Mean concentrations of radioactivity, Xal activity, and IIal activity in organ and plasma samples after subcutaneous administration of $^{3}$H-tinzaparin and $^{3}$H-heparin to pregnant female New Zealand White rabbits.

| Source | Maternal/ Fetal | Radioactivity, DPM x $10^3$ per mL or gram | | Xal, U/mL | | IIal, U/mL | |
|---|---|---|---|---|---|---|---|
| | | Tinzaparin | Heparin | Tinzaparin | Heparin | Tinzaparin | Heparin |
| Plasma | Mother before fetal sampling | 254 | 253 | 2.8 | 3.0 | 1.05 | 2.9 |
| | Mother after fetal sampling | 201 | 274 | 3.5 | 4.1 | 1.12 | 4.5 |
| | Fetus 1 + 2 | 13 | 5.6 | 0.0 | 0.0 | 0.0 | 0.0 |
| | Fetus 3 + 4 | 14 | 5.7 | - | - | - | - |
| Liver | Mother | 58 | 165 | - | - | - | - |
| | Fetus 1 + 2 | 5.9 | 4.5 | - | - | - | - |
| | Fetus 3 + 4 | 6.6 | 4.7 | - | - | - | - |
| Kidney | Mother | 1664 | 1770 | - | - | - | - |
| | Fetus 1 + 2 | 13 | 6.3 | - | - | - | - |
| | Fetus 3 + 4 | 17 | 6.9 | - | - | - | - |

Mean concentration of radioactivity (DPM x $10^3$/mL or g) in plasma, organs, and complete fetuses after subcutaneous administration of $^{3}$H-tinzaparin to pregnant female Wistar rats at a dose of 25 mg/kg.

| Analysis | Mean |
|---|---|
| Maternal Plasma | 212 |
| Maternal Liver | 56 |
| Maternal Kidney | 1356 |
| Fetal Liver | 6.8 |
| Fetus | 5.4 |

## Dogs

### Binding of $^{3}$H-Tinzaparin to Dog Plasma Proteins (Report No. HRC/NV069/88927).

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

**Results**: $^{3}$H-tinzaparin was not found to be extensively bound to dog plasma proteins as binding was generally <64%.

Binding of $^{3}$H-Tinzaparin to Dog 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 Drug-Related Radioactivity In Dogs Following Subcutaneous Administration of $^{3}$H-Tinzaparin (Report No. HRC/NV069/88927).**

**Methods:** The tissue distribution of drug-related radioactivity was examined in dogs following subcutaneous administration of 1 mg/kg. One dog/sex was sacrificed at 1.5, 24, and 120 hr after dosing. Blood was collected for determination of radioactivity concentration and plasma radioactivity concentration, anti-Factor Xa activity, and activated partial thromboplastin time. The following organs and tissues were collected for determination of radioactivity concentration: adrenal gland, aorta, bile, bone marrow, brain, eyes, fat, stomach, small intestine, large intestine, heart, kidneys, liver, lungs, lymph nodes, muscle, ovaries, pancreas, pituitary, prostate (males), spleen, testes (males), thymus, thyroid (and parathyroid), uterus (females), vena cava, and injection site.

**Results:**

1. **Tissue Concentration of Radioactivity Expressed on µg/g Basis:** At 1.5 hr after drug administration, the highest concentrations of drug-related radioactivity were found in the kidney (4.09/4.73 µg/g) for both male and female dogs and the prostate gland (4.89 µg/g); although, levels declined significantly at 24 and 120 hr after dosing. Peak concentrations of radioactivity for the liver (2.68/2.93 µg/g) and lymph nodes (3.58/4.85 µg/g) were observed at 24 hr after dosing. Significant concentrations of radioactivity were still observed in the liver (1.77/2.07 µg/g) and lymph nodes (0.82/1.24 µg/g) at 120 hr after dosing.

2. **Tissue Content Expressed as a Percent of the Administered Dose:** At 1.5 hr after dosing, greater than 40% of the administered dose was found at the injection site; however, by 24 hr, less than 0.4% remained. At 1.5 hr after dosing, the highest levels of systemic radioactivity were observed in the muscle (4.47/6.41%), plasma (4.30/4.55%), and whole blood (3.26/4.86%); although, levels declined significantly at 24 and 120 hr after dosing. Peak levels of radioactivity for the liver (7.34/7.08%) and lymph nodes (3.21/4.42%) were observed at 24 hr after dosing. Significant levels of radioactivity were still observed in the liver (5.15/5.58%) and lymph nodes (0.97/1.23%) at 120 hr after dosing.

3. **Tissue: Plasma Ratios:** At 1.5 hr after dosing, tissue: plasma ratios for drug-related radioactivity were highest for the kidney (4.71/5.21) and lymph nodes (1.54/2.82) in both male and female dogs and in the prostate gland (5.59) for male dogs. At 24 and 120 hr after dosing, several tissues had ratios >2 due to low plasma radioactivity levels, which could not be accurately measured.

Proportions of drug-related radioactivity (% dose administered) in tissues and at the site of injection in dogs sacrificed at 1.5, 24, and 120 hr following subcutaneous administration of $^{3}$H-tinzaparin at 1 mg/kg.

| Sacrifice Time, hr | 1.5 hr | | 24 hr | | 120 hr | |
|---|---|---|---|---|---|---|
| Animal | Male | Female | Male | Female | Male | Female |
| Tissue Total* | 15.59 | 22.53 | 13.85 | 15.31 | 8.22 | 9.30 |
| Injection Site | 40.30 | 42.90 | 0.38 | 0.53 | 0.35 | 0.29 |
| Total | 55.89 | 65.43 | 14.23 | 15.84 | 8.57 | 9.59 |

* Excludes plasma, bone marrow, bile, stomach, small intestine, and large intestine.

## Metabolism

### Rats and Dogs

**Urinary Metabolites of $^{3}$H-Tinzaparin in Rats and Dogs (Report No. HRC-NV070-88853 and HRC/NV069/88929).**

**Methods**: The nature of drug-related radioactivity present in rat and dog urine samples was determined by high performance liquid chromatography using gel permeation columns. Rats or dogs received $^{3}$H-tinzaparin by the subcutaneous or intravenous route at 1 mg/kg. Urine samples were collected at intervals of 0-6, 6-24, 24-48, 48-72, 72-96, and 96-120 hr after dosing.

**Results**: Urine samples were found to contain a similar range of compounds with molecular weights similar to those present in $^{3}$H-tinzaparin suggesting that little metabolism of this group of compounds occurred in rats or dogs.

## Excretion

### Rats

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

**Methods**: Excretion of total and drug-related radioactivity in the urine and feces were examined in male Sprague-Dawley rats following intravenous treatment with $^{3}$H-tinzaparin for 1 or 21 days. Following treatment with a single dose, urine and feces were collected from rats for 7 days after dosing. For rats treated for 21 days, urine and feces were collected daily. One-half of the rats treated for 21 day were sacrificed 24 hr after the last dose, while remaining animals were followed for 7 days. For rats sacrificed 24 hr after the last dose, the left kidney was used for assessment of radioactivity content, while the right kidney was used for determination of anti-FXa activity.

Results: Following a single intravenous dose, drug-related radioactivity was primarily excreted in the urine (85.61%). During a 21-day treatment period with $^{3}$H-tinzaparin at 1 mg/kg/day, radioactivity was primarily excreted in the urine. The % of dose excreted in the urine from days 1 to 21 ranged from 63.82 to 76.66%, while the % of dose excreted in the feces ranged from 0.79 to 2.39%. From days 22 to 28, the % of dose excreted in the urine declined from 23.34 to 7.15%, while the % of dose excreted in the feces ranged from 0.52 to 2.12% during this period.

Excretion of drug-related radioactivity in urine and feces following a single intravenous administration of 1 mg/kg $^{3}$H-tinzaparin to rats.

| Interval | % of Dose Administered | |
|---|---|---|
| | Urine | Feces |
| Day 1 | 73.71 | 1.52 |
| Day 2 | 5.04 | 0.83 |
| Total (Days 1-7) | 85.61 | 4.18 |

**Excretion of $^{3}$H-Tinzaparin by Rats Following Subcutaneous or Intravenous Administration and by Bile Duct-Cannulated Rats Following Intravenous Administration (Report No. HRC-NV070-88853).**

Methods: Excretion of drug-related radioactivity by male and female Sprague-Dawley rats was determined following subcutaneous or intravenous administration of $^{3}$H-tinzaparin at 1 mg/kg. Excretion of drug-related radioactivity by bile duct-cannulated male and female Sprague-Dawley rats was determined following intravenous administration of $^{3}$H-tinzaparin at 1 mg/kg. There were 3 rats/sex/group. Urine was collected at intervals of 0-6, 6-24, 24-48, 48-72, 72-96, and 96-120 hr after dosing. Fecal samples were collected in 24 hr intervals up to 120 hr after dosing. For bile duct-cannulated rats, bile, urine, and fecal samples were collected as follows: bile was collected at intervals of 0-1, 1-2, 2-3, 3-4, 4-5, 5-6, 6-24, and 24-48 hr; urine was collected at intervals of 0-6, 6-24, and 24-48 hr; and fecal samples were collected from 0-48 hr. Cages were rinsed and cage wash samples were retained for measurement of radioactivity. Expired air was passed through two traps of distilled water in order to collect volatile radioactivity.

Results: Following subcutaneous or intravenous administration of $^{3}$H-tinzaparin to male and female rats, >75% of the drug-related radioactivity was excreted in the urine. For bile duct-cannulated rats that received $^{3}$H-tinzaparin by the intravenous route, similarly >75% of drug-related radioactivity was excreted in the urine, while only 0.16% was excreted in the bile and 1.34% was associated with the liver and walls of the gastrointestinal tract.

Percent recovery of drug-related radioactivity following single subcutaneous or intravenous doses of $^{3}$H-Tinzaparin to normal rats and single intravenous doses to bile duct-cannulated rats.

| | Normal Rats/ Subcutaneous Route | Normal Rats/ Intravenous Route | Bile Duct-Cannulated Rats/ Intravenous Route |
|---|---|---|---|
| Urine | 77.56 | 81.38 | 75.32 |
| Cage Wash | 0.13 | 0.12 | 2.03 |
| Feces | 3.06 | 2.26 | 1.91 |
| Injection Sites | 0.31 | - | - |
| Carcass | 3.13 | 2.67 | 5.39 |
| Total | 84.18 | 86.43 | (84.65) |
| Bile | - | - | 0.16 |
| Liver and GI Tract Walls | - | - | 1.34 |
| Total | - | - | 86.14 |

## Dogs

**Excretion of Drug-Related Radioactivity Following Subcutaneous or Intravenous Administration of $^{3}$H-Tinzaparin to Dogs (Report No. HRC/NV069/88929).**

**Methods**: Urinary and fecal excretion of drug-related radioactivity examined in dogs following subcutaneous or intravenous administration of 1 mg/kg $^{3}$H-tinzaparin. Urine was collected at intervals of 0-6, 6-24, 24-48, 48-72, 72-96, and 96-120 hr after dosing. Fecal excretions were collected in 24 hr intervals up to 120 hr after dosing. Cages were rinsed at 24 hr intervals.

**Results**: Following subcutaneous or intravenous administration of $^{3}$H-tinzaparin at a dose of 1 mg/kg, drug-related radioactivity was primarily excreted in the urine.

Excretion of drug-related radioactivity in dogs that received $^{3}$H-tinzaparin by the subcutaneous or intravenous route at a dose of 1 mg/kg.

| Source | Subcutaneous Route | | | Intravenous Route | | |
|---|---|---|---|---|---|---|
| | Male | Female | M + F | Male | Female | M + F |
| Urine | 77.59 | 86.99 | 82.29 | 77.00 | 87.20 | 82.10 |
| Feces | 1.73 | 0.53 | 1.13 | 1.87 | 0.51 | 1.19 |
| Cage wash | 4.43 | 2.53 | 3.48 | 4.62 | 0.87 | 2.75 |
| Total | 83.75 | 90.05 | 86.90 | 83.50 | 88.58 | 86.04 |

The absorption, distribution, metabolism, and excretion of tinzaparin were assessed in rats, rabbits, and dogs. Following administration of $^{3}$H-tinzaparin by the subcutaneous or intravenous route at doses of 1 or 4 mg/kg to rats or dogs, plasma AUC values increased in a dose proportional manner. Following subcutaneous administration of tinzaparin at 4 or 25 mg/kg to rabbits, based upon either plasma levels of drug-related radioactivity, anti-Factor Xa activity or anti-Factor IIa activity, AUC and $C_{max}$ values increased in a dose proportional manner. Bioavailability of tinzaparin administered by the subcutaneous route to rats or dogs, as assessed by plasma drug-related radioactivity was approximately 100%. In contrast, bioavailability, as assessed by anti-Factor Xa activity, was approximately 70%. Clearance of tinzaparin in rats (0.65-0.75 L/hr/kg), based upon plasma drug-related radioactivity or anti-Factor Xa

activity was between the glomerular filtration (0.2 L/hr/kg) and renal plasma flow (1.3 L/hr/kg). Clearance of tinzaparin in dogs (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. Clearance following intravenous administration of 4500 IU tinzaparin to healthy human volunteers was 1.7 L/hr and the primary route of elimination was renal. For rats, rabbits, and dogs, volume of distribution values for tinzaparin, 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; however, volume of distribution values, based upon plasma levels of radioactivity, exceeded blood volume suggesting distribution into tissue. For healthy human volunteers, the volume of distribution as indicated by anti-Factor Xa activity was approximately equivalent to the blood volume (i.e., central compartment). Differences in pharmacokinetic parameters based upon plasma drug-related radioactivity and anti-Factor Xa/anti-Factor IIa activity most likely reflect the broad distribution of molecular weights for the components of tinzaparin. Tissue distribution studies in rats following subcutaneous or intravenous administration of $^{3}$H-tinzaparin using whole body autoradiography or measurement of tissue radioactivity suggested a widespread distribution of radioactivity. Initially, tissue radioactivity concentrations were highest in the kidney, which was consistent with this organ's primary role in excretion. At later time points, high concentrations of radioactivity were observed in the liver. Studies with pregnant female rats and rabbits suggested that small amounts of drug-related radioactivity could cross the placenta and enter fetal tissues. Studies with lactating female rats indicated that low levels of drug-related radioactivity were excreted into the milk; although, there were negligible levels of radioactivity evident in suckling pups. Chromatographic examination of urine excreted from rats and dogs treated with $^{3}$H-tinzaparin indicated no significant metabolism of the test article. Excretion studies in rats and dogs indicated that 70 to 80% of drug-related radioactivity was excreted in the urine. Negligible levels of drug-related radioactivity were excreted in the feces or bile.

Summary of pharmacokinetic parameters following a single subcutaneous of tinzaparin to healthy human volunteers.

| Parameter | Subcutaneous Dose | |
|---|---|---|
| | 4,500 IU (0.9 mg/kg) | 175 IU/kg (1.75 mg/kg) |
| $C_{max}$ (IU/mL) | 0.25 | 0.87 |
| $T_{max}$ (hr) | 3.7 | 4.7 |
| $AUC_{0-\infty}$ (IU * hr/mL) | 2.0 | 9.6 |
| Half-life (hr) | 3.4 | 3.9 |

**TOXICOLOGY:**

**ACUTE TOXICITY**

| Species | Report Number | Testing Laboratory | Date Started | Date Completed | Drug Batch & Activity |
|---|---|---|---|---|---|
| NMRI mice | 0786 | [redacted] | 02-13-86 | 09-22-86 | F85010 [redacted] |
| NMRI mice | 884 | | 04-25-84 | 09-27-84 | F84001 [redacted] |
| NMRI mice | 0686 | | 02-11-86 | 09-17-86 | F85010 [redacted] |
| Mice | 784 | | 04-03-84 | 09-26-84 | F84001 [redacted] |
| Wistar rats | 0586 | | 02-04-86 | 12-9-86 | F85010 [redacted] |
| Rats | 4584 | | 07-17-84 | 09-27-84 | F84001 [redacted] |
| Wistar rats | 0486 | | 02-06-86 | 09-16-86 | F85010 [redacted] |
| Rats | 3184 | | 06-26-84 | 09-26-84 | F84001 [redacted] |

**Methods**: The acute toxicity of tinzaparin was examined in mice and rats. Drug was administered to mice by the intravenous route at dose volumes of 10 or 25 mL/kg, or by the subcutaneous route at dose volumes ranging from 1 to 25 mL/kg. Drug was administered to rats by the intravenous route at a dose volume of 10 mL/kg, or by the subcutaneous route at a dose volume of 1 or 20 mL/kg. For all studies, the vehicle was [redacted] Animals were monitored for clinical signs of toxicity and moribundity/ mortality for 15 days after dosing. Following 15 day observation periods, animals were submitted to gross pathological examination.

**Results**: Following intravenous administration of tinzaparin to NMRI mice at doses ranging from 25 to 2000 mg/kg, clinical signs of toxicity during the first 2 hr included decreased motor activity, unsteady gait/high gait/abducted legs, blue tail, bleeding from injection site, closed eyes, and piloerection. After 2 hr, clinical signs at doses ≥250 mg/kg/day included paleness, closed eyes, and convulsions. Other clinical signs included bleeding from injection site, blue tail, decreased motor activity, piloerection, and unsteady gait/high gait. Gross pathological findings at doses ≥250 mg/kg included blood in the gastrointestinal tract, anemic internal organs, and hemothorax. Following subcutaneous administration of tinzaparin to NMRI mice at doses ranging from 300 to 1000 mg/kg, clinical signs of toxicity during the first 30 min after dosing included

hematoma formation and bleeding at the injection site. Clinical signs after 30 min included hematoma formation and bleeding at the injection site, decreased motor activity, piloerection, and ptosis. Following intravenous administration of tinzaparin to Wistar rats at doses ranging from 300 to 5000 mg/kg, clinical signs within the first 30 min were principally confined to the high dose group and included increased respiration, ptosis, piloerection, and decreased motor activity. At 2 hr dosing, clinical signs observed in all treatment groups included bleeding from the injection site, piloerection, and decreased motor activity. Clinical signs observed in only the high dose group were decreased respiration, ptosis, and high gait. From 2 hr to day 15, clinical signs were confined to the 3000 and 5000 mg/kg groups and included decreased motor activity, piloerection, high gait, and anemia. Gross pathological findings for animals at 3200 mg/kg included bleeding in the thymus, blood from the injection site, blood in the gastrointestinal tract, and anemic internal organs. Following subcutaneous administration of tinzaparin to Wistar rats at doses ranging from 100 to 1000 mg/kg, clinical signs within 2 hr after dosing included hematoma formation and bleeding at the injection site. From 2 hr to day 15 in all treatment groups, general signs included anemia, piloerection, decreased motor activity, and unsteady gait. Signs localized to the injection site in all treatment groups included hematomas, wounds, alopecia, and firm swelling.

Acute toxicity of tinzaparin in mice and rats by the intravenous and subcutaneous routes.

| Species and Route | | Dose mg/kg | Dose IU/kg | Maximum nonlethal dose, mg/kg | | Minimum lethal dose, mg/kg | | $LD_{50}$, mg/kg | | Time to Death |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | M | F | M | F | M | F | |
| NMRI mice 5/sex/dose (0786) | IV | 0<br>100<br>300<br>1000 | 0<br>7320<br>22000<br>73200 | | | | | | | - |
| NMRI mice 5/sex/dose (884)<br>Tinzaparin<br><br>Heparin<br><br>Chemical LMWH | IV | 0<br>25<br>70<br>185<br>250<br>500<br>1000<br>1500[a]<br>2000[b] | 0<br>1900<br>5400<br>14200<br>19200<br>38300<br>76700<br>115000<br>153000 | | | | | | | Within 2 days |
| NMRI mice 5/sex/dose (0686) | SC | 0<br>300<br>1000<br>3000 | 0<br>22000<br>73200<br>220000 | | | | | | | 1 to 5 days |
| Mice 5/sex/dose (784)<br>Tinzaparin<br><br>Heparin<br><br>Chemical LMWH | SC | 0<br>250[c]<br>500<br>680<br>1000<br>1850<br>2000<br>5000 | 0<br>19200<br>38300<br>52100<br>76700<br>142000<br>153000<br>383000 | | | | | | | 1 to 3 days |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Wistar rats 5/sex/dose (0586) | IV | 0<br>300<br>1000<br>3000<br>5000 | 0<br>22000<br>73200<br>220000<br>366000 | | | | 1 to 5 days |
| Rats 5/sex/dose (4584)<br>Tinzaparin<br><br>Heparin<br><br>Chemical LMWH | IV | 0<br>25<br>60[d]<br>100<br>400<br>800<br>1600<br>3200 | 0<br><br><br>7700<br><br>61300<br>123000<br>245000 | | | | |
| Wistar rats 5/sex/dose (0486) | SC | 0<br>100<br>300<br>1000 | 0<br>7300<br>22000<br>73200 | | | | <20 hr to day 5 |
| Rats 5/sex/dose (3184)<br>Tinzaparin<br><br>Heparin<br><br>Chemical LMWH | SC | 0<br>25<br>62[f]<br>100<br>400<br>1600<br>3200 | 0<br>1900<br>4800<br>7700<br>15300<br>30700 | | | | |

a. The highest dose of heparin administered to mice was 1500 mg/kg.
b. The highest dose of tinzaparin or chemical LMW heparin administered to mice was 2000 mg/kg/day.
c. Dose levels have only been provided for tinzaparin treatment groups.
d. The dose of 60 mg/kg was only used with heparin.
e. The dose of 1600 mg/kg was apparently not administered to female rats.
f. The dose of 62 mg/kg was only used with heparin.

The acute toxicity of tinzaparin was examined in mice and rats by the intravenous and subcutaneous routes of administration. Following intravenous administration, the maximum nonlethal doses in male and female mice were 185 and 250 mg/kg, respectively. The minimum lethal doses in male and female mice were 250 and 500 mg/kg, respectively. $LD_{50}$ values in male and female mice were 185 and 1000 mg/kg, respectively. Following subcutaneous administration, the maximum nonlethal doses in male and female mice were 250 and 500 mg/kg, respectively. The minimum lethal doses in male and female mice were 500 and 300 mg/kg, respectively. Following intravenous administration, the maximum nonlethal doses in male and female rats were 800 and 1600 mg/kg. The minimum lethal doses in male and female rats were 1600 and 3200 mg/kg, respectively. Following subcutaneous administration, the maximum nonlethal and minimum lethal doses in rats were 25 and 100 mg/kg/day, respectively.

The acute toxicity of tinzaparin in mice by the intravenous and subcutaneous routes were relatively similar. In contrast for rats, tinzaparin administered by the subcutaneous route was significantly more toxic than by the intravenous route. Symptoms of acute toxicity included hematoma formation and bleeding at the injection site, anemia, decreased motor activity, unsteady gait, piloerection, and ptosis.

## SUBACUTE/SUBCHRONIC/CHRONIC TOXICITY

## RATS

### Four Week Intravenous Toxicology Study in Rats (Study #5785).

**Methods**: In a study conducted by ——— on 9/24 and 11/8/85, groups of 12M and 12F Wistar rats were injected I.V. with HFN-1 (Batch 524651) for 28 consecutive days at levels of 0, 10, 30, and 100 mg/kg. The volumetric dose was 5 ml/kg in each case and the concentration of the injected solutions were 0.2, 0.6, and 2% at the low, mid, and high drug groups, respectively. In a 14-day intravenous toxicology study, rats received tinzaparin at doses of 0, 3, 10, 30, 100, and 300 mg/kg/day (Study #2285). A no effect dose was not established. Anemia was evident at 100 mg/kg/day. Body weight gain was by >10% in all treatment groups. Discoloration of the tail was evident at doses >10 mg/kg/day. Pale organs were evident in animals at 300 mg/kg/day.

**Results**:

No drug related deaths occurred at any of the three test dosages. Dose related discoloration of the injected tail occurred in all drug groups, plus bleeding from the injection site at the upper two levels. Two animals from the mid dose and seven at the top dose developed hematomas in areas of the body other than the injection site. Overall, body weight and food consumption were unaffected. Hematology revealed dose related 8 to 21% reductions in RBCs in females ($p = <0.05$ in low dose females and $<0.01$ in mid and high dose females) and dose related 3-12% reductions in both sexes of Hb ($p = <0.05$ and $<0.01$ in high dose males and females respectively), a dose related trend towards increase in neutrophils (due no doubt to infection in traumatized tails) and in lymphocytes. Serum chemistry revealed a general lowering of serum potassium ($p <0.01$ in high dose males.).

Absolute and relative weights were increased, generally in a dose related way, at all drug levels or in both sexes for the spleen, liver and kidneys. Statistical significance ($p = <0.05$ or $<0.01$) was achieved with respect to the liver and spleen, particularly at the top dose.

Macroscopic inspection revealed focal hemorrhage in the thymus and hemorrhages in lymph glands in a few animals from each drug level and contusions in 7/12 at the high dose. Comprehensive microscopic exam of control and high dose animals revealed extrahematopoiesis in the liver, and spleen and hemorrhage in various internal tissues at the high dose; examination of lymph gland tissue at all levels revealed reactive lymphoid hyperplasia that was dose related in frequency (0/24 controls vs 3/24, 8/24, 10/24 at the L,M, and H dose groups). The latter is no doubt a consequence of the internal hemorrhage that the drug induced. Although the bones (femur) were examined microscopically, there is no mention of pathology at this site.

The study demonstrated that the toxicity of Logiparin is directly and indirectly due to its anticoagulant properties.

Addendum: Tinzaparin Batch number F84001 with a specific activity of 76.7 anti-Factor Xa IU/mg was used in this study. Doses were equivalent to 0, 800, 2300, and 7700 anti-Factor Xa IU/kg/day. RBC counts and hemoglobin levels for male rats at 100 mg/kg/day were decreased to 83.1 and 87.6% of control values ($7.7 \times 10^6$/µL and 145 g/L), respectively. RBC counts for female rats at 10, 30, and 100 mg/kg/day were decreased to 92.5, 91.25, and 78.75% of the control ($8.0 \times 10^6$/µL), respectively. Hemoglobin levels for female rats at 100 mg/kg/day was decreased to 91.6% of the control (143 g/L). Mean corpuscular volume for female rats at 100 mg/kg/day was increased to 112.7% of the control (63 cµ). WBC counts for male rats at 30 and 100 mg/kg/day were increased to 131.9 and 133.6% of the control ($11.6 \times 10^3$/µL), respectively. Neutrophil counts for male rats at 30 and 100 mg/kg/day were increased to 129.4 and 158.8% of the control ($1.7 \times 10^3$/µL), respectively. WBC counts for female rats at 100 mg/kg/day were increased to 124.6% of the control ($12.2 \times 10^3$/µL). Potassium levels for male rats at 100 mg/kg/day were decreased to 90.8% of the control (6.5 mmol/L). Absolute and relative spleen weights for male rats at 100 mg/kg/day were increased to 130.9 and 138.4% of the control (692 mg and 2610), respectively. Absolute spleen weights for male rats at 30 and 100 mg/kg/day were increased to 117.1 and 158% of the control (503 mg), respectively. Relative spleen weights for female rats at 30 and 100 mg/kg/day were increased to 114.1 and 154.3% of the control (2703), respectively. Absolute liver weights for female rats at 30 and 100 mg/kg/day were increased to 110.7 and 118.2% of the control (7708 mg), respectively. Relative liver weights for female rats at 30 and 100 mg/kg/day were increased to 109.8 and 117.1% of the control (41), respectively. Relative kidney weights for male rats at 100 mg/kg/day were increased to 109.8% of the control (6825). Contusions were observed for 1 of 12 male rats and 7 of 12 female rats at 100 mg/kg/day.

In a 4-week intravenous toxicology study, rats received tinzaparin at doses of 0, 10, 30, and 100 mg/kg/day. Dose related discoloration of the tail occurred in all treatment groups. Bleeding from the injection site was evident at 30 and 100 mg/kg/day. Animals at 30 and 100 mg/kg/day developed hematomas in body areas other than the injection site. Examination of the liver and spleen from animals at 100 mg/kg/day

revealed extrahematopoiesis. Hemorrhage in various internal tissues was evident at 100 mg/kg/day. A dose-related reactive lymphoid hyperplasia was evident in all treatment groups. Histopathological findings appear to be consequences of the anticoagulant properties of tinzaparin.

**Tinzaparin: Toxicity Study by Intravenous Administration to CD Rats for 26 Weeks Followed by a 4 Week Reversibility Period (LSR Report No. 92/NLP142/0768).**

**Testing Laboratory:** [redacted]

**Date Started:** October 23, 1991 (Animals received)

**Date Completed:** March 8, 1993

**GLP Compliance:** Statements of compliance with GLP Regulations and the Quality Assurance Unit were included.

**Animals:** CD strain male and female rats were used.

**Drug Batch:** Tinzaparin, Batch No. LMW 9101 [redacted]

**Methods:** In a 26-week intravenous toxicology study, rats received tinzaparin by the intravenous route at doses of 0, 5, 10, or 25 mg/kg/day (0, 400, 900, and 2200 anti-Factor Xa IU/kg/day, respectively). There were 15 rats/sex/group. An additional 10 rats/sex/group were assigned to the control and 25 mg/kg/day groups for a 4-week recovery period following the 26-week treatment period. Control animals received the vehicle, [redacted] Vehicle or drug solution was administered by the intravenous route at a dose volume of 1 mL/kg. During the treatment period, animals were observed twice per day for clinical signs of toxicity and moribundity/mortality. During the recovery period, animals were observed once per day. A detailed physical examination of each animal was performed once per week. Body weight was measured once per week during the treatment and recovery periods. Food consumption was measured on a weekly basis. Food conversion efficiencies were calculated for the first 13 weeks of treatment. Water consumption was not measured. Ophthalmic examinations were performed on all animals prior to the start of treatment and during week 24. Blood for determination of hematology and blood chemistry parameters was collected from 10 rats/sex/group after weeks 12 and 24 of the treatment period (prior to dosing) and after week 3 of the recovery period. The female control and 10 mg/kg/day groups were resampled during week 13 due to problems with an excessive number of clotted samples. Urine samples for analysis were collected from 10 rats/sex/group after weeks 11 and 23 of the treatment period and after week 3 of the recovery period. At the end of the treatment and recovery periods, animals were sacrificed and subjected to gross pathological examination. Absolute and relative

weights were determined for the adrenal glands, brain, epididymides, heart, kidneys, liver, lungs with mainstem bronchi, ovaries, pituitary, prostate, submandibular salivary glands, seminal vesicles, spleen, testes, thymus, thyroids with parathyroids (after fixation), and uterus with cervix. Tissues and organs were preserved as follows: any abnormalities, adrenal glands, brain, caecum, colon, duodenum, epididymides, eyes and optic nerves, femoral bone marrow, Harderian gland (left), heart, ileum, jejunum, kidneys, liver, lungs with mainstem bronchi, lymph nodes (mandibular and mesenteric), mammary gland (caudal), esophagus, ovaries, pancreas, injection sites (tail), pituitary, prostate, rectum, salivary gland (submandibular, left), sciatic nerve (left), seminal vesicles, skin, spinal cord, spleen, sternum, stomach (keratinized and glandular), testes, thymus, thyroid with parathyroids, tongue, trachea, urinary bladder, uterus with cervix, and vagina. Tissues and organs were collected and preserved, but not examined as follows: aorta (thoracic), Harderian gland (right), mammary gland (cranial), salivary gland (submandibular, right), sciatic nerve (right), and skeletal muscle (thigh). Femoral bone smears were prepared. All tissues were subjected to histopathological examination for the control and 25 mg/kg/day groups at the end of the treatment and recovery periods and for all animals that died or were sacrificed in a moribund condition.

**Results**:

1. **Observed Effects**: Observations at injection sites for tinzaparin-treated groups included bruising, swelling, encrustation, exfoliation, erythema, and prolonged bleeding; however, these changes were not evident by the 3rd week of recovery. The incidence of bruising during the treatment period was as follows: 5 mg/kg/day, 0-5% for male rats and 0-15% for female rats; 10 mg/kg/day, 0-15% for male rats and 0-25% for female rats; and 25 mg/kg/day, 0-45% for both male and female rats. During week 1 of recovery, bruising was evident for male and female rats at 25 mg/kg/day with incidences of 5 and 35%, respectively; however, no bruising was evident at week 4 of recovery. The incidence of swelling during the treatment period was as follows: 5 mg/kg/day, 0-5% for female rats; 10 mg/kg/day, 0-5% for male rats and 0-10% for female rats; and 25 mg/kg/day, 0-30% for male rats and 0-35% for female rats. During week 1 of recovery, swelling was evident for male and female rats at 25 mg/kg/day with incidences of 5 and 35%, respectively; however, no swelling was evident at week 4 of recovery. The incidence of encrustation during the treatment period was as follows: 5 mg/kg/day, 0-5% for male rats; 10 mg/kg/day, 0-25% for male rats and 0-10% for female rats; and 25 mg/kg/day, 0-25% for both male and female rats. During week 1 of recovery, encrustation was evident for male and female rats at 25 mg/kg/day with incidences of 10 and 5%, respectively; however, no encrustation was evident at week 4 of recovery. The incidence of exfoliation during the treatment period was as follows: control, 0-13.3% for female rats; 5 mg/kg/day, 0-6.7% for male rats and 0-13.3% for female rats; 10 mg/kg/day, 0-20% for male rats and 0-13.3% for female rats; and 25 mg/kg/day, 0-20% for male rats and 0-73.3% for female rats. The incidence of erythema during the treatment period was as follows: 10 mg/kg/day, 0-13.3% for male rats; and 25 mg/kg/day, 0-35% for male rats and 0-73.3% for female rats. The incidence of prolonged bleeding during the treatment period was as follows: 10 mg/kg/day, 0-13.3% for male rats; and 25 mg/kg/day, 0-13.3% for male rats and 0-6.7% for female rats.

2. Mortality: Deaths during the treatment period included 1 male rat (#37) at 5 mg/kg/day (day 64), 1 male rat (#44) at 10 mg/kg/day (day 161), and 3 male rats (#57, #61, and #75) at 25 mg/kg/day (days 179, 178, and 152). Two female rats (#131 and #132) at 10 mg/kg/day were sacrificed in a moribund condition (days 94 and 179). The sponsor considered the deaths of 2 male rats at 25 mg/kg/day during the treatment period to be test article-related; although, no rationale was provided for defining which deaths were treatment-related and which were not. Analysis of histopathological findings suggests that treatment-related deaths occurred for 1 male rat and 2 female rats at 10 mg/kg/day and 2 male rats at 25 mg/kg/day. There were no histopathological findings for male #37 at 5 mg/kg/day due to severe autolysis. Findings for male rat #44 at 10 mg/kg/day included alveolar edema, alveolar hemorrhage, and congestion for the lung, hemorrhage at the injection site and in the pancreas and thymus, and abnormal contents in the stomach consisting of dark red and caseous material. Findings for male rat #57 at 25 mg/kg/day consisted of a malignant lymphoma. Findings for male rat #061 at 25 mg/kg/day included hemorrhage at the injection site and in the brain (multifocal) and thymus (diffuse), erthrocytes and erythrophagocytosis (sinuses) in the mesenteric lymph node, alveolar edema and congestion in the lungs, and red pulp and reduced blood content in the spleen. Findings for male rat #75 included hemorrhage in the thymic lymph node and thymus, extramedullary hematopoiesis in the liver (multifocal) and spleen, interstitial and alveolar edema in the lung (multifocal), and a hematoma in the skeletal muscle. Finding for female #131 at 10 mg/kg/day included hemorrhage at the injection site and in the skeletal muscle, thymus, and lungs (alveolar, multifocal), erythrocytes and erythrophagocytosis (sinuses) in the mesenteric lymph node, cortical hypertrophy in the left adrenal cortex, and diffuse hypertrophy in the right adrenal cortex. Findings for female #132 at 10 mg/kg/day included extramedullary hematopoiesis in the adrenal cortex, liver (multifocal), and spleen (marked), and a benign fibroadenoma in the mammary gland.

3. Body Weight and Food Consumption: There were no treatment-related effects on body weight gain or food consumption. Body weights for main controls during weeks 0, 26, and 30 were 174, 737 (724 g for recovery animals), and 767 g, respectively. Body weight gains for male rats at 5, 10, and 25 mg/kg/day during the treatment period were 99.6, 99.9, and 92.5% of the control, respectively. Body weight gain for male rats at 25 mg/kg/day during the 4 week recovery period were 77.6% of the control. Body weights for female controls during weeks 0, 26, and 30 were 150, 370 (353 g for recovery animals), and 358 g, respectively. Body weight gains for female rats at 5, 10, and 25 mg/kg/day during the treatment period were 110.2, 104.4, and 107.6% of the control, respectively. Body weight gains for female rats at 25 mg/kg/day during the 4 week recovery period were 78.2% of the control. Food conversion efficiencies for male and female treatment groups during the first 13 weeks were unaffected.

4. Hematology and Blood Coagulation: Small decreases in red blood cell (RBC) counts, hemoglobin levels, and hematocrit were observed throughout the treatment period for male and female rats at 25 mg/kg/day. Increased platelet counts were observed for male and female treatment groups throughout the treatment period, and were still evident at week 3 of the recovery period.

**Week 12**: RBC counts, hemoglobin levels, and hematocrit for male rats at 25 mg/kg/day were decreased to 91.1, 90.5, and 91.1% of control values (8.74 x $10^6$/µL, 15.8 g%, and 45%), respectively. Platelet counts for male treatment groups were increased to 110.7-121.5% of the control (922, units were 1000/µL). Hematocrit for female rats at 10 and 25 mg/kg/day were both decreased to 95.3% of the control (43%). Platelet counts for female rats at 25 mg/kg/day were increased to 131.2% of the control (1362, units were 1000/µL), respectively.

**Week 24**: RBC counts, hemoglobin levels, and hematocrit for male rats at 25 mg/kg/day were decreased to 92.2, 90.2, and 93.3% of control values (9.25 x $10^6$/µL, 16.3 g%, and 45%), respectively. Platelet counts for male treatment groups were increased to 117.8-136.8% of the control (976, units were 1000/µL), respectively. RBC counts, hemoglobin levels, and hematocrit for female rats at 25 mg/kg/day were decreased to 92.9, 92.1, and 92.9% of control values (7.86 x $10^6$/µL, 15.2 g%, and 42%), respectively. Platelet counts for female treatment groups were increased to 122.4-138.6% of the control (1002, units were 1000/µL).

**Week 25**: Hemoglobin levels and hematocrit for male rats at 10 mg/kg/day were decreased to 94.9 and 95.3% of control values (15.6 g% and 43%), respectively. RBC counts, hemoglobin levels, and hematocrit for male rats at 25 mg/kg/day were decreased to 92.7, 91.0, and 93% of control values (8.91 x$10^6$/µl, 15.6 g%, and 43%), respectively. Platelet counts for male rats at 10 and 25 mg/kg/day were increased to 127.4 and 129.2% of control values (1122, units were 1000/µL), respectively.

**Week 3 of Recovery**: Platelet counts for male and female rats at 25 mg/kg/day were increased to 109.2 and 119.6% of control values (928 and 848, units were 1000/µL), respectively.

5. **Blood Biochemistry and Urinalysis**: Albumin were generally decreased while globulin levels were increased for male and female treatment groups. Urinalysis revealed no treatment-related changes.

**Week 12**: Triglyceride levels for male treatment groups were decreased to 72.8-79.4% of the control value (136 mg%). Phospholipid levels for male treatment groups were decreased to 84.8-91.4% of the control value (105 mg%), respectively. Total protein levels for male treatment groups were increased to 103-106.1% of control values (6.6 g%). Albumin levels for male and female rats at 25 mg/kg/day were decreased to 90.3 and 92.1% of the control values (3.1 and 3.8 g%). $\alpha_1$-Globulin levels for male treatment groups were increased to 115.4% of the control value (1.3 g%). $\alpha_1$-Globulin levels for female rats at 25 mg/kg/day were increased to 118.2% of the control value (1.1 g%). $\alpha_2$-Globulin levels for male treatment groups were increased to 125% of the control value (0.4 g%). β-globulin levels for male rats at 10 and 25 mg/kg/day were both increased to 113.3% of the control value (1.5 g%). β-globulin levels for female rats at 25 mg/kg/day were increased to 120% of the control value (1.5 g%), respectively. The albumin to globulin ratio for male rats at 5, 10, and 25 mg/kg/day were decreased to 88.9, 88.9, and 77.8% of control values (0.9), respectively.

The albumin to globulin ratio for female rats at 25 mg/kg/day was decreased to 81.8% of the control value (1.1).

**Week 24**: Total protein levels for male treatment groups were increased to 104.4-105.8 of the control value (6.9 g%). Albumin levels for male rats at 10 and 25 mg/kg/day were both decreased to 87.1% of the control value (3.1 g%). Albumin levels for female treatment groups were decreased to 87.5-92.5% of the control value (4.0 g%). $\alpha_1$-Globulin levels for male treatment groups were increased to 106.2-112.5% of the control value (1.6 g%). $\alpha_2$-Globulin levels for male treatment groups were increased to 125% of the control value (0.4 g%). $\alpha_2$-Globulin levels for female rats at 25 mg/kg/day were increased to 150% of the control value (0.4 g%). β-globulin levels for male rats at 5, 10, and 25 mg/kg/day were increased to 111.7-123.5% of the control value (1.7 g%). β-globulin levels for female rats at 10 and 25 mg/kg/day were increased to 120 and 133.3% of the control value (1.5 g%), respectively. The albumin to globulin ratio for male treatment was decreased to 75-87.5% of the control value (0.8). The albumin to globulin ratio for female treatment was decreased to 66.7-83.3% of the control value (1.2).

**Week 3 of Recovery**: Albumin levels and the albumin to globulin ratio for male rats at 25 mg/kg/day were decreased to 90.6 and 77.8% of control values (3.2 g% and 0.9), respectively. The albumin to globulin ratio for female rats at 25 mg/kg/day was decreased to 81.8% of the control value (1.1). $\alpha_1$-Globulin levels for male rats at 25 mg/kg/day were increased to 120% of the control value (1.5 g%). β-globulin levels for female rats at 25 mg/kg/day were increased to 118.75% of the control value (1.6 g%).

6. **Ophthalmic Examination**: Ophthalmic examinations revealed no treatment-related changes.

7. **Organ Weights**: With the possible exception of the kidney, organ weight changes has no relationships to histopathological changes. Absolute kidney weights for female rats at 25 mg/kg/day were increased to 110% of the control (2.83 g). Relative kidney weights for male rats at 25 mg/kg/day were increased to 109% of the control (0.679 g). Following the recovery period, absolute and relative kidney weights for male rats at 25 mg/kg/day were increased to 113.1 and 118.5% of control values (4.64 g and 0.614%), respectively.

8. **Gross Pathology**: Gross pathological changes were observed at a low incidence and revealed no treatment-related effects. Swollen liver was observed for 2 male rats at 5 mg/kg/day, 1 male rat at 10 mg/kg/day, and 3 male rats at 25 mg/kg/day.

9. **Histopathology**: Histopathological changes were observed at low incidences and did not appear to reveal a target organ of toxicity. Progressive nephropathy was observed for 17.9% of rats at 25 mg/kg/day. Evidence of the anticoagulant effect of tinzaparin was observed at 25 mg/kg/day and included erythrocytes and erythrophagocytosis in sinuses for the mandibular and mesenteric lymph nodes, pelvic hemorrhage in the kidney, presence of blood in the lumen of the bronchi, hemorrhage at the injection site, and hemosiderosis in the mesenteric lymph node. At the end of the 4 week recovery

period, most of these changes were not observed. One female rat (#153) at 25 mg/kg/day was found with a β-granulosa-thecal cell tumor. Potential evidence of the anticoagulant effect of tinzaparin was observed for rats at 25 mg/kg/day after the recovery period as follows: Female #0159, erythrocytes and erythrophagocytosis in the sinuses and hemosiderosis in the pancreatic lymph node; Male #72, erythrocytes and erythrophagocytosis in the sinuses in the renal lymph node; and Male #079, hemosiderosis in the pancreatic lymph node.

Histopathological changes for rats that received tinzaparin by the intravenous route at doses of 0 and 25 mg/kg/day for 26 weeks.

| Organ/Tissue | 0 mg/kg/day | | 5 mg/kg/day | | 10 mg/kg/day | | 25 mg/kg/day | |
|---|---|---|---|---|---|---|---|---|
| | M | F | M | F | M | F | M | F |
| Kidneys | | | | | | | | |
| -n = | 15 | 15 | 2 | 0 | 1 | 1 | 13 | 15 |
| -progressive nephropathy | 0 | 0 | 0 | 0 | 1 | 0 | 3 | 2 |
| -dilated cortical tubules | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| -pelvic hemorrhage | 0 | 1 | 0 | 0 | 0 | 0 | 2 | 0 |
| Bronchi | | | | | | | | |
| -n = | 15 | 15 | 0 | 0 | 0 | 0 | 13 | 15 |
| -presence of blood in the lumen | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| Mandibular lymph node | | | | | | | | |
| -n = | 14 | 15 | 2 | 0 | 1 | 0 | 13 | 15 |
| -erythrocytes and erythrophagocytosis in sinuses | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| -plasmacytosis | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| Mesenteric lymph node | | | | | | | | |
| -n = | 15 | 15 | 0 | 0 | 0 | 1 | 13 | 15 |
| -erythrocytes and erythrophagocytosis in sinuses | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| -parafollicular hyperplasia | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| -hemosiderosis | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 0 |
| Injection site (Tail) | | | | | | | | |
| -n = | 15 | 15 | 0 | 0 | 0 | 0 | 13 | 15 |
| -chronic inflammation | 1 | 1 | 0 | 0 | 0 | 0 | 2 | 4 |
| -hemorrhage | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |

In a 26-week intravenous toxicology study, rats received tinzaparin by the intravenous route at doses of 0, 5, 10, or 25 mg/kg/day. There were 15 rats/sex/group. An additional 10 rats/sex/group were assigned to the control and 25 mg/kg/day groups for a 4-week recovery period following the 26-week treatment period. The no effect dose was 5 mg/kg/day. Treatment-related mortality appeared to occur at doses of 10 and 25 mg/kg/day. Histopathological changes were observed at low incidences and did not appear to reveal a target organ of toxicity. Progressive nephropathy was observed for 17.9% of rats at 25 mg/kg/day. Evidence of the anticoagulant effect of tinzaparin was observed at 25 mg/kg/day and included erythrocytes and erythrophagocytosis in sinuses for the mandibular and mesenteric lymph nodes, pelvic hemorrhage in the kidney, presence of blood in the lumen of the bronchi, hemorrhage at the injection site, and hemosiderosis in the mesenteric lymph node. At the end of the 4 week recovery

period, most of these changes were not observed. Observations at injection sites for tinzaparin-treated groups included bruising, swelling, encrustation, exfoliation, erythema, and prolonged bleeding; however, these changes were not evident by the 3rd week of recovery.

**One-Year Chronic Toxicity Study in the Rats Followed by a 6-Week Reversibility Period (Final Report, LSR #89/NLP031/0142).**

**Conducting Lab:** [redacted]

**Dates of Conduct:** Initiated 5/26/87, completed 7/8/88.

**GLP Statement:** In compliance with the FDA's GLPs.

**Chemical:** LHN-1, lot #s BN 100487, 110487, 120487, and Heparin lot #150487.

**Animals:** Rats, Sprague-Dawley, [redacted] 64-92 g, 25/sex/group.

**Doses:** 0 (control vehicle), 4, 10, and 25 mg/kg/day LHN-1, and Heparin at 12.5 mg/kg/d, in 0.5 mL/kg volume, injected by the subcutaneous route at different sites, once daily, 7 days/week for 52 weeks. The animals did not receive any further treatment for additional 6 weeks but were observed for recovery (called reversibility phase).

**Methods:** The control vehicle or the test agent (LHN-1 or Heparin) were injected into each animal, once daily for 52 weeks. The animals were observed for morbidity, and mortality throughout the study and also for additional 6 weeks (reversionary phase) for recovery. The body weights and food intake were recorded once weekly; ophthalmoscopy on prior to treatment, and during weeks 5, 12, 24, and 50 on control and high dose LHN-1 rats; all rats during week 51; and during week 3 of recovery of control and high dose rats; hematology and clinical chemistry from 10 rats/sex/group during weeks of pre-treatment, 5, 12, 24, 51 of treatment and during week 3-5 of recovery; and finally urinalyses were performed during weeks 4, 11, 23, and 50, and during week 3-5 of recovery. At termination of the experiment, all the rats were autopsied and examined macro- and microscopically.

**Results:**

**Observed Effects:** Swellings and hemorrhages at injection sites were common findings in most treated rats.

**Mortality:** A total of 32 rats died or had to be killed because they were moribund: 2 (1M, 1F); 18 (6M,12F); 2 (1M, 1F); and 10 (4M, 6F) in the control, Heparin and mid and high dose LHN-1 groups, respectively. Five of these 32 were considered not treatment-related. Further, most (18/32) rats were in the Heparin treatment group.

Food Consumption: The food consumption of the LHN-1-treated male but not of the female rats was higher than the controls throughout treatment, and during recovery. The water consumption remained normal.

Body Weights: The body weight gain by the high dose male rats was lower than the controls by 13% (controls, 630±94; Heparin, 520±98; low dose, 628±97, mid dose, 600±89; high dose LHN-1, 548±108 g, significant at $p < 0.05$) inspite of increased food intake with no clear explanation for this effect. However, they recovered much faster during the recovery period.

Ophthalmoscopy: Of significance was the finding of lenticular cataracts in 17 rats. Of the 25 rats of each sex/dose, there were 3, 5, and 7 male rats in the control, Heparin and high dose LHN-1 groups, respectively; and one female rat each in the control and Heparin group with lenticular cataracts. Three male rats (1 Heparin and 2 high dose LHN-1) assigned to the recovery phase did not recover but the cataracts progressed from radial to total cataract. It is not clear why all 17 rats with cataracts were not followed for recovery since this was a significant finding.

Hematology: LHN-1 and Heparin produced significant ($p \leq 0.05$-001) but marginal decreases in packed cell volume (4-5%), Hb concentration (g%), erythrocyte counts (4%), and increased platelet counts (up to 20%). However, these changes were not dose-dependent, and the rats recovered during recovery. These changes were possibly related to hemorrhages/hematomas at injection sites as a result of the anticoagulant effects of the test agents. The prothrombin times were normal.

Clinical Chemistry: There were sporadic changes, increases and decreases in the plasma electrolytes levels in the high dose LHN-1 rats, but not consistently and thus cannot be attributed to LHN-1 treatment. Alkaline phosphatase levels were decreased, and remained low during the recovery phase. However, the significance of this decrease remains unclear. Blood glucose levels in the treated animals were not different from the controls.

Urinalyses: The total reducing substances and glucose were present in the urine of 3 Heparin males, one mid dose and 3 high dose male rats during treatment and also during recovery phase, suggestive of impaired carbohydrate metabolism. However, the number of rats affected was small.

Organ weights: The relative weights (organ/body weight) of the liver, kidneys and spleen were increased almost dose-dependently. However, significant changes were as follows: livers of both male and female rats increased up to 32% in the males with high dose (25 mg/kg/d), 12% with mid dose (10 mg/kg/d); and up to 12 to 17% in the females with these doses. The kidney weights increased up to 11% each at mid and high dose in the females; the spleen weights increased by 36% and 10% at high and mid doses only in the male rats. During recovery phase, most Heparin and LHN-1 treated animals' spleen weights returned to normal, while those of the liver and kidneys, did not.

**Histopathology**: The increase in the organ weights of the liver, kidneys and spleen was not accompanied by any treatment-related significant microscopical changes. In the animals that died or those that had to be sacrificed during the study, there were occasional dark areas and masses at injection sites; generalized pallor of internal organs (suggestive of blood loss), fluid in the abdomen or thorax and dark gastrointestinal contents. No other significant pathology was evident as a result of treatment with Heparin or LHN-1 at doses of up to 25 mg/kg/d.

**Bone Analysis**: The extent of change in the bone density with Heparin was between 9-11% in the male and female rats, and 1-2% in the female and male rats, respectively, with 25 mg/kg/d of LHN-1; while the bone ash (% of femur) decreased by 14-19% with Heparin and 4-17% with 25 mg/kg/d of LHN-1 in the male and female rats, respectively.

In summary, LHN-1 at subcutaneous doses of 4, 10, and 25 mg/kg/d or Heparin at 12.5 mg/kg/d for one year produced hemorrhages/hematomas at the injection sites in almost all animals, resulting in a few deaths (10 of 50) at the high dose (25 mg/kg/d), but higher number (18 of 50) of rats died in the Heparin group. LHN-1 also caused about 13% decrease in the body weight gain of 25 mg/kg/d rats inspite of increased food consumption with no clear cut explanation for such a change. The high dose LHN-1 and Heparin produced irreversible lenticular cataracts, more in the male rats. Whether this was a possible diabetogenic effect of the agent, is not clear. There were decreases in the packed cell volume (4.5%), Hb concentration (g%), and RBCs (4%) and increases in the platelet counts (up to 20%) at 10 and 25 mg/kg/d doses of LHN-1 and Heparin, but not at 4 mg/kg/d. However, the animals recovered after the discontinuation of the treatment. The weights of the liver, kidneys and spleen in most treated rats increased with treatment; but those of the spleen, but not of the kidneys and liver returned to normal during recovery. The organ weight increases were not accompanied by any significant changes in the liver or renal function or any histopathological findings, and thus the significance of such organ weight increases remains unclear. No significant toxicity was evident at the lowest dose, 4 mg/kg/d and that could be considered as the no-toxic dose for the rats.

**Addendum**:

**Testing Laboratory**: [redacted]

**Drug Batch**: Tinzaparin bulk drug batch F668A ([redacted] anti-Factor Xa IU/mg) was supplied for the study as a preformulated solution containing sodium metabisulfite in glass vials (Lot 100487, Lot 110487, and Lot 120487).

**Doses**: Doses were equivalent to 0, 300, 700, and 1800 anti-Factor Xa IU/kg/day.

**Mortality:** For the reference standard group receiving heparin at 12.5 mg/kg/day, treatment was suspended after the first administration due to adverse reactions, which included large hematoma formation at the injection site and death of 2 animals. Eleven animals (6 male and 5 female rats) from this group were replaced due to either death or large hematoma formation, and treatment was resumed on day 15. As a result, animals receiving heparin were only treated for a total of 50 weeks as compared to 52 weeks for other groups. Deaths observed during the treatment period were as follows: control group, 1 male rat; heparin at 12.5 mg/kg/day, 6 male and 12 female rats (Note: these numbers do not include the 6 male and 5 female rats removed from the study); tinzaparin at 10 mg/kg/day, 1 male rat and 1 female rat; and tinzaparin at 25 mg/kg/day, 4 male and 6 female rats. Deaths observed during the recovery period were as follows: control group, 1 female rat; and tinzaparin at 25 mg/kg/day, 1 male rat. Deaths for animals receiving heparin at 12.5 mg/kg/day or tinzaparin at 10 or 25 mg/kg/day appeared to be generally related to marked hemorrhage at the injection site. Five deaths were reported to be unrelated to treatment. Three rats (1 control male rat, 1 control female rat, and 1 heparin-treated female rat) died or were killed during routine blood sampling. Two rats (1 heparin-treated female rat and 1 female rat that received tinzaparin at 25 mg/kg/day) were killed after sustaining accidental injuries.

**Ophthalmic Examination:** Ophthalmic examinations were performed on all animals prior to the start of treatment and at week 50/51. Ophthalmic examinations were performed on animals that received the vehicle-control, heparin at 12.5 mg/kg/day, or tinzaparin at 25 mg/kg/day during weeks 12 and 24 of the treatment period and during week 3 of recovery. It should be noted that prior to the start of treatment, 6 male and 2 female rats were reported to have severe ocular lesions and replaced with spare animals showing no significant abnormalities. At week 50/51, cataracts were observed in 1 male rat that received the vehicle-control, 5 male rats and 1 female rat that received heparin at 12.5 mg/kg/day, and five male rats that received tinzaparin at 25 mg/kg/day. An early cataract was reported at week 24 for 1 male rat that received the vehicle-control; however, at week 50/51, this animal was reported with posterior capsular opacity plaques. Posterior capsular plaques which appear to be either associated with or a consequence of cataract development (Molecular Vision 5:6, 1999), were observed at week 50/51 and week 3 of recovery for 4 male rats that received the vehicle-control, 5 male rats and 1 female rat that received heparin at 12.5 mg/kg/day, 1 male rat and 1 female rat that received tinzaparin at 4 mg/kg/day, 3 male rats that received tinzaparin at 10 mg/kg/day, and 1 male rat at 25 mg/kg/day. Based upon communications with the Division of Anti-inflammatory, Analgesic, and Ophthalmology Drug Products (HFD-550), posterior capsular plaques should be listed as cataracts. The combined incidences of cataracts and posterior capsular plaques at week 50/51 and week 3 of recovery were as follows: 5 male rats that received the vehicle-control, 10 male rats and 2 female rat that received heparin at 12.5 mg/kg/day, 1 male rat and 1 female rat that received tinzaparin at 4 mg/kg/day, 3 male rats that received tinzaparin at 10 mg/kg/day, and 6 male rat at 25 mg/kg/day. Posterior polar opacity plaques, considered a congenital defect, were observed at week 50/51 for 2 male rats that received the vehicle-control. The combined incidences of cataracts and posterior capsular plaques displayed no relationship to tinzaparin treatment.

Results of ophthalmic examinations for rats that received the vehicle-control, heparin at 12.5 mg/kg/day, or tinzaparin at 4, 10, or 25 mg/kg/day by the subcutaneous route.

| Group | # | Sex | Week | Structure | Observation |
|---|---|---|---|---|---|
| **Vehicle-Control** | 5 | Male | 50/51 | Lens | Faint nuclear opacity, **posterior capsular opacity plaques** |
| | 14 | Male | 24 | Lens | **Early cataract** |
| | | | 50/51 | Lens | **Posterior capsular opacity plaques** |
| | 19 | Male | 50/51 | Lens | **Posterior polar opacity plaque** |
| | 20 | Male | 50/51 | Lens | **R- cataract** |
| | | | | | L- faint nuclear opacity |
| | | | | Iris | R-irititis |
| | | | | Ocular fundus | R- not observable |
| | 29 | Male | 50/51 | Lens | **Posterior polar opacity plaque** |
| | | | 3-Rec | Lens | **Posterior polar opacity plaque** |
| | 30 | Male | 3-Rec | Lens | **R-posterior capsular opacity plaque** |
| | 35 | Male | 50/51 | Lens | **L-posterior capsular opacity plaque** |
| | | | 3-Rec | Lens | **L-posterior capsular opacity plaque** |
| **Heparin at 12.5 mg/kg/day** | 36 | Male | 50/51 | Lens | **R-posterior capsular opacity plaque** |
| | 37 | Male | 50/51 | Lens | **Radial cataract** |
| | | | | Ocular fundus | Slight pallor, slightly tortuous vessels |
| | | | | Iris | Slight Iritis |
| | 40 | Male | 50/51 | Lens | Faint nuclear opacity. **R-posterior capsular opacity plaques** |
| | | | | Ocular fundus | Slight pallor. L-pre-retinal loop |
| | 44 | Male | | Lens | **R-posterior capsular cataract** |
| | 52 | Male | 50/51 | Lens | **Radial cataract** |
| | | | | Iris | Iritis |
| | | | | Ocular fundus | Not Observable |
| | 55 | Male | 50/51 | Lens | **Radial cataract** |
| | | | | Iris | Iritis |
| | | | | Ocular fundus | Not Observable |
| | | | 3-Rec | Lens | **Total Cataract** |
| | | | | Iris | Iritis |
| | | | | Ocular Fundus | Not Observable |

| | | | | | |
|---|---|---|---|---|---|
| | 57 | Male | 50/51 | Lens<br>Ocular fundus<br>Iris | **Early radial cataract**<br>Slightly tortuous vessels<br><br>Slight iritis |
| | 61 | Male | 3-Rec | Lens | **Posterior capsular opacity plaque** |
| | 67 | Male | 50/51<br><br>3-Rec | Lens<br><br>Lens | **Posterior capsular opacity plaques**<br><br>**Posterior capsular opacity plaque** |
| | 68 | Male | 50/51<br><br>3-Rec | Lens<br><br>Lens | **Posterior capsular opacity plaque**<br><br>**R-nuclear opacity spot and posterior capsular opacity plaques.** |
| | 195 | Female | 50/51 | Lens<br>Ocular fundus<br>General | **L-total cataract**<br>L-not observable<br><br>Slight phthisis |
| | 216 | Female | 50/51<br><br>3-Rec | Lens<br><br>Lens | **Posterior capsular opacity plaque**<br><br>**Posterior capsular opacity plaque** |
| **Tinzaparin at 4 mg/kg/day** | 83 | Male | 50/51 | Lens | **Posterior capsular opacity plaque** |
| | 228 | Female | 50/51 | Lens | **Posterior capsular opacity plaques** |
| **Tinzaparin at 10 mg/kg/day** | 102 | Male | 50/51 | Lens | Nuclear opacity, **posterior capsular opacity plaque** |
| | 109 | Male | 50/51 | Lens | **Small posterior capsular opacity plaque** |
| | 110 | Male | 50/51 | Lens<br><br>Ocular fundus | Faint nuclear opacity, **posterior capsular opacity plaque**<br>L-pre-retinal loop |
| **Tinzaparin at 25 mg/kg/day** | 124 | Male | 50/51 | Lens | Anterior polar opacity, **early radial cataract** |
| | 125 | Male | 50/51 | Lens | R-posterior capsular opacity, **early radial cataract**<br>Anterior capsular opacity |
| | 135 | Male | 50/51 | Lens<br>Iris | **Radial cataract**<br>Iritis |
| | 146 | Male | 3-Rec | Lens | Faint nuclear opacity. **L-posterior capsular opacity plaques** |

| | | | | | |
|---|---|---|---|---|---|
| | 148 | Male | 50/51 | Lens<br>Iris<br>Ocular fundus | **Radial cataract**<br>Iritis<br>Not observable |
| | | | 3-Rec | Lens<br>Iris<br>Ocular fundus | **Total cataract (L-lens with cataract partly protrudes into the anterior chamber)**<br>Iritis<br>Not observable |
| | 152 | Male | 50/51 | Lens<br>Iris<br>Ocular fundus | **Radial cataract**<br>Iritis<br>Not observable |
| | | | 3-Rec | Lens<br>Iris<br>Ocular fundus | **Total cataract**<br>Iritis<br>Not observable |

**Histopathology**: Microscopic examination revealed tissue damage at the injection sites for animals receiving tinzaparin or heparin. Changes included acute and chronic inflammation, hemorrhage, siderophages and fibrosis. Following the 6-week recovery period, tissue damage at injection sites had generally resolved.

Histopathological changes for rats that received the vehicle-control, heparin at 12.5 mg/kg/day, or tinzparin at 4, 10, or 25 mg/kg/day by the subcutaneous route for 52 weeks.

| Organ/Tissue | 0 mg/kg/day | | Heparin, 12.5 mg/kg/day | | Tinzaparin, 4 mg/kg/day | | Tinzaparin, 10 mg/kg/day | | Tinzaparin, 25 mg/kg/day | |
|---|---|---|---|---|---|---|---|---|---|---|
| | M | F | M | F | M | F | M | F | M | F |
| Injection sites | | | | | | | | | | |
| n = | 24 | 25 | 21 | 18 | 25 | 25 | 24 | 24 | 22 | 20 |
| -Acute inflammation | 0 | 1 | 1 | 2 | 4 | 4 | 3 | 5 | 5 | 2 |
| -Chronic inflammation | 4 | 3 | 12 | 14 | 3 | 6 | 8 | 13 | 12 | 14 |
| -Granulomas | 1 | 0 | 1 | 1 | 2 | 1 | 1 | 0 | 0 | 1 |
| -Fibrosis | 1 | 1 | 9 | 14 | 4 | 1 | 3 | 7 | 1 | 11 |
| -Hemorrhage | 1 | 3 | 13 | 17 | 4 | 12 | 5 | 19 | 8 | 15 |
| -Perivascular inflamm. | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| -Perivascular fibrosis | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| -Siderophages | 0 | 0 | 12 | 18 | 2 | 4 | 3 | 9 | 11 | 17 |
| -Epithelial inclusion cyst | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 |
| -Hematoma | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| -Superficial eschar | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 2 | 0 |
| -Fibrous-walled hematoma | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| -Superficial ulceration | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 |

## DOGS

### Four Week Intravenous Toxicology Study in Beagle Dogs.

**Methods**: Groups of 3M and 3F beagles were injected I.V. daily at a constant volume of 1 mL/kg with 0 (0.9% saline), 10 ,30, and 50 mg/kg of LFN-1 (Batch F85010) in physiological saline for 28 days. The concentration injected was 1,3, and 5% at the 10, 30, and 50 mg/kg levels. The study was conducted by [redacted], starting on July 18, 1985 and ending on August 20, 1985. The study was said to have been completed in accordance with "international" GLPs.

**Results**: No dogs died, but the usual irritant and hemorrhagic local effects were evident at all doses, especially at the top dose. ECG (performed at baseline and at 15 minutes and 24 hours post-drug on day 28) revealed no drug-related electrical disturbances or heart rate changes. Blood pressure determination (made immediately after ECG) were also normal. Hematology was undisturbed (including RBC and Hb parameters) and serum chemistry was normal except for an increase in serum creatinine in the males at the mid ($p = 0.01$) and high ($p = 0.001$) doses.

Weight of the spleen was increased in one mid dose and one high dose animal. The results of macroscopic and microscopic studies gave a strong impression that the incidence and severity of local tissue changes, e.g. discoloration, thickening of vein, hemorrhage, edema, and fibrosis in the subcutis at the injection sites, were increased in a dose related way among drug treated vs. saline injected controls. Because the data are not tabulated however, this conclusion is a subjective estimate only. The bones of all dogs were examined microscopically; there was no mention of histopathology at this site. As in rats, the toxicity of LHN-1 hinges on its anticoagulant properties.

**Addendum**: Tinzaparin batch number F85010 with a specific activity of [redacted] anti-Factor Xa IU/mg was used in this study. Doses were equivalent to 0, 700, 2200, and 3700 anti-Factor Xa IU/kg/day. Body weights for female control dogs on days 0 and 28 were 8.3 and 8.7 kg, respectively. Body weight gain for female dogs at 50 mg/kg/day was decreased to 67.7% of the control. Serum creatinine levels in male dogs at 30 and 50 mg/kg/day were increased to 112.5 and 137.5% of the control (0.8 mg%), respectively. Serum calcium levels for male treatment groups were elevated to 107.4-109.3% of the control (5.4 mEq/L), respectively. Absolute spleen weights for male dogs at 10, 30, and 50 mg/kg/day were increased to 124.6, 121.3, and 182% of the control (61 g), respectively. Relative spleen weights for male dogs at 10, 30, and 50 mg/kg/day were increased to 130.2, 128.3, and 184.9% of the control (0.53), respectively. Relative kidney weights for male dogs at 30 and 50 mg/kg/day were increased to 125.6 and 120.9% of the control (0.43), respectively. Histopathologic analysis of administration sites found an increased incidence of hemorrhage and edema in male and female treatment groups.

Histopathological findings for beagle dogs that received tinzaparin by the intravenous route at doses of 0, 10, 30, and 50 mg/kg/day for 28 days.

| Histopathological Findings | Male Dogs | | | | Female Dogs | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 10 | 30 | 50 | 0 | 10 | 30 | 50 |
| Left cephalic admin. site | | | | | | | | |
| -subcutis, hemorrhage | 0 | 1 | 2 | 3 | 0 | 1 | 3 | 1 |
| -subcutis, edema | 0 | 1 | 0 | 3 | 0 | 2 | 1 | 2 |
| Right cephalic admin. site | | | | | | | | |
| -subcutis, hemorrhage | 1 | 1 | 3 | 2 | 0 | 2 | 1 | 3 |
| -subcutis, edema | 0 | 0 | 3 | 1 | 0 | 3 | 2 | 2 |
| -subcutis, fibrosis | 0 | 0 | 1 | 2 | 0 | 3 | 3 | 1 |
| Right Saphenous admin. site | | | | | | | | |
| -subcutis: chronic inflammatory cells | 0 | 3 | 1 | 2 | 1 | 1 | 2 | 1 |
| -subcutis: hemorrhage | 1 | 3 | 2 | 3 | 1 | 2 | 1 | 2 |
| -subcutis: edema | 0 | 3 | 0 | 1 | 0 | 0 | 3 | 2 |

In a four-week intravenous toxicology study, beagle dogs received tinzaparin at doses of 0, 10, 30, and 50 mg/kg/day. Localized irritant and hemorrhagic effects were observed at injection sites for treatment groups. Macroscopic changes (i.e., discoloration, thickening of vein, hemorrhage, edema, and fibrosis in the subcutis) at the injection sites were increased in a dose-related manner regarding incidence and severity for tinzaparin treatment groups.

**52-Week Intravenous Toxicology Study in Beagle Dogs Following a 4-Week Recovery Period (Final Report LSR #93/NLP141/0245).**

**Testing Laboratory:** [redacted]

**Date Started:** January 4, 1992 (Start of treatment)

**Date Completed:** October 6, 1993

**GLP Compliance:** Statements of compliance with GLP Regulations and the Quality Assurance Unit were included.

**Animals:** Pure-bred beagle dogs were used in the present study. After arrival of animals at the testing laboratory, a number of dogs developed a minor respiratory infection characterized by ocular and nasal discharge, coughing, and increased leukocyte counts. Animals were allowed to recover from this infection before the start of treatment. The acclimatization period lasted for a total of 17 weeks. Animals were randomized into test groups based upon total leukocyte counts. The dogs were approximately 32 to 36 weeks of age at the start of treatment.

**Drug Batch:** Tinzaparin batch number LMW 9101 with a specific activity of [redacted] anti-Factor Xa IU/mg was used in this study.

**Methods**: In a 52-week intravenous toxicology study, beagle dogs received tinzaparin by the intravenous route at doses of 0, 4, 10, or 25 mg/kg/day (equivalent to 0, 300, 900, and 2200 anti-Factor Xa IU/kg/day, respectively). Animals in the control group received the vehicle, [redacted] There were 4 dogs/sex/group for control, 4 mg/kg/day, and 10 mg/kg/day groups. The 25 mg/kg/day group was composed of 6 dogs/sex/group. For the control and 25 mg/kg/day groups, there were an additional 2 dogs/sex/group for a 4-week recovery period following the 52-week treatment period. The sponsor's dose selection was based upon 4-week intravenous and 52-week subcutaneous toxicology studies in dogs. In the 4-week intravenous toxicity study, doses ≤50 mg/kg/day did not produce any toxicity. In the 52-week subcutaneous toxicology study, a dose of 25 mg/kg/day was found to produce mortality due to excessive hemorrhage. In the present study, vehicle or drug solutions were administered as a bolus by the intravenous route into the left cephalic vein, right cephalic vein, or right saphenous vein using a dose volume of 0.5 mL/kg. The left saphenous vein was not used and served as a within-animal control. Dogs were monitored daily for clinical signs of toxicity and moribundity/mortality. Kennels were inspected daily for evidence of vomit, blood, and loose feces. Body weights were measured weekly during the treatment and recovery periods. Food consumption was measured daily during the treatment and recovery periods. Animals were submitted to veterinary examinations prior to the start of treatment, after weeks 12, 24, 37, and 50 of the treatment period, and after week 3 of the recovery period. Ophthalmic examinations were performed on all dogs prior to the start of treatment and after weeks 12, 24, and 50 of the treatment period. Blood for determination of hematology and clinical chemistry parameters was collected prior to the start of treatment and after weeks 12, 24, and 51 of the treatment period. Urine samples for analysis were collected after weeks 11, 23, and 49 of the treatment period. Blood for determination of plasma anti-Factor Xa activity was collected on days 1, 7, and 361 of the treatment period at 0 (predose), 10 min, 1 hr, and 4 hr after dosing. At completion of the treatment or recovery periods, dogs were sacrificed and subjected to a gross pathological examination. Bone marrow samples were obtained at necropsy from all animals at the end of either the treatment or recovery period, processed, and examined by counting 100 nucleated cells. Absolute and relative organ weights were determined for the adrenal glands, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary gland, prostate, salivary gland, spleen, testes, thymus, thyroid gland (with parathyroid glands), and uterus with cervix. Organs and tissues were collected and preserved as follows: abnormalities, adrenal glands, aorta (thoracic), brain, bronchi, cecum, colon, duodenum, epididymides, eyes and optic nerves, femoral bone, gall bladder, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (bronchial, mesenteric, and retro-pharyngeal), mammary glands (caudal and cranial), nictitans glands, esophagus, ovaries, pancreas, injection sites (treated and control), pituitary gland, prostate, rectum, salivary glands (submandibular), sciatic nerve, skeletal muscle (thigh), skin, spinal cord, spleen, sternal bone and marrow, stomach (fundus and pylorus), testes, thymus, thyroid gland (with parathyroid glands), tongue, trachea, urinary bladder, uterus with cervix, and vagina. Tissues and organs from all animals were subjected to microscopic examination.

**Results:**

1. **Observed Effects:** Observations at injection sites (i.e., left cephalic vein, right cephalic vein, or right saphenous vein) for dogs at 4, 10, or 25 mg/kg/day during the 52-week treatment period included thickening, bruising, scabbing, swelling, and reddening. By the end of the 4-week recovery period, these changes, with the exception of reddening, were no longer evident in rats that had received 25 mg/kg/day.

Incidence range (%) of observed changes at injection sites (i.e., left cephalic vein, right cephalic vein, or right saphenous vein) for dogs that received tinzaparin by the intravenous route at doses of 0, 4, 10, or 25 mg/kg/day during the 52-week treatment period.

| Observations | 0 mg/kg/day | | 4 mg/kg/day | | 10 mg/kg/day | | 25 mg/kg/day | |
|---|---|---|---|---|---|---|---|---|
| | Male | Female | Male | Female | Male | Female | Male | Female |
| Thickening | 0% | 0-5% | 0-85% | 0-100% | 0-100% | 0-100% | 0-100% | 0-100% |
| Bruising | 0% | 0-10% | 0-30% | 0-15% | 0-45% | 0-45% | 0-55% | 0-40% |
| Scabbing | 0-15% | 0-20% | 0-20% | 0-30% | 0-35% | 0-25% | 0-30% | 0-25% |
| Swelling | 0-5% | 0% | 0-20% | 0-5% | 0-20% | 0-25% | 0-20% | 0-20% |
| Reddening | 0-5% | 0-5% | 0-15% | 0-10% | 0-10% | 0-15% | 0-40% | 0-15% |

2. **Mortality:** None.

3. **Body Weight and Food Consumption:** There were no treatment-related effects on body weight or food consumption. Body weights for male controls at week 0, week 52, recovery week 0, and recovery week 4 were 11.5, 14.9, 16.6, and 17.1 kg, respectively. After the 52 week treatment period, body weights for male dogs at 0, 4, 10, and 25 mg/kg/day were increased by 29.6, 16.7, 14.3, and 17.1% of initial body weights at week 0, respectively. After the 4-week recovery period, body weights for male dogs at 0 and 25 mg/kg/day were increased by 3 and 0% of initial body weights at recovery week 0, respectively. Body weights for female controls at week 0, week 52, recovery week 0, and recovery week 4 were 10.1, 12.0, 10.9, and 11.4 kg, respectively. After the 52 week treatment period, body weights for female dogs at 0, 4, 10, and 25 mg/kg/day were increased by 18.8, 19.6, 25.7, and 18.1% of initial body weights at week 0, respectively. After the 4-week recovery period, body weights for female dogs at 0 and 25 mg/kg/day were increased by 4.6 and 5.5% of initial body weights at recovery week 0, respectively.

4. **Hematology and Blood Coagulation:** At week 24, platelet counts for male rats at 25 mg/kg/day were elevated to 128% of the control (376, units were 1000 cmm). At week 51, platelet counts for male rats at 25 mg/kg/day were elevated to 131.3% of the control (294, 1000 cmm). By week 3 of recovery, platelet counts for male rats at 25 mg/kg/day had returned to control levels.

5. **Blood Biochemistry and Urinalysis:** There were no treatment-related changes of blood biochemistry or urinalysis parameters.

6. **Ophthalmic Examination:** Ophthalmic examinations found no treatment-related ocular effects.

7. **Organ Weights**: Observed organ weight changes had no relationships to histopathological findings.

8. **Gross Pathology**: An increased incidence of gross findings (i.e., subcutis thickened, encrustations, vein lumen enlarged, area(s) of change) at injection sites were reported for male and female treatment groups. An increased incidence of swollen spleen was observed for male and female treatment groups; however, there was no histopathological correlation. Findings for the injection sites and spleen were still evident at the end of the recovery period.

Gross pathological findings for rats that received tinzaparin by the intravenous route at doses of 0, 4, 10, or 25 mg/kg/day for 52 weeks.

| Organ/Tissue | 0 mg/kg/day | | 4 mg/kg/day | | 10 mg/kg/day | | 25 mg/kg/day | |
|---|---|---|---|---|---|---|---|---|
| | M | F | M | F | M | F | M | F |
| Injection Sites | | | | | | | | |
| -subcutis thickened | 0 | 0 | 1 | 0 | 1 | 2 | 3 | 2 |
| -encrustations | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 |
| -vein lumen enlarged | 0 | 0 | 1 | 0 | 0 | 1 | 2 | 1 |
| -area(s) of change | 1 | 3 | 4 | 3 | 3 | 4 | 6 | 5 |
| Spleen | | | | | | | | |
| -swollen | 0 | 2 | 1 | 2 | 1 | 2 | 2 | 3 |

Gross pathological findings for rats that received tinzaparin by the intravenous route at doses of 0 or 25 mg/kg/day following a 4-week recovery period.

| Organ/Tissue | 0 mg/kg/day | | 25 mg/kg/day | |
|---|---|---|---|---|
| | Male | Female | Male | Female |
| Injection Site | | | | |
| -subcutis thickened | 0 | 0 | 1 | 0 |
| -encrustation(s) | 0 | 0 | 1 | 0 |
| Spleen | | | | |
| -swollen | 0 | 1 | 2 | 0 |
| -appears large | 0 | 0 | 2 | 0 |
| -area(s) of change | 0 | 0 | 1 | 0 |
| Popliteal lymph node | | | | |
| -dark | 0 | 0 | 0 | 1 |

9. **Histopathology**: Histopathological changes were primarily confined to the injection sites (i.e., hemorrhage, reactive inflammation/fibrosis) following the 52-week treatment period. Some changes at injection sites (i.e., hemorrhage) were still evident following the 4-week recovery period.

Histopathological findings for rats that received tinzaparin by the intravenous route at doses of 0, 4, 10, or 25 mg/kg/day for 52 weeks.

| Organ/Tissue | 0 mg/kg/day | | 4 mg/kg/day | | 10 mg/kg/day | | 25 mg/kg/day | |
|---|---|---|---|---|---|---|---|---|
| | M | F | M | F | M | F | M | F |
| Injection Site<br>-hemorrhage<br>-reactive inflammation/fibrosis | <br>1<br>3 | <br>2<br>2 | <br>2<br>3 | <br>4<br>3 | <br>4<br>4 | <br>4<br>3 | <br>4<br>5 | <br>5<br>6 |
| Parathyroids (L&R)<br>-cystic lesions | 1 | 1 | 1 | 2 | 1 | 1 | 2 | 3 |
| Epididymides (L&R)<br>-focal lymphocytic infiltrate | 0 | 2 | 1 | 2 | - | - | - | - |

Histopathological findings for rats that received tinzaparin by the intravenous route at doses of 0 or 25 mg/kg/day following a 4-week recovery period.

| Organ/Tissue | 0 mg/kg/day | | 25 mg/kg/day | |
|---|---|---|---|---|
| | Male | Female | Male | Female |
| Injection Sites<br>-hemorrhage | 1 | 0 | 2 | 0 |
| Popliteal lymph node<br>-hemosiderosis | 0 | 0 | 0 | 1 |

10. **Plasma Drug Levels**: Plasma $C_{max}$ and AUC values for tinzaparin, expressed in anti-factor Xa activity, were slightly less than proportional to dose on days 1, 7, and 361. Than were no gender-related differences in $C_{max}$ or AUC values, so values for male and female rats have been pooled. On days 1 and 7, half-life values observed with doses of 10 and 25 mg/kg/day were greater than those observed for 4 mg/kg/day; however, on day 361, half-life values were comparable at all three doses. On days 1 and 7, elimination of tinzaparin at doses of 10 and 25 mg/kg/day may have been slower than at 4 mg/kg/day.

Toxicokinetic parameters for plasma tinzaparin (expressed as anti-factor Xa activity) in rats that received tinzaparin by the intravenous route at doses of 0, 4, 10, or 25 mg/kg/day (male and female rats have been pooled together).

| Dose mg/kg | $C_{max}$, anti-factor Xa units/mL | | | AUC Anti-factor Xa U/mL * minute | | | Half-life, minutes | | |
|---|---|---|---|---|---|---|---|---|---|
| | Day 1 | Day 7 | Day 361 | Day 1 | Day 7 | Day 361 | Day 1 | Day 7 | Day 361 |
| 4 | 4.7 | 4.7 | 4.7 | 480 | 493 | 479 | 87 | 83 | 92 |
| 10 | 9.8 | 9.7 | 10.3 | 1122 | 1085 | 1150 | 138 | 115 | 92 |
| 25 | 17.2 | 15.2 | 17.9 | 2087 | 1751 | 1967 | 133 | 119 | 95 |

In a 52-week intravenous toxicology study, beagle dogs received tinzaparin by the intravenous route at doses of 0, 4, 10, or 25 mg/kg/day. For the control and 25 mg/kg/day groups, there were additional dogs for a 4-week recovery period following the treatment period. The maximum tolerated dose was 25 mg/kg/day. Histopathological changes were primarily confined to the injection sites (i.e., hemorrhage, reactive inflammation/fibrosis) following the 52-week treatment period. Some changes at injection sites (i.e., hemorrhage) were still evident following the 4-week recovery period.

**One-Year Chronic Subcutaneous Toxicity Study in Beagle Dogs (Final Report, LSR #88/NLP026/0843).**

**Conducting Lab:** [redacted]

**Dates of Conduct:** Initiated 5/21/87, completed 5/24/88.

**GLP Statement:** In compliance with the FDA's GLPs.

**Chemical:** LHN-1, lot #s BN 170487, 808330, 180487. 190487, and Heparin lot # 200487.

**Animals:** Beagle dogs, 24-27 wk old, 4/sex/group.

**Doses:** 0 (control vehicle), 4, 10, and 25 mg/kg/day LHN-1, and Heparin at 12.5 mg/kg/d, in 0.1 mL/kg volume, injected by the subcutaneous route at different sites, once daily, 7 days/week for 52 weeks. Due to excessive mortality, the 25 mg/kg/d dose of LHN-1 was reduced to 16.7 mg/kg/d, and that of Heparin was reduced to 5 mg/kg/d during the week 19 of the study, and the recovery phase was abandoned.

**Methods:** The control vehicle or the test agent (LHN-1 or Heparin) were injected into each animal, once daily, after feeding, for 52 weeks. The animals were further observed for morbidity and mortality throughout the study. The animals dying or killed prematurely were subjected to a full necropsy. The body weight, food and water consumption were recorded once weekly; ophthalmoscopy was performed on all the dogs prior to treatment, and during weeks 6, 12, 24, and 50; hematology and clinical chemistry from each dog before commencement and during weeks of 6, 12, 24, 50 of treatment; and finally urinalyses were performed before commencement and during weeks 5, 11, 23, and 49 of treatment. No EKGs were recorded, however, the heart and lungs were examined by auscultation. To assure absorption of the drug, during the week 10 of the study, blood samples were taken from all dogs immediately before dosing, 30 min and 4 hr after dosing, and prothrombin time, and activated thromboplastin time were determined. At termination, all the dogs were autopsied and examined macro- and microscopically. Bone marrow smears were also prepared.

**Results:**

**Observed Effects:** Swellings and hemorrhages at injection sites were common findings in most treated dogs.

**Mortality:** Ten dogs died or had to be killed for various reasons: Heparin, 3 (1M, 2F); mid dose, 1F; high dose, 6 (2M, 4F). One of the Heparin treated female dogs that died on day 5 of the treatment had severe focal hemorrhage in the spleen (considered as pre-existing condition exacerbated by treatment). The mid dose female dog died from an accident unrelated to treatment. The other eight dogs had severe hemorrhages, hematomas, and edema at the injection sites .

**Food Consumption**, **Body Weights**, and **Ophthalmoscopy** were essentially unremarkable in the treated animals.

**Hematology**: There were no significant treatment-related changes in the packed cell volume, Hb concentration, erythrocyte counts, platelets, and other hematological parameters in the LHN-1 and Heparin treated animals. There were no significant effects on the prothrombin times either. Further, bone marrow smears of the dead or killed dogs indicated higher erythroid cell activity, which may be a response to severe hemorrhage in the animal.

**Clinical Chemistry**: There were sporadic changes, increases and decreases in the plasma electrolytes levels in the Heparin-treated dogs (a maximum decrease of 7% in the calcium levels with Heparin to a similar decrease at other times); a decrease of up to 7% in calcium levels and a maximum of 20% decrease in the phosphorus levels in the LHN-1 treated dogs, but there was no clear and consistent trend attributable to LHN-1 treatment.

**Urinalyses** were unremarkable.

**Organ Weights**: There were no drug-related changes in any organ weight that could be considered significant as compared to controls.

**Histopathology**: The findings at the injection site were similar as reported earlier, that is, hemorrhage, edema, acute and chronic inflammation were seen in many treated dogs. In the spleen of 1M, 2F dogs in the high dose group, and 1F dog in the mid dose group, there was contraction of the sinuses, probably related to blood loss seen in these animals. There were no other significant findings in any organ that could be attributed to LHN-1 treatment.

In summary, subcutaneous doses of LHN-1 at 4, 10, and 25 (later reduced to 16.7 mg/kg/d) mg/kg/d or Heparin at 12.5 mg/kg/d (later reduced to 5 mg/kg/d) for one year produced hemorrhages/hematomas, edema etc. at the injection sites in almost all animals, resulting in 6 deaths at 16.7 mg/kg/d, and 3 in the Heparin group. The body weight gains of the treated dogs were normal. The hematology was essentially unaffected. No organ weight changes occurred in any group. The contraction of the sinuses in the spleen of 1M, 2F dogs in the 16.7 mg/kg/d group, probably related to blood loss seen in these animals, could be considered drug-related. Similar splenic change was seen in the 10 mg/kg/d female dog that died accidentally during the study and thus cannot be attributed to LHN-1 treatment. Because no significant toxicity was evident at 4 and 10 mg/kg/d doses, the latter dose could be considered as the no-toxic and maximum tolerated dose for the dog.

**Addendum:**

**Testing Laboratory:** [redacted]

**Drug Batch:** Tinzaparin bulk drug batch F668A [redacted] was supplied for use in the study as a solution in vials containing sodium metabisulfite. The solution batch numbers were 808330, 170487, 180487, and 190487.

**Doses:** Doses were equivalent to 0, 300, 700, and 1800/1200 anti-Factor Xa IU/kg/day.

**Hematology:** At week 6, platelet counts for high dose male dogs were increased to 121% of the control (228, units were 1000/cmm). At week 12, platelet counts for male dogs in the mid and high dose groups were increased to 123.15 and 131.5% of the control (216), respectively. At week 24, platelet counts for male dogs at the mid and high doses were increased to 126.9 and 129.6% of the control (186), respectively. At week 24, the APPT for male tinzaparin treatment groups was increased to 108.7-113% of the control (11.5 sec), respectively. At week 24, platelet counts for female tinzaparin treatment groups were increased to 115.8-132.6% of the control (190), respectively. At week 50, platelet counts for male dogs in the mid and high dose groups were both increased to 125.45% of the control (165), respectively. At week 50, platelet counts for female tinzaparin treatment groups were increased to 114-142.7% of the control (171).

**Clinical Chemistry:**

**Week 6:** Total protein levels for male dogs in the mid and high dose groups were decreased to 96.55 and 93.1% of the control (5.8 g%), respectively. Albumin levels for male dogs in the high dose group were decreased to 82.4% of the control (3.4 g%). Phosphorus levels for male dogs in the mid and high dose groups were decreased to 89.2 and 86.5% of the control (3.7 mEq/L), respectively. Phospholipid levels for female dogs in the mid and high dose groups were increased to 113 and 129% of the control (289 mg%), respectively. Total cholesterol levels for female dogs in the mid and high dose groups were increased to 118.4 and 136.0% of the control (125 mg%), respectively.

**Week 12:** Phospholipid levels for male dogs in the high dose group were decreased to 84% of the control (337 mg%). Albumin levels for male dogs in the high dose group were decreased to 83.3% of the control (3.6 g%). $\alpha_2$-Globulin levels for male dogs in the mid and high dose groups were both increased to 133.3% of the control (0.6 g%). Triglyceride levels for female dogs in the mid and high dose groups were increased to 134.3 and 145.7% of the control (35 mg%), respectively. Phospholipid levels for female tinzaparin treatment groups were increased to 113.6-128.7% of the control (272 mg%).

**Week 24**: Phospholipid levels for female dogs in the mid and high dose groups were increased to 117.5 and 134.6% of the control (292 mg%), respectively. Total cholesterol levels for female dogs in the mid and high dose groups were increased to 132.8 and 180.5% of the control (128 mg%), respectively.

**Week 50**: Triglyceride levels for female dogs in the high dose group were increased to 148.7% of the control (39 mg%). Phospholipid levels for female treatment groups were increased to 110.8-166.3% of the control (306 mg%). Total cholesterol level for female treatment groups were increased to 122.7-195.4 of the control (132 mg%).

## Reproductive Toxicology

### Rats

### Segment I: Intravenous Fertility and Reproductive Performance Study in the Rat (LSR Report #92/NLP137/0528).

**Testing Laboratory**: [redacted]

**Date Started**: November 4, 1991

**Date Completed**: November 12, 1992

**GLP Compliance**: Statements of compliance with GLP Regulations and the Quality Assurance Unit were included.

**Animals**: Sprague-Dawley rats (CD strain) were used in the present study. At the start of treatment, male rats were 7-8 weeks of age and had a body weight range of 184-221 g. Female rats were approximately 10-11 weeks of age and had a body weight range of 219-254 g.

**Drug Batch**: Tinzaparin, Lot No. LMW 9101 [redacted]

**Methods**: In an intravenous Segment I fertility and reproductive performance study, rats received tinzaparin at doses of 0, 5, 15, and 50 mg/kg/day (equivalent to 0, 400, 1300, and 4300 anti-Factor Xa IU/kg/day, respectively). Control animals received the vehicle, 0.9% sodium chloride solution. The sponsor's dose selection was based upon an intravenous Segment I/III dose range finding study in which rats received tinzaparin at doses of 0, 25, 50, 75, or 100 mg/kg/day (LSR Report No.: 92/NLP134/0093). Male rats were treated for 71 days prior to pairing. Female rats were treated for 15 days prior to pairing. Treatment was continued throughout the mating, gestation, and lactation periods to day 4 postpartum. Mortality or moribund sacrifice occurred at doses of 50, 75, and 100 mg/kg/day. All animals at 100 mg/kg/day died or were sacrificed in a

moribund condition. Necropsy findings for animals at 50, 75, and 100 mg/kg/day included internal organs, enlargement of the spleen, and thin blood. Observed effects at doses ≥50 mg/kg/day included dose-related incidences of pallor and pale eyes, reduced activity, piloerection, hunched posture, irregular respiration, and bleeding from the vagina or injection sites. Body weight gain from days 0 to 20 of gestation for female dams at 25, 50, and 75 mg/kg/day was impaired by 79.9, 46.25, and 47.3% of the control, respectively. Body weight gain from days 1 to 4 postpartum was unaffected. In the present study, there were 24 rats/sex/group. Animals received the vehicle or drug solution by the intravenous route using a dose volume of 1 mL/kg. Male rats were treated for 71 days prior to pairing, throughout the mating period, and up to termination after necropsy of female rats. Female rats were treated for 15 days prior to pairing, throughout the mating period, and from day 0 to 7 of gestation. Animals were monitored daily for clinical signs of toxicity and moribundity/mortality. Body weights for male rats were measured weekly. Body weights for female rats were measured twice weekly until evidence of mating was detected and on days 0 to 8, 10, 13, 17, and 20 of gestation. Food consumption of male and female rats was measured weekly until animals were paired for mating. Food consumption for female rats was also measured on days 0 to 7 of gestation and thereafter, twice weekly until termination. For female rats, estrous cycles were monitored for 10 days prior to pairing. Male and female rats were paired on a 1 to 1 basis. The time between initial pairing and detection of mating was recorded. On day 20 of gestation, female rats were sacrificed and submitted to gross examination. The reproductive tract including ovaries was assessed for number of corpora lutea in each ovary, number of implantation sites, number of resorption sites, and number and distribution of fetuses in each uterine horn. Fetuses were weighed, sexed, and examined for any external abnormalities. Individual placental weights and placental abnormalities were recorded. The neck and thoracic cavities for approximately one-half of the fetuses of each litter were dissected and examined for abnormalities. Remaining fetuses were processed and examined for visceral abnormalities. Eviscerated fetuses were processed and examined for skeletal abnormalities. It should be noted that examination of fetuses for external, visceral, and skeletal malformations and variations were performed despite the fact that drug treatment did not occur during the period of organogenesis. Following necropsy examinations of female rats, male rats were sacrificed and submitted to gross examinations. Organ weights were determined for the testes, epididymides, prostate gland, and seminal vesicles.

**Results:**

1. **Observed Effects of $F_0$ Male and Female Rats:** There were treatment-related incidences of bruising at the injection sites and localized tail damage, including swelling, thickening, and reddening. Exfoliation and encrustations were observed at injection sites in a few animals at 15 and 50 mg/kg/day.

2. **Mortality for $F_0$ Male and Female Rats:** One male at 15 mg/kg/day and 3 male rats and 1 male rat at 50 mg/kg/day were found dead or sacrificed in moribund condition. Male #59 at 15 mg/kg/day was found dead after week 12. A necropsy examination revealed a blood clot surrounding the right kidney and ureter. Male #74 at 50 mg/kg/day was sacrificed in a moribund condition after 12 weeks of treatment. This animal