CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

20-484

PHARMACOLOGY REVIEW

PHARMACOLOGIST'S REVIEW OF NDA 20,484
(Amendment Dated May 15, 2000)

KEY WORDS: Tinzaparin Sodium
Reviewer Name: Timothy W. Robison, Ph.D.
Division Name: Gastrointestinal and Coagulation Drug Products
HFD# 180
Review Completion Date: June 14, 2000

IND/NDA number: 20,484
Serial number/date/type of submission: Amendment dated May 15, 2000
Date of HFD-180 Receipt: May 16, 2000
Information to sponsor: Yes ( ) No (X)
Sponsor (or agent): Dupont Pharmaceuticals Company
Wilmington, DE
Manufacturer for drug substance: Same

Drug: Tinzaparin sodium
Code Name: LHN-1
Generic Name: Tinzaparin sodium
Trade Name: Unknown

Drug Class: Anticoagulant/Low Molecular Weight Heparin.

Introduction and drug history: The sponsor received an Approvable letter dated April 28, 2000 from the Office of Drug Evaluation III for the indication of "the [redacted] treatment of acute symptomatic deep vein thrombosis when administered in conjunction with warfarin sodium".

Studies reviewed within this submission: The sponsor has responded to the April 28, 2000 Approvable letter from the Office of Drug Evaluation III regarding the Agency's proposed revisions of drug product labeling. The Agency's revised product labeling from the April 28, 2000 Approvable letter is listed below followed by the sponsor's response within quotations, an evaluation of this response, and a recommended version, if necessary.

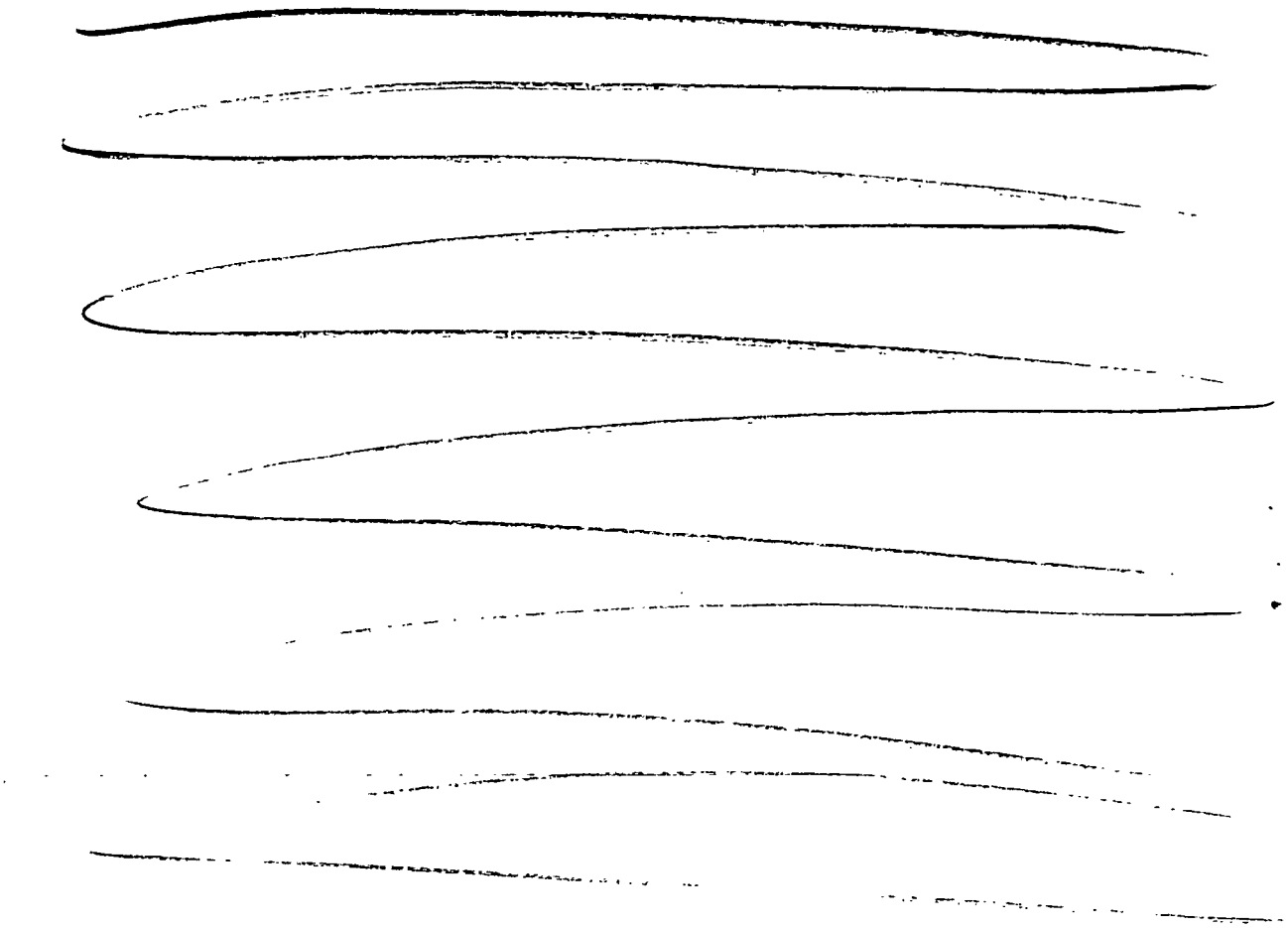
1. Carcinogenesis, Mutagenesis, Impairment of Fertility:

Agency's Version:

No long-term studies in animals have been performed to evaluate the carcinogenic potential of tinzaparin. Tinzaparin displayed no genotoxic potential in an *in vitro* bacterial cell mutation assay (AMES test), *in vitro* Chinese hamster ovary cell forward gene mutation test, *in vitro* human lymphocyte chromosomal aberration assay, and *in vivo* mouse micronucleus assay. Tinzaparin at subcutaneous doses up to 1800

IU/kg/day in rats (about 2 times the maximum recommended human dose based on body surface area) was found to have no effect on fertility and reproductive performance.

Sponsor's Version:



2. Pregnancy:

Agency's Version:

Teratogenic Effects: Pregnancy Category B: Teratogenicity studies have been performed in rats at subcutaneous doses up to 1800 IU/kg/day (about 2 times the maximum recommended human dose based on body surface area) and in rabbits at subcutaneous doses up to 1900 IU/kg/day (about 4 times the maximum recommended human dose based on body surface area) and have revealed no evidence of impaired fertility or harm to the fetus due to tinzaparin. There are however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are

2 page(s) of revised draft labeling has been redacted from this portion of the review.

[redacted]

3. Nursing Mothers:

Agency's Version:

In studies where tinzaparin was administered subcutaneously to lactating rats, very low levels of tinzaparin sodium were found in breast milk. It is not known whether TRADENAME is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when TRADENAME is administered to nursing women.

[redacted]

4. Overdosage:

Agency's Version:

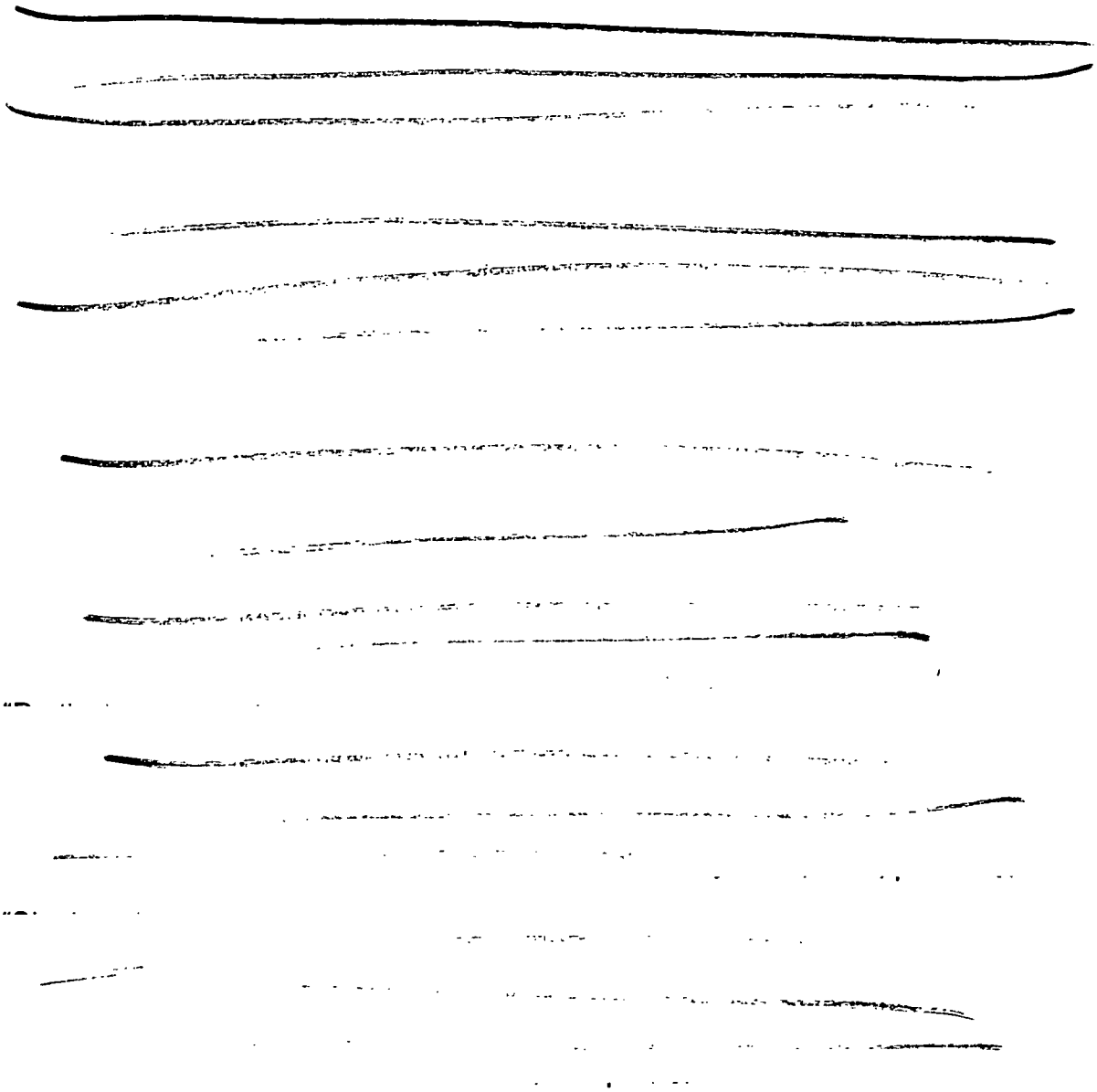
[redacted]

Sponsor's Version:

"Symptoms/Treatment: Accidental overdosage of TRADENAME may lead to bleeding complications. Nosebleeds, blood in urine or tarry stools may be noted as the first signs of bleeding. Easy bruising or petechial hemorrhages may precede frank bleeding. In case of minor bleeding, the patient should be monitored for signs of more severe bleeding."

"Of patients known to have received an overdose of tinzaparin sodium in clinical trials, defined as one or more doses >200 IU/kg for the treatment of DVT or >100 IU/kg for the prevention of DVT, approximately 16% experienced a bleeding complication."

"Of spontaneous reports of probable overdosing with tinzaparin sodium, approximately,



Evaiuation: From a preclinical standpoint, the sponsor's version appears to be acceptable.

/S/
Timothy W. Robison, Ph.D.
Pharmacologist

6-14-2000
Date

Comments:

/S/ — 6/21/00

Jasti B. Choudary, B.V.Sc., Ph.D. Date
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REVIEW # 1

FEB 22 2000

Reviewer: Timothy W. Robison, Ph.D.
Pharmacologist, HFD-180

Date of Submission: Original: June 30, 1999
Amendment: December 15, 1999

Date of HFD-180 Receipt: Original: July 1, 1999
Amendment: December 16, 1999

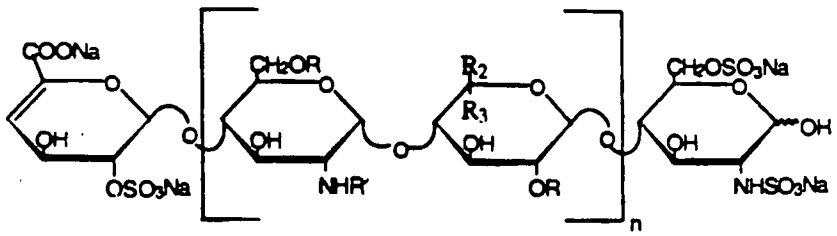
Date of Review: February 14, 2000

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

ORIGINAL SUMMARY

Drug: INNOHEP® (Tinzaparin Sodium; LHN-1); 10000 and 20000 anti-Xa IU/mL

Chemical Name and Structure: Tinzaparin sodium is the sodium salt of a low molecular heparin that is obtained by controlled enzymatic depolymerization of heparin from porcine intestinal mucosa using heparinase from *Flavobacterium heparinum*. The majority of the components have a 2-O-sulpho-4-enepyranosuronic acid structure at the non-reducing end and a 2-N,6-O-disulpho-D-glucosamine structure at the reducing end of their chain.



n= 1 to 25, R = H or SO_3Na, R' = H or SO_3Na or $COCH_3$
R_2 = H and R_3 = COONa or R_2 = COONa and R_3 = H

Molecular Weight: The mass-average molecular mass ranges between 5500 and 7500 Dalton. The mass percentage of chains between 2000 and 8000 ranges between 60 and 72 percent. The mass percentage of chains above 8000 ranges between 22 and 36 percent. The mass percentage of chains below 2000 is less than 10%.

Formulation:

Table 5.1. INNOHEP 10,000 anti-Xa IU/mL in 2 mL Vial

Component	Quantity per mL	Quantity per 30 L batch	Function
Tinzaparin sodium			
Benzyl alcohol, Ph. Eur./USP			
Sodium metabisulfite, Ph. Eur./USP			
Sodium hydroxide, Ph. Eur./USP			
Water for Injections, Ph. Eur./USP			
Nitrogen, NF			

[a]Weight adjusted based on 100% tinzaparin sodium potency and includes a 2% manufacturing excess.
[b]Corresponding to 2.0 mg/mL sodium bisulfite.
na=Not applicable

Table 5.2. INNOHEP 20,000 anti-Xa IU/mL in 2 mL Vial

Component	Quantity per ml	Quantity per 30 L batch	Function
Tinzaparin sodium			
Benzyl alcohol, Ph. Eur./USP			
Sodium metabisulfite, Ph. Eur./USP			
Sodium hydroxide, Ph. Eur./USP			
Water for Injections, Ph. Eur./USP			
Nitrogen, NF			

[a]Weight adjusted based on 100% tinzaparin sodium potency and includes a 2% manufacturing excess.
[b]Corresponding to 3.4 mg/mL sodium bisulfite.
na=Not applicable

Category: Anticoagulant/Low Molecular Weight Heparin.

Related Drugs/INDs/NDAs/MFs:

Proposed Marketing Indication: INNOHEP® is indicated for the treatment of acute symptomatic deep vein thrombosis with and without pulmonary embolism when administered in conjunction with warfarin sodium.

Dose: For treatment of deep vein thrombosis with and without pulmonary embolism, the dose is 175 anti-Xa IU/kg administered by the subcutaneous route once daily for at least 6 days and until the patient is adequately anti-coagulated with warfarin (International Normalized Ratio at least 2.0 for two consecutive days).

APPEARS THIS WAY
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Preclinical Studies and Testing Laboratories:

STUDY	Report No.	TESTING LABORATORY	DRUG BATCH	PAGE #
PHARMACOLOGY:				7-17
ADME:				
ABSORPTION				
Rats				
Pharmacokinetics of ^{3}H-Tinzaparin following single and repeated administration.	91100 91101 91102 92078 92079			17-18
Pharmacokinetics of ^{3}H-Tinzaparin a single subcutaneous or intravenous administration.	HRC-NV070-88853			18-19
Rabbits				
Pharmacokinetics of ^{3}H-Tinzaparin a single subcutaneous or intravenous administration.	10188			19-20
Dogs				
Pharmacokinetics of ^{3}H-Tinzaparin a single subcutaneous or intravenous administration.	HRC-NV069-88927			20-21
DISTRIBUTION:				
Rats				
Binding of ^{3}H-Tinzaparin to plasma proteins.	HRC-NV070-88853			21-22
Tissue distribution of ^{3}H-Tinzaparin following subcutaneous or intravenous administration by whole body autoradiography.	HRC-NV070-88853			22
Tissue distribution of ^{3}H-Tinzaparin after single and repeated intravenous administration.	91100 91101 91102 92078 92079			22-23
Tissue distribution of ^{3}H-Tinzaparin following subcutaneous administration to male rats and non-pregnant and pregnant rats.	HRC-NV070-88853			23-24
Transplacental passage of ^{3}H-Tinzaparin in the pregnant rat.	91100 91101 91102 92078 92079			24-25
Excretion of ^{3}H-Tinzaparin into the milk of lactating female rats following intravenous administration.	91100 91101 91102 92078 92079			26
Distribution of ^{3}H-Tinzaparin into the milk of lactating female rats following subcutaneous administration.	HRC-NV070-88853			26-27

Rabbits				
Determination of placental transfer of ^{3}H-Tinzaparin after subcutaneous administration to pregnant rabbits.	10788			27-28
Dogs				
Binding of ^{3}H-Tinzaparin to dog plasma proteins	HRC/ NV069/ 88927			28
Tissue distribution of drug-related radioactivity following subcutaneous administration of ^{3}H-Tinzaparin.	HRC/ NV069/ 88927			29-30
METABOLISM				
Rats & Dogs				
Urinary metabolites of ^{3}H-Tinzaparin.	HRC-NV070-88853 and HRC/ NV069/ 88929			30
EXCRETION				
Rats				
Excretion of ^{3}H-Tinzaparin after single and repeated intravenous administration.	91100 91101 91102 92078 92079			30-31
Excretion of ^{3}H-Tinzaparin following subcutaneous or intravenous administration and by bile duct-cannulated rats following intravenous administration.	HRC-NV070-88853			31-32
Dogs				
Excretion of drug-related radioactivity following subcutaneous or intravenous administration of ^{3}H-Tinzaparin.	HRC/ NV069/ 88929			32
TOXICOLOGY:				
ACUTE TOXICITY	0786 884 0686 784 0586 4584 0486 3184	[redacted]	F85010 F84001 F85010 F84001 F85010 F84001 F85010 F84001	34-37
SUBACUTE/SUBCHRONIC/CHRONIC TOXICITY				
RATS				
4-Week Intravenous Study.	5785	[redacted]	F84001	37-39
26-Week intravenous study followed by a 4-week reversibility period.	92/NLP 142/07 68	[redacted]	LMW 9101	39-45
1-Year subcutaneous study followed by a 6-week reversibility period.	89/NLP 031/ 0142	[redacted]	F668A	45-51

DOGS				
4-Week intravenous study.			F85010	52-53
52-Week intravenous study followed by a 4-week recovery period.	93/NLP 141/02 45		LMW 9101	53-57
1-Year subcutaneous study.	88/NLP 026/08 43		F668A	58-61
REPRODUCTIVE TOXICOLOGY				
Rats				
Segment I intravenous fertility and reproductive performance study.	92/NLP 137/05 28		LMW 9101	61-65
Segment I subcutaneous fertility and reproductive performance study.	88/NLP 027/ 458		F668A	65-72
Segment II intravenous teratology study.	92/NLP 138/ 0361		LMW 9101	72-78
Segment II intravenous teratology study.	92/NLP 150/ 0828		LMW 9101	78-85
Segment II subcutaneous teratology study.	88/NLP 025/ 100		F668A	85-90
Rabbits				
Segment II intravenous teratology study.	92/NLP 140/ 0183		LMW 9101	90-94
Segment II subcutaneous teratology study.	88/NLP 063/ 360		F668A	94-99
Segment II subcutaneous teratology study.	88/NLP 083/ 178		F704Y	100-105
Rats				
Segment III intravenous perinatal and postnatal development study.	92/NLP 139/ 0524		LMW 9101	105-111
Segment III subcutaneous perinatal and postnatal development study.	88/NLP 030/ 245		F668A	111-115

GENOTOXICITY				
Bacterial reverse mutation assay with strains TA1535, TA1537, TA100, and TA98 (Ames Test).	85/NLP 006/ 462	[redacted]	F85010	115
Bacterial reverse mutation assay with strains WP2 and WP2 uvrA.	88/NLP 081/ 0649		F547	115-116
Mouse micronucleus test.	85/NLP 007/ 726		F85010	116-117
Human lymphocyte chromosomal aberration assay.	87/NLP 032/ 734		F668A	117
Chinese hamster ovary forward mutation assay (CHO/HGPRT).	87/NLP 033/ 768		F668A	117-118
SPECIAL TOXICITY STUDIES				
Active anaphylaxis in guinea pigs.	92/NLP 060/ 0344		F682X	118-119
Passive cutaneous anaphylaxis assay in guinea pigs.	88/NLP 061/ 0401		F682X	120-121
Passive hemagglutination assay in rabbits.	88/NLP 062/ 0403		F682X	121-122
Local irritation in rabbits after intramuscular injection.	3785		F84001	123

Pharmacology Reviews of [redacted] with document room dates of September 21, 1988 and June 12, 1989 have been incorporated into the present review.

PHARMACOLOGY:

Tinzaparin is an enzymatically depolymerized heparin with an average molecular weight of approximately 5000 daltons determined by gel filtration chromatography. The molecular weight distribution of tinzaparin is broad ranging from about 600 (disaccharides) to above 15,000 daltons. Tinzaparin has the same chemical structure as heparin except for the terminal unsaturated hexuronic acid residues ($\Delta_{4,5}$ iduronic acid) produced during the depolymerization process. These unsaturated iduronic residues, which absorb light UV light at 235 nm occur in about two-thirds of the molecules. Pharmacology studies examined the antithrombotic properties of tinzaparin in comparison with conventional heparin.

A Comparison of the Antithrombotic and Hemorrhagic Effects of Tinzaparin and Heparin (Report Number Hep 88081).

Both tinzaparin and heparin were able to inhibit thrombus formation in a New Zealand male white rabbit model of experimental thrombosis following intravenous administration. Bleeding time assessed with the use of male Wistar rat tail transsection model was prolonged following administration of either heparin (0.5 and 1 mg/kg) or tinzaparin (1.2 and 2.4 mg/kg).

Weight of thrombi in experimental thrombosis in rabbits following intravenous administration of tinzaparin or heparin.

Saline		Tinzaparin		Heparin	
Dose, mg/kg	Weight of thrombi, mg	Dose, mg/kg	Weight of thrombi, mg	Dose, mg/kg	Weight of thrombi, mg
0	56.9	-	-	-	-
-	-	0.13	25.3	0.07	27.1
-	-	0.19	17.8	0.11	14.2
-	-	0.26	19.1	0.15	8.3
		0.38	5.9	0.22	1.7
-	-	1.54	0.0	0.89	0.0

Antithrombotic Effect in Rats Following Subcutaneous Administration (Study No. 4586).

The antithrombotic effects of tinzaparin and heparin were compared at subcutaneous doses of 1.25, 2.5, and 5 mg/kg in a stasis model using female Wistar rats between 2 and 3 hr after administration. Tinzaparin produced a dose-dependent inhibition of thrombus formation with complete inhibition observed at 5 mg/kg. On a weight basis, tinzaparin was found to be less potent than heparin as the ratio for doses with the same antithrombotic effect was 2 to 1. However, this corresponded to a 1 to 1 ratio based on anti-Xa units.

Weight of thrombi corresponding to mean rank number at 2 to 3 hr after subcutaneous administration of tinzaparin or heparin to female Wistar rats.

Dose, mg/kg	Weight of thrombi (mg) with Tinzaparin	Weight of thrombi (mg) with Heparin
0	9.2	-
1.25	12.3	3.9
2.5	2.0	2.0
5	0	0

Hemorrhagic Effect in Rats Following Subcutaneous Administration (Study No. 4986).

Bleeding times in female Wistar rats were assessed using the tail transection technique at 2 hr after subcutaneous administration of tinzaparin or heparin at doses of 1.25, 2.5, or 5 mg/kg. For tinzaparin, no significant hemorrhagic effect was found as compared to controls. For heparin at 5 mg/kg, the total bleeding time was >15 min in all rats, which was significantly different from the controls. Comparison of bleeding time obtained with tinzaparin were not significantly different from heparin.

Total bleeding time (corresponding to mean rank number) after subcutaneous administration of tinzaparin or heparin to female Wistar rats.

Dose, mg/kg	Total bleeding time (min) with Tinzaparin	Total bleeding time (min) with Heparin
0	6.5	-
1.25	5	5
2.5	5	10.5
5	6.5	>15

Comparison of Antithrombotic and Hemorrhagic Effects of Heparin (Batch numbers 314 and 1167) with Tinzaparin (Batch numbers F85030, F53711, and F547) (Report number 1987.09.18-Buchanan).

The antithrombotic and hemorrhagic effects of unfractionated heparin (batch number 314 and 1167) and tinzaparin (batch numbers F85030, F53711, and F547) were compared relative to an international heparin standard (SH) to assess batch-to-batch variation. For inhibition of tissue thromboplastin-induced thrombus formation using a jugular vein rabbit stasis model, the three different batches of tinzaparin were determined to be as effective as unfractionated heparin. Further, anti-factor Xa activities measured after dosing were comparable, while considerable variation occurred between the different batches of unfractionated heparin. In a study assessing hemorrhagic effects (i.e., blood loss) in rabbits, the three tinzaparin batches enhanced blood loss at 40 times the antithrombotic dose; although, blood loss was lower than observed with unfractionated heparin batches. The sponsor concluded that greater variations in biological properties occurred between different batches of unfractionated heparin. Further, the three batches of tinzaparin had similar properties and were as effective as unfractionated heparin.

Efficacy of Tinzaparin and Sodium Heparin USP During Extracorporeal Circulation (Report 1993.03-16-Sorenson).

The anticoagulant properties of tinzaparin and heparin were compared following intravenous administration in an open heart surgery model in dogs using a heart-lung machine (i.e., extra-corporeal circulation). In the first study, these two agents were compared at a dose of 3 mg/kg (267 and 534 anti Xa U/kg for tinzaparin and heparin, respectively). Further, the ability of protamine sulfate at dose of 3 mg/kg to neutralize the anticoagulant effects of tinzaparin and heparin were compared. In the second study, tinzaparin and heparin were compared at equal anti-factor Xa doses of 100 and 175 anti-Factor Xa U/kg. In the first study, blood for determination of platelet count and APTT assay was collected prior to dosing, during by-pass, after discontinuation of by-pass, and when protamine sulfate was administered. In the second study, blood for determination of platelet counts, anti-factor Xa activity and APTT was collected prior to dosing, during by-pass, and following the discontinuation of by-pass. For both studies, the clotting status of each dog was monitored by measurement of the activated whole blood clotting time (AWBCT). No clotting was evident with tinzaparin or heparin at doses ≥175 mg/kg/day. Minor clots were evident with tinzaparin and heparin at a dose of 100 anti Xa U/kg. These clots were rich in granular materials and poor in fibrin, which indicates that clots were initiated by platelet deposition. At a dose of 3 mg/kg, both

tinzaparin and heparin prolonged the AWBCT and APTT; although, heparin was more potent. Heparin produced peak elevations of AWBCT and APTT to 408-449 sec and >1100 sec, respectively, from 10 min after the start of bypass to 5 min after discontinuation of by-pass as compared to baseline values of 100 sec and 16.8 sec. Tinzaparin produced elevations of AWBCT and APTT over the same time period; although, peak values of 213 sec and >500 sec, respectively, observed at 10 min after dosing were smaller. Neither agent had any effects on platelet counts. Infusion of protamine sulfate neutralized tinzaparin and heparin-induced elevations of AWBCT and APTT. Both agents administered at equal anti-factor Xa doses produced comparable changes in AWBCT; however, heparin had more pronounced effects on APTT. Heparin at 100 and 175 anti-Factor Xa U/kg produced peak elevations in AWBCT to 346 and 254 sec, respectively, at 10 min after dosing as compared to a baseline value of 97-102 sec. Tinzaparin at 100 and 175 anti-Factor Xa U/kg produced peak elevations in AWBCT to 168.5 and 456 sec, respectively, at 10 min after dosing. Heparin at 100 and 175 anti-Factor Xa U/kg produced peak elevations of APTT to >300 sec at 10 min after dosing as compared to a baseline value of 15-17 sec. Tinzaparin at 100 and 175 anti-Factor Xa U/kg produced peak elevations of APTT to 46 and 74 sec, respectively, at 10 min after dosing. The half-life of tinzaparin appeared to be independent of dose, while the half-life of heparin decreased with dose. The sponsor concluded that tinzaparin was as effective as heparin in preventing coagulation during extra-corporeal circulation.

Neutralization of Tinzaparin and Heparin by Protamine Sulfate in Rats (Report No. 88082).

Neutralization of tinzaparin and heparin by protamine sulfate in terms of APTT and anti-factor Xa activity was examined in male Wistar rats. Further, neutralization of the hemorrhagic and antithrombotic effects of these agents were examined by using [redacted] cut/tail transection and stasis models, respectively. Tinzaparin or heparin was administered by the intravenous route at doses of 7.2 and 3 mg/kg, respectively, followed 5 min later by intravenous administration of protamine sulfate at progressively increasing doses. Heparin-induced increases of APTT and anti-factor Xa activity were both neutralized by protamine sulfate at 3.5 mg/kg. In contrast, the tinzaparin-induced increase of APTT was neutralized by protamine sulfate at 5 mg/kg; however, increased anti-factor Xa activity was neutralized by protamine sulfate at 13 mg/kg. The heparin-induced increase in bleeding time was neutralized by protamine sulfate at 3.5 mg/kg. The tinzaparin-induced increase in bleeding time was neutralized by protamine sulfate at doses of 5 or 13 mg/kg. The antithrombotic effect of tinzaparin was unaffected by protamine sulfate at a dose of 5 mg/kg; however, a dose of 13 mg/kg was found to neutralize this effect. For tinzaparin, anti-factor Xa activity and antithrombotic effects were neutralized by a significantly higher dose of protamine sulfate as compared to hemorrhagic effects.

APTT, anti-factor Xa activity, thrombus, and thrombus frequency in rats treated with saline, heparin, or tinzaparin followed by saline or protamine sulfate.

1st Treatment	2nd Treatment	APTT (sec)	Anti-Factor Xa (U/mL)	Thrombus Weight (mg)	Thrombus Frequency (%)
Saline	Saline	22	0	15.2	83
Heparin, 3 mg/kg	Saline	>200	>3	-	-
Tinzaparin, 7.2 mg/kg	Saline	>200	>3	-	-
Heparin, 3 mg/kg	Protamine sulfate, 3.5 mg/kg	23	0.04	7.2	83
Tinzaparin, 7.2 mg/kg	Protamine sulfate, 5 mg/kg	29	0.80	0	0
Tinzaparin, 7.2 mg/kg	Protamine sulfate, 13 mg/kg	34	0.19	6.5	100

Both tinzaparin (0.13-1.54 mg/kg) and heparin (0.07-0.89 mg/kg) were able to inhibit thrombus formation in the rabbit model of experimental thrombosis. Bleeding time was prolonged following administration of either heparin (0.5 and 1 mg/kg) or tinzaparin (1.2 and 2.4 mg/kg). In a stasis model using female Wistar rats, tinzaparin or heparin at doses of 1.25 to 5 mg/kg produced a dose-dependent inhibition of thrombus formation with complete inhibition observed at 5 mg/kg. Tinzaparin at subcutaneous doses of 1.25 to 5 mg/kg had no effects on bleeding times as assessed by the tail transection technique in female Wistar rats. In contrast, heparin at 5 mg/kg significantly prolonged bleeding times. Administration of heparin by the intravenous route at a dose of 3 mg/kg to male Wistar rats produced increases of APTT and anti-factor Xa activity, which were both neutralized by protamine sulfate at 3.5 mg/kg. In contrast, tinzaparin administered by the intravenous route at 7.2 mg/kg produced an increase of APTT, which was neutralized by protamine sulfate at 5 mg/kg; however, increased anti-factor Xa activity was neutralized by protamine sulfate at 13 mg/kg. The heparin-induced increase in bleeding time was neutralized by protamine sulfate at 3.5 mg/kg; however, the tinzaparin-induced increase in bleeding time was neutralized by protamine sulfate at doses of 5 or 13 mg/kg. For tinzaparin, anti-factor Xa activity and antithrombotic effects were neutralized by a significantly higher dose of protamine sulfate as compared to hemorrhagic effects.

SAFETY PHARMACOLOGY:

Effect of Tinzaparin on Spontaneous Motor Activity in Mice (Report No. 91279).

The effect of tinzaparin at intravenous doses of 0, 100, 1000, and 10000 anti-Factor I.U./kg on spontaneous motor activity was examined in NMRI mice (4 mice/sex/group). ,Heparin at an intravenous dose of 10000 anti-Factor I.U./kg was used as a reference standard. Chlorpromazine at an intravenous dose of 3 mg/kg was used as a positive control. Spontaneous locomotor activity and rearing were assessed for a 10-min period beginning 5-min after drug administration. Tinzaparin at intravenous doses ≤10000 anti-Factor I.U./kg had no effect on spontaneous motor activity.

Effect of Tinzaparin on Pentylenetetrazol-Induced Seizures in Rats (Report No. 91268).

The effect of tinzaparin at intravenous doses of 0, 100, 1000, and 10000 anti-factor Xa I.U./kg was examined on pentylenetetrazol-induced seizures in female Sprague-Dawley rats (5 rats/group). Heparin at an intravenous dose of 10000 anti-factor Xa I.U./kg was used as a reference standard. Diazepam, an anticonvulsant, and FG 7142, a proconvulsant, at intravenous doses of 0.5 and 5 mg/kg, respectively, were used as positive controls. Five min after treatment with the test article, intravenous infusion of 5 mg/mL pentylenetetrazol solution at 95 mL/hr was started and the time until occurrence of the first myoclonic jerk was recorded and the dose of pentylenetetrazol/kg was calculated for each rat. Tinzaparin had no effect on pentylenetetrazol-induced seizures in female Sprague-Dawley rats.

Effect of Tinzaparin in the Mouse Paw Formalin Test (Report No. 91231).

The effect of tinzaparin at intravenous doses of 0, 100, 1000, and 10000 anti-factor Xa I.U./kg was examined for possible antinociceptive effects in the mouse paw formalin test (6 female NMRI mice/group). Heparin at an intravenous dose of 10000 anti-factor Xa I.U./kg was used as a reference standard. Morphine at an intravenous dose of 3 mg/kg was used as a positive control. Mice received the test article 5 min prior to subcutaneous administration of formalin in the plantar surface of one hindpaw. Tinzaparin at intravenous doses ≤10000 anti-Factor I.U./kg displayed no antinociceptive properties in female NMRI mice.

The Cardiovascular and Respiratory Effects of Tinzaparin After Intravenous Administration in the Anesthetized Cat (Report No. 91281).

The effects of tinzaparin at an intravenous dose of 10000 anti-factor Xa I.U./kg were examined on blood pressure, heart rate, electrocardiogram, carotid occlusion, injection of noradrenaline, blood flow, blood gases, and respiratory rate in anesthetized female cats. Heparin at an intravenous dose of 10000 anti-factor Xa I.U./kg was used as a reference standard. There were 3 cats/group. Neither tinzaparin nor heparin at an intravenous dose of 10000 anti-factor Xa I.U./kg displayed any effect on blood pressure, heart rate, blood flow, sinus- or carotid-driven vascular reflexes, electrocardiogram, respiratory rate, and blood gases in anesthetized cats.

Cardiovascular and Respiratory Effects of Tinzaparin in the Anesthetized Cat (Report No. 1987).

The effects of tinzaparin at intravenous doses of 100 and 1000 I.U./kg on blood pressure, heart rate, electrocardiogram, carotid occlusion, injection of noradrenaline, and respiratory rate was examined in anesthetized female cats. Each cat was treated consecutively at 20 min intervals (5 min prior to carotid occlusion) with placebo, 100 I.U./kg tinzaparin, and 1000 I.U./kg tinzaparin. Tinzaparin at intravenous doses ≤1000 I.U./kg had no effect on blood pressure, carotid- or sinus-driven vascular reflexes, heart rate, electrocardiogram, and respiratory rate.

The Cardiovascular Effects of Tinzaparin in the Anesthetized Pig (Report No. 2087).

The effects of tinzaparin at intravenous doses of 100 and 1000 I.U./kg on cardiac output, systemic arterial blood pressure (SAP), pulmonary arterial blood pressure (PAP), central venous blood pressure (CVP), heart rate, and hematocrit were examined in anesthetized female pigs. Each pig received in a consecutive manner by intravenous bolus administration: placebo, 100 I.U./kg tinzaparin, 1000 I.U./kg tinzaparin, and 1 µg/kg adrenaline. The interval between treatments was not specified. Cardiac output was measured at 2, 5, and 10 min after administration of the test substance. SAP, PAP, and pulse rate were measure continuously. Recording of CVP was interrupted by measurements of cardiac output. Tinzaparin at intravenous doses of 100 and 1000 I.U./kg had no effects on cardiac output, systemic arterial blood pressure, pulmonary arterial blood pressure, central venous blood pressure, heart rate, and hematocrit.

Effect of Tinzaparin on Gastrointestinal Transit in Mice (Report No. 91284).

The effects of tinzaparin at intravenous doses of 0, 100, 1000, and 10000 anti-factor Xa I.U./kg on gastrointestinal transit time was examined in female NMRI mice. Heparin at an intravenous dose of 10000 anti-factor Xa I.U./kg was used as a reference standard. Morphine at an intravenous dose of 3 mg/kg was used as a positive control. The intestinal transit rate of a charcoal suspension was examined at 30 min after oral administration. Tinzaparin at intravenous doses ≤10000 anti-factor Xa I.U./kg had no effects on gastrointestinal transit.

Effect of Tinzaparin on Diuresis and Electrolyte Excretion in Waterloaded Conscious Rats (Report No. 91225 and 1887).

Effects of tinzaparin at intravenous doses of 100, 1000, and 10000 anti-factor Xa I.U./kg on urinary excretion of Na^+, K^+, and Cl^- were examined in water-loaded, conscious, female Sprague-Dawley rats over a 3 hr period after administration. Heparin at identical intravenous doses was used as a reference standard. Furosemide and vasopressin at intravenous doses of 10 mg/kg and 0.5 I.U./kg, respectively, were used as positive controls. Tinzaparin and heparin at doses ≥100 anti-factor Xa I.U./kg decreased urinary Na^+ excretion; however, observed effects did not occur in a dose-related manner for either drug. Tinzaparin at doses ≥100 anti-factor Xa I.U./kg and heparin at a dose of 10000 anti-factor Xa I.U./kg increased urinary K^+ excretion approximately 1.5 to 2 times the control (0.30 and 0.58 mmol/3 hr/kg). Tinzaparin at doses of ≥100 anti-factor Xa I.U./kg decreased urinary Cl^- excretion to approximately one-half of control values (0.32 and 0.58 mmol/3 hr/kg). Similarly, heparin at doses ≥100 anti-factor Xa I.U./kg decreased urinary Cl^- excretion to 62.1-70.7 of the control. Urinary excretion of the water load over the 3 hr study period was unaffected by intravenous treatment with tinzaparin or heparin. Both tinzaparin and heparin at doses ≥100 anti-factor Xa I.U./kg decreased urinary Na^+ and Cl^- excretion. Tinzaparin at doses ≥100 anti-factor Xa I.U./kg and heparin at 10000 anti-factor Xa I.U./kg increased urinary K^+ excretion.

Effects of Tinzaparin on Diuresis and Electrolyte Excretion in Conscious Rats (Report No. 7187).

Effects of tinzaparin at intravenous doses of 100 and 1000 anti-factor Xa I.U./kg on urinary excretion of Na^+, K^+, and Cl^- were examined in water-loaded, conscious, female Sprague-Dawley rats over a 3 hr period after administration. Heparin at identical intravenous doses was used as a reference standard. Tinzaparin at doses of 100 and 1000 anti-factor Xa I.U./kg decreased urinary Na^+ excretion to 28.1 and 21.9% of the control (0.32 mmol/3 hr/kg), respectively. Heparin at doses of 100 and 1000 anti-factor Xa I.U./kg decreased Na^+ excretion to 56.25 and 31.25% of the control, respectively. Tinzaparin at doses of 100 and 1000 anti-factor Xa I.U./kg decreased urinary K^+ excretion to 60.9 and 71.9% of the control (0.64 mmol/3 hr/kg), respectively. Heparin at doses of 100 and 1000 anti-factor Xa I.U./kg decreased K^+ excretion to 73.4 and 78.1% of the control, respectively. Tinzaparin at doses of 100 and 1000 anti-factor Xa I.U./kg decreased urinary Cl^- excretion to 32.8 and 35.9% of the control (0.64 mmol/3 hr/kg), respectively. Heparin at doses of 100 and 1000 anti-factor Xa I.U./kg decreased Cl^- excretion to 60.9 and 26.6% of the control, respectively. Urinary excretion of the water load over the 3 hr study period and urinary pH were unaffected by intravenous treatment with tinzaparin or heparin. Treatment with tinzaparin or heparin at doses of 100 or 1000 anti-factor Xa I.U./kg decreased urinary excretion of Na^+ or Cl^-. The observed increases of urinary K^+ excretion found in other studies with tinzaparin was not found in the present study.

Effects of Tinzaparin and Conventional Heparin on the Release of Lipases and the Clearance of Lipids in Rats (Report No. 93001.PB0).

The effects of tinzaparin were compared with standard heparin on the activities of plasma lipoprotein lipase (LPL) and hepatic lipase (HL) as well as clearance of chylomicron labeled with [^{14}C]-triglyceride and [^{3}H]-retinol in rats. Tinzaparin or heparin were administered by the intravenous route to rats at doses of 50 and 250 anti-factor Xa U/kg. Tinzaparin and heparin at 50 U/kg caused a transient reduction of LPL activity, which was correlated with a decreased clearance of chylomicrons. Heparin at 250 U/kg increased chylomicron clearance as compared to tinzaparin at 250 U/kg, which was correlated to a higher plasma LPL activity. Tinzaparin and heparin had no effect on available HL activity, which suggested that HL displaced into the circulation returned to its binding sites when tinzaparin or heparin disappeared from the blood. Difference between tinzaparin and heparin with regard to plasma LPL activity and clearance of chylomicrons were due to the kinetics of these processes.

Effect of Tinzaparin on Isolated Guinea Pig Ileum (Report Nos. 3387 and 91269).

Effects of tinzaparin on isolated guinea pig ileum contractions were examined at concentrations of 1, 10, and 100 anti-factor Xa units/mL. Tinzaparin alone at concentrations of 1 and 10 anti-factor Xa units/mL had no effects on guinea pig ileum contractions. Tinzaparin at concentrations of 1 and 10 anti-factor Xa unit/mL had no effects on agonist-induced contractions; however, 100 anti-factor Xa units/mL slightly potentiated histamine- and acetylcholine-induced contractions by 7.9 and 4.3%, respectively.

Effect of Tinzaparin and Unfractionated Heparin on Platelet Aggregation (Report No. 91089).

Effects of tinzaparin on unstimulated platelets and after stimulation with collagen, ADP, and ristocetin were compared with unfractionated heparin. Tinzaparin and heparin were examined at final concentrations ranging from 0.11 to 9 anti-factor Xa IU/mL. Platelets were obtained from healthy human volunteers. Both tinzaparin and heparin stimulated collagen and ADP-induced platelet aggregation; although, heparin was more potent as illustrated by lower K_d values in the table below. Both agents weakly stimulated ristocetin-induced platelet aggregation. Addition of tinzaparin or heparin to unstimulated platelets produced significant aggregation; although, there were no significant differences between these two agents.

Table I.
E_{max} and K_d of UH and LHN-1 obtained from concentration-effect curves by use of 4 different concentration units.

ADP INDUCED AGGREGATION					
	E_{MAX}		K_d		
HEPARINS	UH	LHN-1	UH	LHN-1	(K_d LHN-1)/(K_d UH)
UNITS					
XaIU/ml	59.8%	56.8%	0.057	0.29	5.1
µg/ml	59.8%	56.8%	0.031	3.34	10.8
IIaIU/ml	59.8%	56.8%	0.057	0.18	3.2
nmol/ml.	59.8%	56.8%	0.025	0.86	34.4
COLLAGEN INDUCED AGGREGATION					
	E_{MAX}		K_d		
HEPARINS	UH	LHN-1	UH	LHN-1	(K_d LHN-1)/(K_d UH)
UNITS					
XaIU/ml	36.9%	24.7%	0.028	0.064	2.3
µg/ml	36.9%	24.7%	0.15	0.74	4.9
IIaIU/ml	36.9%	24.7%	0.028	0.041	1.46
nmol/ml.	36.9%	24.7%	0.012	0.189	15.5

An Assessment of the Effects of Tinzaparin on Human Platelet Aggregation (Report No. 3187).

The effect of tinzaparin on in vitro platelet aggregation were assessed at concentrations of 1 and 10 anti-factor Xa I.U./mL. Platelets were obtained from healthy male and female human volunteers. Tinzaparin at concentrations ≤10 anti-factor Xa I.U./mL did not induce aggregation of human platelets or interfere with ADP-induced platelet aggregation.

Effect of Tinzaparin on the Duration of Hexobarbital-Induced Sleep in Mice (Report No. 91234).

The effects of tinzaparin at intravenous doses of 0, 100, 1000, and 10000 anti-Factor Xa I.U./kg were examined on the duration of hexobarbital-induced sleep time in NMRI mice (3 mice/sex/group). Heparin at an intravenous dose of 10000 anti-Factor Xa I.U./kg was used as a reference standard. Chlorpromazine at an intravenous dose of 1 mg/kg was used as a positive control. Tinzaparin at intravenous doses ≤10000 anti-factor Xa I.U./kg had no effect on the duration of hexobarbital-induced sleep time in mice.

Effect of Tinzaparin on the Duration of Ethanol-Induced Sleep in Mice (Report Nos. 91233 and 3087).

The effects of tinzaparin at intravenous doses of 0, 100, 1000, and 10000 anti-Factor Xa I.U./kg were examined on the duration of ethanol-induced sleep time in NMRI mice. Heparin at an intravenous dose of 10000 anti-Factor Xa I.U./kg was used as a reference standard. Chlorpromazine at an intravenous dose of 1 mg/kg was used as a positive control. Tinzaparin at intravenous doses ≤10000 anti-factor Xa I.U./kg had no effect on the duration of ethanol-induced sleep time in mice.

Effect of Tinzaparin on the Duration of Hexobarbital-Induced Sleep in Mice (Report No. 2987).

The effects of tinzaparin at intravenous doses of 0, 100, and 1000 anti-Factor Xa I.U./kg were examined on the duration of hexobarbital-induced sleep time in NMRI mice (5 mice/sex/group). Chlorpromazine at an intravenous dose of 1 mg/kg was used as a positive control. Tinzaparin at intravenous doses ≤1000 anti-factor Xa I.U./kg had no effect on the duration of hexobarbital-induced sleep time in mice.

Interaction with the Glucose Metabolism and Cardiovascular Effects of Tinzaparin in Glucose Loaded and Anesthetized Rats (Report No. 1787).

The effects of tinzaparin at intravenous doses of 0, 100, and 1000 anti-factor Xa I.U./kg were examined on blood pressure, heart rate, and the plasma glucose concentration in female Sprague-Dawley rats that were anesthetized with pentobarbital and intravenously loaded with glucose. Adrenaline was used as a positive control.

Tinzaparin at intravenous doses ≤1000 anti-factor Xa I.U./kg administered to anesthetized rats had no effects on blood pressure, heart rate, or capacity to metabolize an intravenous load of glucose.

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION:

For studies with radiolabeled drug, tinzaparin was radiolabeled with tritium by catalytic hydrogenation of the terminal aldehyde group or of the conjugated double bond. This labeling procedure is expected to occur randomly in susceptible groups. The radioactivity distribution is expected to be similar to the distribution of molecules and not to the mass of molecules (i.e., small molecules carry the same amounts of radioactivity as large molecules). In radioactivity measurements, the small molecules with little or no activity play a major role. In general, anti-Factor Xa and anti-Factor IIa activity represent larger molecules with antithrombin III binding properties, with anti-Factor IIa activity representing the largest molecules. In ADME studies reviewed below, drug-related radioactivity refers to radioactivity remaining after removal of all exchanged tritium (i.e., ^3H-H_2O) from each sample.

Absorption

Rats

Pharmacokinetics of ^3H-Tinzaparin in the Rat After Single and Repeated Intravenous Administration (Report Nos. 91100, 91101, 91102, 92078, and 92079).

Methods: Radiolabeled tinzaparin was administered by the intravenous route to male and female Sprague-Dawley rats at a dose of 1 mg/kg/day for 1, 7, or 21 days. To assess the effects of increased body weight over the 21-day treatment period, one group of rats was treated with saline for 20 days and on day 21 received intravenous treatment with ^3H-tinzaparin. Blood for measurement of plasma levels of total radioactivity and drug-related radioactivity was collected at 0.083, 0.25, 0.5, 1, 2 , 3, 4, and 6 hr after dosing on days 1, 7, or 21.

Results: Maximal plasma concentrations at 5 min after dosing on days 1, 7, and 21 were 4.45 ± 0.36, 4.20 ± 0.55, and 4.06 ± 0.45 µg/mL, respectively. For the group that received saline treatment for 20 days followed by treatment with ^3H-tinzaparin on day 21, the maximal plasma concentration at 5 min after dosing on day 21 was 3.91 ± 0.73 µg/mL. There was no significant difference in the maximal plasma drug concentration at 5 min between this group and the group receiving one treatment despite a 40% difference in body weight. Individual plasma concentrations were similar in the respective treatment groups yielding a coefficient of variation of 20%. Vc was larger than the plasma volume (7.8 mL).

Pharmacokinetic parameters for plasma drug-related radioactivity in rats that received treatment with ^{3}H-tinzaparin at 1 mg/kg/day for 1, 7, or 21 days. These pharmacokinetic parameters were obtained by fitting all plasma concentration data from treated for 1, 7, or 21 days.

Days 1, 7, and 21	$C_{5\ min}$, µg/mL	AUC µg*hr/mL	CL mL/min	T½α hr	T½β hr	T½γ hr	Vc, mL
Pooled data	4.41	3.21	1.30	0.254	1.50	37.0	52.8

Pharmacokinetics of ^{3}H-Tinzaparin in the Rat After a Single Subcutaneous or Intravenous Administration (Report No. HRC-NV070-88853).

Methods: Plasma pharmacokinetic studies were performed with Sprague-Dawley rats following subcutaneous or intravenous administration of ^{3}H-tinzaparin at doses of 1 and 4 mg/kg. Blood for determination of plasma drug-related radioactivity concentrations, anti-Factor Xa activities, and APTT values was collected at time points between 0.08 and 120 hr following intravenous administration or 0.25 and 120 hr following subcutaneous administration. Three rats/sex were used for each time point.

Results: AUC values following subcutaneous or intravenous administration of tinzaparin increased in a dose proportional manner. Bioavailability of tinzaparin administered by the subcutaneous route, determined using plasma drug-related radioactivity, was approximately 100%. Bioavailability of tinzaparin administered by the subcutaneous route, determined using anti-Factor Xa activity, was less than obtained with administration by the intravenous route. Plasma C_{max} values obtained with intravenous administration were greater than those obtained with subcutaneous administration. Clearance of tinzaparin (0.65-0.75 L/hr/kg) was smaller than renal plasma flow (1.3 L/hr/kg); although, significantly greater than glomerular filtration rate (0.2 L/hr/kg). The volume of distribution (0.65-0.85 L/kg) was greater than blood volume (0.054 L/kg) indicating distribution into tissues as demonstrated in studies below.

Pharmacokinetic parameters derived from the concentration of drug-related radioactivity in the plasma of rats following administration of ^{3}H-tinzaparin by the subcutaneous or intravenous routes at doses of 1 and 4 mg/kg.

Parameters	1 mg/kg S.C.		1 mg/kg I.V.		4 mg/kg S.C.		4 mg/kg I.V.	
	Male	Female	Male	Female	Male	Female	Male	Female
T_{max}, hr	0.75	0.50	0.08	0.08	0.50	0.50	0.08	0.08
C_{max}, µg/mL	0.86	0.96	2.66	3.03	3.52	4.60	11.95	13.45
T½, hr (λ_2 phase)	0.50	0.56	0.75	0.91	0.74	0.70	0.73	0.70
AUC∞	1.06	1.11	1.16	1.32	5.57	5.96	5.16	6.03
Clearance, L/hr/kg	-	-	0.75	0.65	-	-	0.75	0.65
Vd, L/kg	-	-	0.81	0.85	-	-	0.79	0.65
Bioavailability, %	91	84	-	-	108	99	-	-

Pharmacokinetic parameters derived from the levels of anti-Factor Xa activity (corrected for baseline activity) in the plasma of rats following administration of ^{3}H-tinzaparin by the subcutaneous or intravenous routes at doses of 1 and 4 mg/kg.

Parameters	1 mg/kg S.C.		1 mg/kg I.V.		4 mg/kg S.C.		4 mg/kg I.V.	
	Male	Female	Male	Female	Male	Female	Male	Female
T_{max}, hr	0.75	0.50	0.08	0.25	0.75	1.00	0.08	0.08
C_{max}, Ui/mL	0.10	0.16	0.38	0.45	0.42	0.70	2.81	3.23
T½, hr (λ_2 phase)	-	-	0.42	-	-	-	0.833	0.918
AUC∞	0.08	0.22	0.13	0.19	1.15	1.38	1.60	2.01
Clearance, L/hr/kg	-	-	0.45	-	-	-	0.17	0.14
Vd, L/kg	-	-	0.27	-	-	-	0.21	0.15
Bioavailability, %	62	116	-	-	72	69	-	-

Rabbits

Pharmacokinetics of Tinzaparin and Conventional Heparin After Subcutaneous and Intravenous Administration in the Conscious Rabbit (Report No. 10188).

Methods: Pharmacokinetics of radiolabeled tinzaparin were determined in female New Zealand White rabbits following subcutaneous administration of 4 or 25 mg/kg and intravenous administration of 1 mg/kg. For comparison, pharmacokinetics of radiolabeled heparin were determined following subcutaneous administration of 12.5 mg/kg/day or intravenous administration of 0.5 mg/kg. Following subcutaneous administration, blood samples were collected at time points between 0 and 24 hr for measurements of plasma levels of radioactivity and anti-Factor Xa and anti-Factor IIa activities. Following intravenous administration, blood samples were collected at time points between 0 and 3 hr after dosing. Plasma radioactivity levels were measured with a liquid scintillation counter. Plasma anti-Factor Xa and IIa activities were measured using amidolytic assays.

Results: Given that radioactive doses for tinzaparin at 4 or 25 mg/kg were 8.6 x 10^6 and 10.1 x 10^6 dpm/kg, respectively, AUC and C_{max} values increased in approximate dose proportional manner. Following subcutaneous administration of tinzaparin at 4 or 25 mg/kg, based upon either plasma levels of anti-Factor Xa or anti-Factor IIa activities, AUC and C_{max} values increased in approximate dose proportional manners. Based upon plasma levels of anti-Factor Xa activities, AUC and C_{max} values for tinzaparin at a subcutaneous dose of 25 mg/kg (1805 Xal U/kg) and heparin at a subcutaneous dose of 12.5 mg/kg (2350 Xa I/kg) were approximately equivalent. Based upon plasma levels of anti-Factor IIa activities, AUC and C_{max} values for heparin at a subcutaneous dose of 12.5 mg/kg (2350 IIa I/kg) exceeded those observed for tinzaparin at a subcutaneous dose of 25 mg/kg (1150 IIal U/kg). Half-life values based upon plasma levels of radioactivity or anti-Factor Xa and anti-Factor IIa activities were relatively similar. Based upon plasma levels of anti-Factor Xa and anti-Factor IIa activities, volume of distribution values for tinzaparin were approximately equivalent to blood volume (66 mL/kg). However, based upon plasma levels of radioactivity, the volume of distribution of tinzaparin exceeded blood volume suggesting distribution into tissue. A similar pattern for volume of distribution values for heparin was observed. Differences observed between radioactivity and anti-Factor Xa or anti-Factor IIa activities for tinzaparin most likely reflect the broad distribution of molecular weights.

AUC and C_{max} values for plasma levels of radioactivity and anti-Factor Xa and anti-Factor IIa activities in female New Zealand White rabbits following subcutaneous administration of ^{3}H-tinzaparin at doses of 4 or 25 mg/kg and ^{3}H-heparin at a dose of 12.5 mg/kg/day.

Compound	Radioactivity			Anti-Factor Xa Activity			Anti-Factor IIa Activity		
	Dose DPM/kg	AUC, DPM x hr/mL	C_{max} DPM/mL	Dose Xai/kg	AUC, Xai U x hr/mL	C_{max} Xai U/mL	Dose IIai U/kg	AUC, IIai U x hr/mL	C_{max} IIai U/mL
Tinzaparin 4 mg/kg	8.6×10^6	3.3×10^3	6200	288	5.3	0.8	184	1.2	0.22
Tinzaparin 25mg/kg	10.1×10^6	3.8×10^3	9000	1805	85	7.6	1150	38	3.9
Heparin 12.5 mg/kg	7.1×106	6.0×10^3	4300	2350	95	7.0	2350	98	7.7

Half-life and Volume of Distribution values based upon plasma levels of radioactivity and anti-Factor Xa and anti-Factor IIa activities in female New Zealand White rabbits following intravenous administration of ^{3}H-tinzaparin at 1 mg/kg or ^{3}H-heparin at 0.5 mg/kg.

Compound	Dose	T½, min	Vd, mL/kg
Tinzaparin, 1 mg/kg	1.6×10^6 dpm/kg	18	236
	70 Xai U/kg	24	41
	46 IIai U/kg	22	60
Heparin, 0.5 mg/kg	1.3×10^6 dpm/kg	20	137
	94 Xai U/kg	24	52
	94 IIai U/kg	19	64

Dogs

Pharmacokinetics of ^{3}H-Tinzaparin in the Dog After a Single Subcutaneous or Intravenous Administration (Report No. HRC-NV069-88927).

Methods: Plasma pharmacokinetic studies were performed with dogs following subcutaneous or intravenous administration of ^{3}H-tinzaparin at doses of 1 and 4 mg/kg. Blood for determination of plasma drug-related radioactivity concentrations, anti-Factor Xa activities, and APTT values was collected at time points between 0.08 and 120 hr following intravenous administration or 0.25 and 120 hr following subcutaneous administration.

Results: AUC values following intravenous administration increased in a dose proportional manner. AUC values following subcutaneous administration increased with dose; although, increases were greater than proportional to dose. Bioavailability of tinzaparin administered by the subcutaneous route at 4 mg/kg, determined using either plasma drug-related radioactivity of anti-Factor Xa activity, was approximately 100%. Bioavailability of tinzaparin administered by the subcutaneous route at 1 mg/kg ranged from 51 to 73%. Plasma C_{max} values obtained with intravenous administration were greater than those obtained with subcutaneous administration. Clearance of tinzaparin (0.21-0.25 L/hr/kg), based upon drug-related radioactivity was comparable to the glomerular filtration rate (0.21 L plasma/hr/kg). However, clearance, based upon anti-Factor Xa activity, was significantly less than the glomerular filtration rate. The volume of distribution (0.30-0.35 L/kg), based upon drug-related radioactivity, was greater than

blood volume (0.09 L/kg). The volume of distribution (0.063-0.13 L/kg), based upon anti-Factor Xa activity, was comparable to blood volume. Difference in clearance and volume of distribution based upon drug-related radioactivity as opposed to anti-Factor Xa activity may be related to low molecular weight components of tinzaparin, that contain radiolabel but are devoid of any anticoagulant activity, that are freely filterable at the kidney glomerulus and able to distribute widely beyond the blood volume.

Pharmacokinetic parameters derived from the concentration of drug-related radioactivity in the plasma of dogs following administration of ^{3}H-tinzaparin by the subcutaneous or intravenous routes at doses of 1 and 4 mg/kg.

Parameters	1 mg/kg S.C.		1 mg/kg I.V.		4 mg/kg S.C.		4 mg/kg I.V.	
	Male	Female	Male	Female	Male	Female	Male	Female
T_{max}, hr	1.50	1.50	0.08	0.08	1.50	1.50	0.08	0.08
C_{max}, µg/mL	0.80	0.96	5.31	5.95	4.22	4.79	22.24	22.28
T½, hr (λ_2 phase)	1.31	1.33	0.88	0.89	1.67	1.64	1.078	1.139
AUC λ_2	1.02	1.08	3.90	4.33	9.81	11.84	17.13	18.30
Clearance, L/hr/kg	-	-	0.25	0.23	-	-	0.22	0.21
Vd, L/kg	-	-	0.32	0.30	-	-	0.35	0.35
Bioavailability, %	72	73	-	-	100	103	-	-

Pharmacokinetic parameters derived from the levels of anti-Factor Xa activity (corrected for baseline activity) in the plasma of dogs following administration of ^{3}H-tinzaparin by the subcutaneous or intravenous routes at doses of 1 and 4 mg/kg.

Parameters	1 mg/kg S.C.		1 mg/kg I.V.		4 mg/kg S.C.		4 mg/kg I.V.	
	Male	Female	Male	Female	Male	Female	Male	Female
T_{max}, hr	3.0	2.0	0.08	0.17	3.0	3.0	0.08	0.17
C_{max}, Ui/mL	0.109	0.215	1.143	1.165	1.190	1.533	3.634	3.275
T½, hr	2.37	2.27	0.67	0.83	3.10	2.88	1.65	2.23
AUC∞	0.63	0.94	1.23	1.44	8.54	10.33	7.14	7.64
Clearance, L/hr/kg	-	-	0.065	0.056	-	-	0.04	0.04
Vd, L/kg	-	-	0.063	0.067	-	-	0.10	0.13
Bioavailability, %	51	65	-	-	120	135	-	-

Distribution

Rats

Binding of ^{3}H-Tinzaparin to Rat Plasma Proteins (Report No. HRC-NV070-88853).

Methods: Binding of ^{3}H-tinzaparin to rat plasma proteins was determined using a micropartition/centrifugation technique. Blood samples were obtained from rats at 0.5 and 2 hr following subcutaneous or intravenous treatment with ^{3}H-tinzaparin at 1 or 4 mg/kg.

Results: ^{3}H-tinzaparin was not found to be extensively bound to rat plasma proteins as binding was generally <80%.

Binding of ^{3}H-Tinzaparin to Rat Plasma Proteins as Determined by Micropartition.

Time after dosing	Subcutaneous Route				Intravenous Route			
	1 mg/kg		4 mg/kg		1 mg/kg		4 mg/kg	
	Male	Female	Male	Female	Male	Female	Male	Female
0.5 hr								
2 hr								

Tissue Distribution of ^{3}H-Tinzaparin Determined by Whole Body Autoradiography (Report No. HRC-NV070-88853).

Methods: Tissue distribution of ^{3}H-tinzaparin was examined in male and pregnant and non-pregnant female Sprague-Dawley rats by whole body autoradiography following subcutaneous or intravenous administration of 4 mg/kg. Rats that received ^{3}H-tinzaparin by the subcutaneous route were sacrificed at 0.5, 2, and 24 hr after dosing (1 male and 1 non-pregnant female rat per time point. Rats that received ^{3}H-tinzaparin by the intravenous route were sacrificed at 10 min, 1 hr, and 24 hr after dosing (1 male rat, 1 non-pregnant female rat, and 1 pregnant female rat per time point).

Results: Following intravenous administration of ^{3}H-tinzaparin to male or non-pregnant female rats, distribution of radioactivity was widespread within 10 min after dosing. The highest contents were present in the bladder (urine). Lower levels were observed in the kidneys and other tissues. Tissue contents of radioactivity declined significantly at 1 and 24 hr as compared to 10 min. Similar findings were observed following subcutaneous administration of ^{3}H-tinzaparin to male or non-pregnant female rats; although, low levels of radioactivity were also observed at the injection site. The distribution of radioactivity in pregnant female rats following intravenous administration of ^{3}H-tinzaparin was similar to that described for male or non-pregnant female rats.

Tissue Distribution of ^{3}H-Tinzaparin in the Rat After Single and Repeated Intravenous Administration (Report Nos. 91100, 91101, 91102, 92078, and 92079).

Methods: The tissue distribution of total and drug-related radioactivity following intravenous administration of ^{3}H-tinzaparin (1 mg/kg) was examined in male Sprague-Dawley rats after 1, 7, 14, and 21 days of treatment and in female Sprague-Dawley rats after 1 day of treatment. On treatment days 1 and 21, rats were sacrificed at 10 min, 1 hr, and 24 hr after dosing. On treatment days 7 and 14, rats were sacrificed at 24 hr after dosing. Blood was collected for determination of blood and plasma content of total and drug-related radioactivity. The following organs and tissues were collected, weighed, and processed for determination of total and drug-related radioactivity: brain, pituitary gland, eyes, Harderian gland, submandibular glands (salivary glands), trachea, thymus, lungs, heart, lymph node from neck region, liver, kidneys, adrenal glands, spleen, pancreas, fat from neck region, mm. gastronecmii, skin from neck region, testis/ovaries, prostate, seminal vesicles, urinary bladder, tibia, bone marrow from the thigh bone, stomach, small intestine, colon, rectum, vena cava, aorta, tail (around the injection site), and carcass.

Results: After a single dose, the highest radioactivity concentrations were found in the kidneys (22.5 µg/g) at 10 min after dosing. At 1 hr after dosing, the highest concentrations of radioactivity were observed in the prostate and kidney. The kidney/plasma ratios at 10 min and 1 hr after dosing were >5. At 24 hr after dosing, the highest radioactivity concentration was found in the kidney and the kidney/plasma ratio was in excess of 200. The high concentrations of radioactivity observed in the kidney may be consistent with its role in excreting tinzaparin. Greater than 70% of drug-related radioactivity was found to be excreted in the urine. After treatment for 21 days, the radioactivity concentration observed in the kidney at 10 min after dosing was 28.2 µg/g. On day 21, significant concentrations of radioactivity were also observed in the liver and thyroid gland.

Tissue Distribution of ^{3}H-Tinzaparin Following Subcutaneous Administration to Male Rats and Non-pregnant and Pregnant Female Rats (Report No. HRC-NV070-88853).

Methods: The tissue distribution of drug-related radioactivity was examined in male and pregnant and non-pregnant female Sprague-Dawley rats following subcutaneous administration of ^{3}H-tinzaparin. Pregnant female rats received seven consecutive daily doses at 1 mg/kg/day starting on day 11 of gestation. Rats were sacrificed at 0.5, 2, and 24 hr following the last dose. Male and non-pregnant female rats received a single subcutaneous dose of 1 mg/kg and were sacrificed at 0.5, 2, and 24 hr after dosing. There were 3 rats per time point. For male and non-pregnant female rats, the following tissues were collected for analysis: adrenal glands, aorta, bone marrow, brain, eyes, fat, gonads (uterus and ovaries or testes), heart, kidneys, lacrimal glands, liver, lungs, lymph nodes, muscle, pancreas, pituitary gland, prostate, spleen, thymus, thyroid and parathyroid, vena cava, and walls of the stomach, small intestines, and large intestines. For pregnant female rats, the following tissues were collected: aorta, bone marrow, heart, kidneys, liver, lungs, mammary tissue, ovaries, placenta, uterus, and vena cava. In addition, the fetuses were removed and fetal livers were dissected out. The site of injection and residual carcasses were retained.

Results:

1. **Tissue Content Expressed as a Percent of the Administered Dose For Male and Non-Pregnant Female Rats**: For male and non-pregnant female rats at 0.5 hr after dosing, the percent of dose found at the injection site exceeded 30%, however, within 2 hr, less than 3% of the dose remained. At 0.5 hr, muscle tissue was found to contain the highest percentages of the administered dose at 6.44 and 4.71%, respectively. Blood for male and non-pregnant rats at 0.5 hr contained 4.24 and 4.11%, respectively. Plasma for male and non-pregnant rats at 0.5 hr contained 3.79 and 3.66%, respectively. Kidney tissue for male and non-pregnant female rats at 0.5 hr contained 2.17 and 1.85%, respectively. Radioactivity contents in the muscle, blood, plasma, and kidney were observed to decline at the 2 and 24 hr sampling times. For the liver in male rats, the radioactivity content of 1.20% at 0.5 hr was observed to increase to 2.05 and 1.80% at later time points, respectively. Similarly for the liver in non-pregnant female rats, the radioactivity content of 0.98% at 0.5 hr was observed to increase to 2.19 and 1.61% at later time points, respectively.

2. **Tissue Content Expressed as a Percent of the Administered Dose For Pregnant Female Rats**: For pregnant female rats, the highest percentages of the administered dose at 0.5 hr were observed in the liver (1.43%) and kidneys (1.17%); however, muscle tissue was not analyzed. The placenta contained <0.10% of the administered dose. Fetuses and fetal livers contained <0.02 and 0.01% of the administered dose, respectively.

3. **Tissue Concentration of Radioactivity Expressed on µg/g Basis**: It should be noted that examination of tissue radioactivity content on a µg/g basis reveals that the kidney (2.31 and 2.33 µg/g for male and non-pregnant rats, respectively, at 0.5 hr) and vena cava (2.10 and 0.83 µg/g for male and non-pregnant rats, respectively, at 0.5 hr) had the highest concentrations of radioactivity. The tissue/plasma ratio was found to be the highest for kidney (ratio of 3 at 0.5 hr). For pregnant female rats, the kidney was found to have the highest tissue concentration of radioactivity at 11.28 µg/g for the 0.5 hr sampling time. Similarly, the tissue/plasma ratio was found to be the highest for kidney (ratio of 10.06 at 0.5 hr). The high concentrations of radioactivity observed in the kidneys are consistent with this organ's primary role in the excretion of tinzaparin.

Proportions of drug-related radioactivity (% of dose administered) in tissues, carcasses, and at the site of injection in male rats and non-pregnant or pregnant female rats sacrificed at 0.5, 2, and 24 hr following subcutaneous administration of ^{3}H-tinzaparin at 1 mg/kg (single dose for male and non-pregnant female rats and 7 consecutive daily doses for pregnant female rats).

	Male Rats			Female Rats			Pregnant Female Rats		
Sacrifice Time (hr)	0.5 hr	2 hr	24 hr	0.5 hr	2 hr	24 hr	0.5 hr	2 hr	24 hr
Tissue total*	9.27	8.85	3.56	8.26	6.56	3.65	4.34	3.77	3.03
Injection site	32.78	3.13	1.18	30.83	3.22	0.79	5.59	1.19	0.94
Carcass	23.83	12.84	6.55	27.57	12.57	8.40	8.40	6.50	5.56
Total	65.88	24.82	11.28	66.66	22.35	12.84	18.34	11.47	9.53

*Excludes bone marrow, fat, lymph nodes, muscle, and plasma.

Transplacental Passage of ^{3}H-Tinzaparin in the Pregnant Female Rat (Report Nos. 91100, 91101, 91102, 92078, and 92079).

Methods: Tissue distribution of total and drug-related radioactivity was determined in pregnant female Sprague-Dawley rats on days 10 and 19 of gestation. On days 10 and 19 of gestation, pregnant female rats were sacrificed at 10 min, 1 hr, and 24 hr after dosing with 1 mg/kg. Blood was collected for determination of blood and plasma levels of total and drug-related radioactivity. The following organs were collected, weighted, and processed for determination of total and drug-related radioactivity content: liver, kidneys, uterus, and ovaries. Fetuses and placentae from each dam were removed and processed for determination of total and drug-related radioactivity content. Amniotic fluid was also collected for assessment of total and drug-related radioactivity content. For dams sacrificed on day 19 of gestation, the fetal liver and kidney were collected and processed for determination of total and drug-related radioactivity content.

Results: In general, the tissue distribution of radioactivity of pregnant female rats was similar to that observed for non-pregnant rats. For pregnant dams, the highest concentrations of radioactivity were observed in the kidneys; although, levels were less than one-half that found in non-pregnant animals. On day 10 of gestation, levels of radioactivity in the placenta were as much as 300 times higher than that observed in the fetus. On day 19 of gestation, levels of radioactivity in amniotic fluid were significantly lower than that observed in maternal plasma. Radioactivity concentrations in the fetal kidney were 25% of concentrations observed in the placenta at 10 min after dosing; however, kidney concentrations declined significantly at 1 and 24 hr after dosing, while placenta concentrations declined more slowly.

Median concentrations of total radioactivity (µg equivalent/mL or g) in maternal tissue on day 10 or 19 of gestation sacrificed at 10 min, 1 hr, and 24 hr following a single intravenous treatment with 1 mg/kg ^{3}H-tinzaparin to pregnant female rats.

Tissues	Day 10 of Gestation			Day 19 of Gestation		
	10 min	1 hr	24 hr	10 min	1 hr	24 hr
Kidneys	9.48	6.49	3.28	9.28	4.42	3.80
Liver	0.58	0.95	0.67	0.90	1.02	0.88
Ovaries	1.07	0.71	0.50	1.04	0.62	0.25
Plasma	3.63	0.94	0.04	4.03	0.72	0.03
Uterus	1.77	0.80	0.34	1.37	0.97	0.19
Whole-blood	1.61	0.21	0.03	1.18	0.25	0.02

TABLE XXVIa. Amounts and concentrations of total radioactivity and drug-related radioactivity in the foetus and placenta on day 10 of the gestational period and in amniotic fluid, placenta, liver, and kidney on day 19 of the gestational period at 10 min, 1 h, and 24 h after a single *i.v.* bolus injection of 250 µg ^{3}H-labelled LHN-1 to pregnant rats. Median.

Tissue	number	Day 10 of the gestational period (N = 8-16)					
		Total radioactivity x 10^{3} µg equivalents			Drug-rel. radioact.		
		10 min	1 h	24 h	10 min	1 h	24 h
Foetus	1	0.34	2.4	0.22	-	-	-
	2	0.36	0.23	0.26	-	-	-
	3	0.24	0.75	0.98	-	-	-
Placenta	1	105	70	18	-	-	-
	2	76	67	17	-	-	-
	3	89	65	19	-	-	-
		Day 19 of the gestational period (N = 3-17)					
		Total radioactivity x 10^{3} µg equivalents/g or ml			Drug-rel. radioact. x 10^{3} µg equivalents/g or ml		
	number	10 min	1 h	24 h	10 min	1 h	24 h
Amniotic fluid	1	7.0	14	19	-	-	-
	2	8.5	13	21	-	-	-
	3	5.6	14	24	-	-	-
Kidneys	1	313	3.6	34	314	< 1	< 1
	2	198	77	2.2	186	< 1	2.4
	3	289	10	5.0	240	8.3	2.1
Liver	1	49	26	25	44	15	9.7
	2	24	22	19	25	20	8.5
	3	33	14	20	23	15	8.3
Placenta	1	822	434	286	748	474	306
	2	886	418	316	848	389	299
	3	935	386	255	980	345	256

Excretion of ^{3}H-Tinzaparin into Milk of Lactating Female Rat (Report Nos. 91100, 91101, 91102, 92078, and 92079).

Methods: Excretion of total and drug-related radioactivity in milk of lactating female rats was examined on day 7 postpartum. One hr prior to drug administration, lactating dams were removed from suckling pups, anesthetized, and a catheter was inserted into the tail artery. The secretion of milk was stimulated 15 min prior to sampling by oxytocin. Rats were milked for three periods of 30 min starting 10 min, 1 hr, and 24 hr after intravenous administration of ^{3}H-tinzaparin. There were 3 dams per time point. Blood samples were collected at 0, 15, and 30 min of each 30 min milk collection period.

Results: Excretion of drug-related radioactivity into milk was low as shown in the table below. Drug-related radioactivity concentrations in milk at 10 min, 1 hr, and 24 hr after dosing were 0.2-0.5%, 2.5-4.2%, and 41.1% of plasma concentration.

TABLE XXVII. Concentrations (μg equivalents/ml) of drug-related radioactivity in plasma and milk on day 7 post partum after a single *i.v.* bolus injection of 250 μg ^{3}H-labelled LHN-1 to nursing rats. Median and range.

	10 min			1 h			24 h		
Relative sampling time	0 min	15 min	30 min	0 min	15 min	30 min	0 min	15 min	30 min
Plasma conc.	3.43	1.92	1.62	0.72	0.51	0.43	0.009	0.009	0.009
Milk conc.	0.0893 (0.0066-0.011)			0.018 (0.014-0.020)			0.0037 (0.0034-0.0042)		
Milk volume	0.63 (0.34-1.01)			1.36 (0.79-1.51)			0.43 (0.17-0.46)		

Distribution of ^{3}H-Tinzaparin in Milk of Lactating Female Rats Following Subcutaneous Administration (Report No. HRC-NV070-88853).

Methods: The distribution of drug-related radioactivity was examined in lactating female Sprague-Dawley rats that received ^{3}H-tinzaparin by the subcutaneous route of administration. Six female rats received ^{3}H-tinzaparin at 1 mg/kg/day starting on day 19 of gestation and continuing until 288 hr (day 11) postpartum. On days 5 and 11 postpartum, milk samples of each dam were obtained by replacement of a portion of the dam's litter (4 of 10) with pups of a similar age, which had been reared by a control dam. The pups were allowed to suckle for 1 hr. Three dams and their pups were sacrificed at 148 or 292 hr postpartum (4 hr after dosing in each case). Milk samples contained in the stomachs of the three control pups were collected and assayed for content of drug-related radioactivity. Blood samples from dams were collected for determination of plasma concentrations of drug-related radioactivity.

Results: The percent of total dose found in whole carcasses of pups from control litters sacrificed after suckling for 1 hr on tinzaparin-treated dams was extremely low (<0.01%). Concentrations of drug-related radioactivity in maternal milk were greater than observed in maternal plasma; although, it should be noted that values represent an extremely small percentage of the total administered dose (<0.002 %dose/g or mL).

Drug-related radioactivity in maternal blood, plasma, and milk (µg equivalent/g or mL), in the whole carcasses of pups from control litters sacrificed after suckling for 1 hr on tinzaparin-treated dams (% total dose administered) at 148 and 292 hr postpartum. In parentheses, the ratio of radioactivity in blood and milk to that in plasma is shown.

Sacrifice time, hr	148 hr	292 hr
Maternal blood (µg equiv./g)	0.049 (1.23)	0.046 (1.18)
Maternal plasma (µg equiv./mL)		
Maternal milk (µg equiv./g)	0.05 (1.28)	0.07 (1.72)
Whole carcasses of pups (% total dose administered)	<0.01 to 0.02	<0.01

Rabbits

Determination of Placental Transfer of Tinzaparin and Conventional Heparin After Subcutaneous Administration in Rabbits and Rats (Report No. 10788).

Methods: A single dose of ^{3}H-tinzaparin at 25 mg/kg or ^{3}H-heparin at 12.5 mg/kg was administered by the subcutaneous route to pregnant female New Zealand white rabbits during the last week of pregnancy. The specific day of gestation was not specified. For comparison, 4 pregnant female Wistar rats received ^{3}H-tinzaparin by the subcutaneous route at a dose of 25 mg/kg. The activity of ^{3}H-tinzaparin was as follows: 69 Xal U/mg and 46 IIal U/mg. The activity of ^{3}H-heparin was as follows: 179 Xal U/mg and 188 IIal U/mg. For pregnant female rabbits, maternal and fetal blood samples (2 fetuses were pooled) were obtained 1 hr after treatment with tinzaparin or heparin. Plasma radioactivity levels were measured with a liquid scintillation counter. Plasma anti-Factor Xa and IIa activities and antithrombin III were measured using amidolytic assays. For pregnant female rats, 1 hr after treatment with tinzaparin, four fetuses were isolated from each dam. Radioactivity levels in two fetal livers and two complete fetuses were measured. Radioactivity levels were also measured in maternal plasma, kidney, and liver.

Results: For rabbits, fetal plasma levels of radioactivity were approximately 6% of maternal plasma levels. Fetal kidney levels of radioactivity were comparable to fetal plasma levels, while fetal liver levels were less than one-half of fetal plasma levels. Anti-Factor Xa and anti-factor IIa activities were not detected in fetal rabbit plasma. Concentrations of antithrombin III in fetal plasma were approximately two-thirds of that found in maternal plasma. Based upon the apparent lack of any anticoagulant activity in rabbit fetal plasma, it appears in general that only low molecular weight components of tinzaparin, devoid of any anticoagulant activity, yet containing radiolabel, were able to cross the placenta into fetal tissues. Similar results were obtained with heparin, which is composed of components with a broad range of molecular weights. For rat fetuses, liver or whole body levels of radioactivity were approximately 2.5 to 3% of maternal plasma levels.