^3), respectively. For female monkeys at 10 mg/kg/day on day 30, the lymphocyte percentage and count were decreased to 52.4 and 56.9% of control values (55.5% and 6.31 x 10^3/mm3), respectively. For female monkeys at 10 mg/kg/day on day 30, the eosinophil percentage and count were decreased to 21.4 and 27.3% of control values (4.2% and 0.44 x 10^3/mm^3), respectively. For female monkeys at 2 and 10 mg/kg/day on day 92, no changes in neutrophil, lymphocyte, or eosinophil percentages or counts were evident. For male monkeys at 2 and 10 mg/kg/day on day 92, neutrophil percentages were increased to 133.6 and 172.8% of the control (23.2%), respectively. For male monkeys at 2 and 10 mg/kg/day on day 92, neutrophil counts were increased to 156.4 and 281.4% of the control (2.04 x 10^3/mm^3), respectively.

Slight alterations in lymphocyte subpopulations were observed principally for female monkeys at 10 mg/kg/day on day 92; although, these change probably had little or no biological significance. T8 lymphocyte percentages for female monkeys at 2 and 10 mg/kg/day were decreased to 77.8 and 82.1% of the control (27.5%), respectively. The T8 lymphocyte count for female monkeys at 10 mg/kg/day was decreased to 73.8% of the control (1.60 x 10^3/mm^3), respectively. The NK lymphocyte percentage and count for female monkeys at 10 mg/kg/day were increased to 166.7 and 137.8% of control values (14.1% and 0.82 x 10^3/mm^3), respectively. No treatment-related changes were found with lymphocyte functional tests.

Blood Coagulation: For female monkeys at 10 mg/kg/day on day 32 before treatment and 1 hr after treatment, fibrinogen levels were increased to 198.3 and 207.8% of control values (2.97 and 2.70 g/L), respectively. No changes in fibrinogen levels for female monkeys at 10 mg/kg/day on day 94 were evident. Prothrombin time (PT) on day 32 at 1 hr after treatment for male monkeys at 0.4, 2, and 10 mg/kg/day were increased to 114.6, 120.3, and 118.5% of the control (10.25 sec), respectively. Activated partial thromboplastin time (APTT) on day 32 at 1 hr after treatment for male monkeys at 0.4, 2, and 10 mg/kg/day were increased to 136.5, 146.6, and 168.6% of the control (27.7 sec), respectively. PT on day 32 at 1 hr after treatment for female monkeys at 0.4, 2, and 10 mg/kg/day were increased to 113.4, 118.3, and 130% of the control (10.23 sec), respectively. APTT on day 32 at 1 hr after treatment for female monkeys at 0.4, 2, and 10 mg/kg/day were increased to 137.6, 158.1, and 174.3% of the control (21.0 sec), respectively. No statistically significant differences in PT or APTT were observed for male or female treatment groups on day 32 before treatment. PT on day 94 at 1 hr after treatment for male monkeys at 0.4, 2, and 10 mg/kg/day were increased to 112.1, 116.9, and 117.1% of the control (10.5 sec), respectively. APTT on day 94 before treatment for male monkeys at 2 and 10 mg/kg/day were increased to 120.9 and 120% of the control (21.5 sec), respectively. APTT on day 94 at 1 hr after treatment for male monkeys at 0.4, 2, and 10 mg/kg/day were increased to 137.6, 158.1, and 174.3% of the control (21.0 sec), respectively. PT on day 94 at 1 hr after treatment for female monkeys at 0.4, 2, and 10 mg/kg/day were increased to 111.6, 114, and 120.5% of the control (10.48 sec), respectively. APTT on day 94 before treatment for female monkeys at 2 and 10 mg/kg/day were increased to 113.7 and 112.8% of the control (21.9 sec), respectively. APTT on day 94 at 1 hr after treatment for female monkeys at 0.4, 2, and 10 mg/kg/day were increased to 135.5, 148.6, and 164.9% of the control (21.4 sec), respectively. No statistically significant differences in PT were observed for male or female treatment groups on day 94 before treatment.

5. **Blood Biochemistry and Urinalysis:** Lactate dehydrogenase levels for male and female treatment groups were elevated on days 30 and 92; although, this enzyme is a nonspecific marker of tissue damage. T_3 levels were decreased on day 92 for male treatment groups and female monkeys at 10 mg/kg/day. Most plasma biochemistry changes were confined to the high dose group and appeared to have no histopathological correlations.

Blood Biochemistry, Day 30: Lactate dehydrogenase activities for male monkeys at 0.4, 2, and 10 mg/kg/day were increased to 130.8, 213.6, and 176% of the control (338 IU/L), respectively. Lactate dehydrogenase activities for female monkeys at 0.4, 2, and 10 mg/kg/day were increased to 156.9, 129, and 172.7% of the control (297 IU/L), respectively. Urea levels for male monkeys were increased to 122.6% of the control (5.30 mM). Globulin levels for male and female monkeys were increased to 123 and 123.6% of control values (34.8 g/L), respectively. The albumin level for male monkeys at 10 mg/kg/day was decreased to 89.6 % of the control (50.8 g/L). Albumin to globulin ratios for male and female monkeys at 10 mg/kg/day were decreased to 77 and 84% of control values (1.47 and 1.26), respectively. Total protein levels for female monkeys at 10 mg/kg/day were increased to 109.45% of the control (82.5 g/L). Chloride levels for male monkeys at 10 mg/kg/day were increased to 105.5% of the control (109.3 mM). Inorganic phosphate levels for male treatment groups were decreased to 63-79% of the control (1.46 mM). IgM levels for male monkeys at 0.4, 2, and 10 mg/kg/day were increased to 149.6, 192, and 181.6% of the control (928 mg/L), respectively.

Blood Biochemistry, Day 92: Lactate dehydrogenase activities for male monkeys at 0.4, 2, and 10 mg/kg/day were increased to 113, 121, and 227% of the control (334 IU/L), respectively. Lactate dehydrogenase activities for female monkeys at 0.4, 2, and 10 mg/kg/day were increased to 150, 142, and 199% of the control (247 IU/L), respectively. Aspartate aminotransferase (i.e., GOT) activities for female monkeys at 0.4, 2, and 10 mg/kg/day were increased to 118, 118, and 135% of the control (17 IU/L), respectively. T_3 levels for male treatment groups were decreased to 73.2-78.8% of the control (3.54 nM). The T_3 to T_4 ratio for male monkeys at 10 mg/kg/day was decreased to 79.4% of the control (0.63). T_3 levels and the T_3 to T_4 ratio for female monkeys at 10 mg/kg/day were decreased to 86.7 and 77.3% of control values (3.09 nM and 0.066), respectively. Albumin levels for male and female monkeys at 10 mg/kg/day were decreased to 89.1 and 90.7% of control values (50.3 and 45.0 g/L), respectively. Globulin levels for male and female monkeys at 10 mg/kg/day were increased to 121.8 and 123.2% of the control (35.3 and 38.0 g/L), respectively. The albumin to globulin ratios for male and female monkeys at 10 mg/kg/day were decreased to 74.8 and 78.5% of the control (1.43 and 1.21), respectively. Triglyceride levels for male monkeys at 10 mg/kg/day were increased to 166.7% of the control (0.30 mM). Triglyceride levels for female monkeys at 2 and 10 mg/kg/day were decreased to 80.4 and 76.8% of the control (0.56 mM), respectively. Total cholesterol levels for female monkeys at 10 mg/kg/day were decreased to 68.1% of the control (3.17 mM). Total bilirubin levels for male monkeys at 10 mg/kg/day were increased to 184% of the control (3.2 μM). IgM levels for male treatment groups were increased to 164-247% of the control (716 mg/L). Inorganic phosphate levels for female monkeys at 0.4, 2, and 10 mg/kg/day were increased to 114.1, 117.7, and 126.3% of the control (1.48 mM), respectively.

Urinalysis, Day 26: Excreted creatinine for female monkeys at 10 mg/kg/day were decreased to 68.5% of the control (623 μmoles).