was observed to have significant blood loss from the injection site for approximately 4 hr after dosing. Male #84 at 50 mg/kg/day was sacrificed in moribund condition after 13 weeks of treatment. This animal had significant blood from a tear in the second digit of the right hind-paw for approximately 6 hr. Male #92 at 50 mg/kg/day was found dead after 10 weeks of treatment. Gross examination of this animal revealed that the tips of all digits were cyanosed, the thymus gland was enlarged and congested, and large amounts of clotted blood in the pericardial sac. Female #192 at 50 mg/kg/day was sacrificed in a moribund condition during week 1 of treatment due to curvature of the spine and an inability to use hind-limbs. Pale red fluid was observed to be seeping from the vagina. Gross examination found no abnormalities of the spine. The cranial lung lobe was found to be dark and congested, and a few hemorrhagic areas were found on the inner walls of the bladder.

3. **Body Weight and Food Consumption for F_0 Male and Female Rats**: There were no treatment-related effects on body weight gain or food consumption. Body weights for male controls at weeks 0 and 14 were 206 and 584 g, respectively. Body weight gains for male rats at 5, 15, and 50 mg/kg/day from weeks 0 to 14 were 101.4, 100.5, and 99.2% of the control, respectively. Body weights for female controls on days 0 and 14 were 237 and 271 g, respectively. Body weight gains for female rats at 5, 15, and 50 mg/kg/day from days 0 to 14 were 102.5, 108.4, and 100% of the control, respectively. Body weights for female controls on days 0 and 20 of gestation were 282 and 472 g, respectively. Body weight gains for female dams at 5, 15, and 50 mg/kg/day from days 0 to 20 of gestation were 103, 100.9, and 109.45% of the control, respectively.

4. **Fertility and Reproductive Performance of F_0 Rats**: There were no treatment-related effects on the estrous cycle or pre-coital interval. There were no treatment-related effects on fertility and reproductive performance in male or female rats.

Mating and fertility in male and female rats that received tinzaparin by the intravenous route at doses of 0, 5, 15, and 50 mg/kg/day.

Parameter	0 mg/kg/day	5 mg/kg/day	15 mg/kg/day	50 mg/kg/day
Number of male rats paired	24	24	24	23
Number of female rats paired	24	24	24	23
Male Mating Index, %	96% (23/24)	100% (24/24)	100% (24/24)	100% (23/23)
Female Mating Index, %	100% (24/24)	100% (24/24)	100% (24/24)	100% (23/23)
Male Conception Rate, %	100% (23/23)	100% (24/24)	100% (24/24)	100% (23/23)
Female Conception Rate, %	100% (24/24)	100% (24/24)	100% (24/24)	100% (23/23)
Male Fertility Index, %	96% (23/24)	100% (24/24)	100% (24/24)	100% (23/23)
Female Fertility Index, %	100% (24/24)	100% (24/24)	100% (24/24)	100% (23/23)

Mating Index = animals mated/animals paired; conception rate = animals that achieved a pregnancy/animals mated; and fertility index = animals that achieved a pregnancy/animals paired.

5. **Litter Data for F_0 Female Dams**: There were no treatment-related effects on numbers of corpora lutea/dam, implantations/dam, viable pups/dam, or resorptions. There were no treatment-related effects on pre-implantation loss, post-implantation loss, or fetal body weight.

Group mean litter data.

Parameter	0 mg/kg/day	5 mg/kg/day	15 mg/kg/day	50 mg/kg/day
Number of pregnant dams	24	24	24	23
Corpora lutea/dam	18.5	19.3	19.4	19.4
Implantations/dam	16.5	18.3	17.7	18.2
Viable pups/dam	15.5	16.7	16.8	17.4*
Viable male pups/dam; Viable female pups/dam	7.6 (M); 7.8 (F)	8.3 (M); 8.4 (F)	8.6 (M); 8.2 (F)	8.5 (M); 8.9 (F)
Resorptions -early -late -total	 1.04 0.04 1.08	 1.46 0.13 1.58	 0.88 0 0.88	 0.74 0.04 0.78
Pre-implantation loss, %	10.8	6.4	8.6	6.7
Post-implantation loss, %	6.5	8.7	4.9	4.3
Fetal body weight, g Male/Female	3.74/3.51	3.71/3.52	3.76/3.57	3.80/3.57

6. **F_1 Male Reproductive Organ Weights**: There were no treatment-related effects on absolute or relative weights of the testes, epididymides, prostate gland, and seminal vesicles.

7. **External, Visceral, and Skeletal Abnormalities for F_1 Fetuses**: There were no treatment-related external, internal, visceral, or skeletal abnormalities in F_1 fetuses; however, it should be noted that drug treatment did not occur during the period of organogenesis.

F_1 fetal observations at necropsy. Expressed as % Incidence/Number of Litters.

Parameter	0 mg/kg/day	5 mg/kg/day	15 mg/kg/day	50 mg/kg/day
Fetuses/Litters	371/24	400/24	404/24	400/23
Large fetus (>4 g)	6.2%/9	6.8%/8	5.4%/7	10.3%/9
Shiny fetus	0	0.3%/1	0.2%/1	0.3%/1
Large placenta (>0.70 g)	1.1%/2	2.5%/5	0.5%/2	3.0%/4
Clotted blood around placenta	0.8%/1	1.0%/1	0	3.8%/5

Internal examination of F_1 fetuses. Expressed as % Incidence/Number of Litters.

Parameter	0 mg/kg/day	5 mg/kg/day	15 mg/kg/day	50 mg/kg/day
Fetuses/Litters	193/24	208/24	206/24	205/23
Clotted blood in abdomen	0	0	0	0.5%/1
Unilateral hydronephrosis	0	0	0	1.0%/2
Bilateral hydronephrosis	0	0	0.5%/1	1.0%/1
Bilateral hydroureter	0	1.4%/2	3.9%/4	3.9%/6

Visceral examination of F_1 fetuses. Expressed as % Incidence/Number of Litters.

Parameter	0 mg/kg/day	5 mg/kg/day	15 mg/kg/day	50 mg/kg/day
Fetuses/Litters	178/24	192/24	198/24	196/23
Head				
Unilateral slight macrophthalmia	0	0	0.5%/1	0.5%/1
Unilateral slight microphthalmia	0	0	0	0.5%/1
Thorax and abdomen				
Localized internal abdominal hemorrhage	0.6%/1	4.2%/4	1.0%/2	4.1%/6
Hemorrhagic abdomen	0	1.0%/2	1.0%/1	1.5%/3
Kidney, slightly enlarged	0	0	0	0.5%/1
Limbs and other				
Subcutaneous hemorrhages, submandibular	0	0	0	0.5%/1

F_1 fetal observations at skeletal observation. Expressed as % Incidence/Number of Litters.

Parameter	0 mg/kg/day	5 mg/kg/day	15 mg/kg/day	50 mg/kg/day
Fetuses/Litters	193/24	208/24	206/24	204/23
Sternebrae and ribs				
Incomplete ossification of 1 sternebra	32.6%/19	25.0%/18	31.1%/21	47.1%/22
One or more sternebrae offset	0.5%/1	0.5%/1	0	1.5%/2
Left 1st & 2nd ribs fused at tips	0	0	0	0.5%/1
Vertebrae				
Incomplete ossification of 1 or more thoracic vertebral centra	4.1%/7	4.8%/8	5.3%/8	6.9%/6
Limbs and girdles				
One or more phalangeal bones ossified	3.1%/5	3.8%/4	5.3%/8	6.4%/7

In an intravenous Segment I fertility and reproductive performance study, rats received tinzaparin at doses of 0, 5, 15, and 50 mg/kg/day. 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. 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. Tinzaparin at doses ≤50 mg/kg/day had no effects on fertility or reproductive performance in rats.

Segment I: Subcutaneous Reproductive Function and Fertility Study in the Rat (LSR Report #88/NLP027/458).

Conducting Lab: [redacted]

Dates of Conduct: Initiated 7/6/87, completed 4/26/88.

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

Chemical: LHN-1, lot #s BN 100486, 120497, and Heparin lot # 150487.

Animals: Rats, Sprague-Dawley, [redacted]; 34 males (7-8 wk old), and 34 females (9-11 wk old), in each treatment group.

Doses: 0 (control vehicle), 4, 10, and 25 mg/kg/day LHN-1, and Heparin at 12.5 mg/kg/d, given subcutaneously in 0.5 mL/kg volume, at different sites, once daily. Doses were selected from the 87/NLP024/729 study, the results of which were not provided.

Methods: Both male and female rats were given LHN-1 at the doses indicated for 71 and 15 days, respectively, prior to breeding and gestation until sacrifice on day 20 instead of day 13 of gestation as per FDA's guidelines for the Segment I reproductive toxicity study. The dams continued receiving the treatment for 25 days duration lactation. The estrus cycles were monitored in the females for 10 days prior to breeding. Dams were observed weekly to twice weekly for signs of morbidity/mortality. Food consumption was recorded once weekly, and body weights of the dams on days 0, 3, 6, 10, 13, 17, and 20 of gestation, and until day 25 postpartum. Gestation length was recorded. About 2/3 dams were sacrificed on day 20 of gestation and the number of corpora lutea, implantation sites, the number of resorptions, and the number and type of distribution of fetuses in each uterine horn were recorded. All remaining dams were allowed to deliver naturally. Offspring (F_1) from all of these dams were tested for developmental and behavioral impairment. In addition, the weight of pups was recorded on postpartum days 1, 4, 7, 11, 14, 18, 21 and 25. The reproductive performance of 26 F_1 males and 26 F_1 females was tested when 9-10 weeks old, and the development of F_2 pups examined. The fetal examinations included: fetal weight, sex, and external abnormalities. Fifty percent of each F_1 and F_2 litter was examined for internal abnormalities prior to fixing for skeletal examinations (by Alizarin techniques) and 50% for internal abnormalities by Bouin's technique.

Results: Six rats (1M, mid dose; 1F, high dose; 1M and 3F Heparin) died during the study. Necropsy of the LHN-treated rats did not reveal any specific cause of death, while the Heparin treated rats had pale organs (suggesting loss of blood). The number of hemorrhages/hematomas at injection sites were observed in most treated dams, more than the control dams. The other common effects were thickening and staining at the injection sites.

The body weight gain and food consumption by the treated male and female rats (dams) were not significantly affected by the treatment with LHN-1 or Heparin. The estrous cycle of the dams was not affected either.

The gestation length, mating performance, fertility index, and pregnancy rate were all comparable with the controls. The number of resorptions in the 4 mg/kg/d group slightly increased resulting in the increased post-implantation loss. In the Heparin group, the number of resorptions were 2.2±1.48 compared to control of 1.0±1.0, and the corresponding post-implantation loss of 12.9% compared to 6.3 in the control, and 10.5, 7.1 and 5.3 in the low, mid and high dose LHN groups, respectively. In the dams that were sacrificed on day 20 of gestation, the other parameters such as corpora lutea, implantations, live embryos etc. were not affected by the treatment.

Fetal examinations (F_1): The number of fetuses with one or more visceral malformations in the control, low, mid, high dose of LHN-1 and Heparin group were 1, 1, 2, 6, and 3, respectively out of 162-168 fetuses/group examined. The type of malformation seen was unilateral microphthalmia, hydrocephaly, absence of innominate artery, absence of tail and imperforate anus. However, the number of fetuses, mentioned above, was not higher than the historical control data from 12,835 fetuses examined from 126 studies. The number of fetuses with skeletal malformations were 3, 1, and 6 in the 4 and 10 mg/kg/d LHN groups, and the Heparin group, respectively. The type of malformations seen were: anomalous vertebrae and ribs, enlarged 14th rib, kinked tail, and anomalous scapulae (5 fetuses in one litter of 10 mg/kg/d group). These numbers were also lower than the historical controls.

Behavioral Performance: The behavioral performance of both F_1 and F_2 offspring was not affected adversely by the treatment with LHN-1 or Heparin at any dose tested.

Reproductive Performance of F_1 pups was not significantly affected by the treatment with either LHN-1 or Heparin.

Physical Development of the pups in both F_1 and F_2 generations was not affected adversely.

In summary, LHN-1 was administered to male and female rats at dose levels of 4, 10, 25 mg/kg/d, and Heparin at 12.5 mg/kg/d prior to mating and throughout gestation. There were hemorrhages/hematomas in all the treated rats. However, this effect is the expected pharmacologic activity of LHN-1 and Heparin and should be of no concern since there were no LHN-1-related deaths. The number of fetuses with skeletal or visceral malformations was not significantly higher than the historical controls. LHN-1 did not reduce fertility and general reproductive performance in the rat at doses of up to 25 mg/kg/d of LHN-1 or Heparin at 12.5 mg/kg/d.

Addendum:

Testing Laboratory: [redacted]

Drug Batch: Tinzaparin bulk drug Batch F668A [redacted] anti-Factor Xa IU/mg) was supplied as solutions containing sodium metabisulfite. The following lot numbers were used: BN100487, BN110487, and BN120487

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

Mating Performance and Fertility of F_0 Rats: Subcutaneous administration of tinzaparin had no effects on mating performance or fertility in rats. Necropsy examination of F_0 dams found that incidences of hemorrhage, hemorrhagic areas, and hematomas at the injection site(s) were increased for treatment groups.

Mating Performance and Fertility of F_0 Generation

Parameter	0 mg/kg/day	4 mg/kg/day	10 mg/kg/day	25 mg/kg/day	Heparin
# Males/Female Paired	34/34	34/34	34/34	33/33	32/32
# Males Mating	34	33	34	33	32
# Females Mating	34	34	34	33	32
# Males producing pregnancy	34	33	34	33	32
# Females pregnant	34	34	34	33	32
%Mating for males	100	97 (33/34)	100	100	100
%Mating for females	100	100	100	100	100
Conception rate (%) for males	100	100	100	100	100
Conception rate (%) for females	100	100	100	100	100
Fertility Index (%) for Males	100	97 (33/34)	100	100	100
Fertility Index (%) for Females	100	100	100	100	100

Necropsy Examination of F_0 Female Rats Sacrificed on Day 20 of Gestation

Parameter	0 mg/kg/day	4 mg/kg/day	10 mg/kg/day	25 mg/kg/day	Heparin
N =	22	22	22	21	21
Hemorrhage at ≥ 1 injection site(s)	0	11	9	13	13
Hemorrhagic areas at ≥ 1 injection site(s)	0	2	2	2	6
Hematoma at ≥ 1 injection site(s)	0	0	3	2	10

Litter Data for F_0 Female Rats Sacrificed on Day 20 of Gestation: Treatment with tinzaparin had no effect on litter data for female rats sacrificed on day 20 of gestation. An increased incidence of resorptions (early and late) and post-implantation were observed for the heparin treatment group.

Group Mean Litter Data for F_0 Female Rats Sacrificed on Day 20 of Gestation.

Parameter	0 mg/kg/day	4 mg/kg/day	10 mg/kg/day	25 mg/kg/day	Heparin
# Pregnant animals	22	22	22	21	21
Total litter loss (%)	0	0	0	0	1 (4.8%)
Corpora lutea/dam	17.5	18.0	17.6	17.8	18.0
Implantations/dam	15.8	16.9	16.1	17.0	17.0
Viable fetuses/dam	14.8	15.1	15.0	16.1	14.8
Resorptions -early -late -total	 0.86 0.14 1.00	 1.50 0.27 1.77	 0.77 0.36 1.14	 0.76 0.14 0.90	 1.20 1.00 2.20
Pre-implantation loss, %	9.8	6.3	9.0	5.8	6.1
Post-implantation loss, %	6.3	10.5	7.1	5.3	12.9
Fetal body weight, g	3.23	3.31	3.31	3.39	3.22
Placental weight, g	0.51	0.50	0.56	0.51	0.52

External, Internal, Visceral, and Skeletal Examination of F_1 Fetuses: Examination of F_1 fetuses found no treatment-related external, internal, visceral, or skeletal abnormalities.

External Examination of F_1 Fetuses. Data expressed as % Incidence/number of litters.

Parameter	0 mg/kg/day	4 mg/kg/day	10 mg/kg/day	25 mg/kg/day	Heparin
Fetuses/Litters	326/22	332/22	329/22	339/21	296/20
Large placenta (>0.70 g)	0.9%/3	1.5%/5	4.0%/5	2.1%/6	1.4%/2
Placenta discolored green	0	0	0	0	4.1%/1
Green/brown edge to placenta	0	0	0	2.9%/1	6.8%/2
Clotted blood around placenta	0	0	0	0	1.0%/1
Amniotic fluid/membranes green/brown	0	0	0	2.9%/1	13.9%/4

Internal Examination of F_1 Fetuses. Data expressed as % Incidence/number of litters.

Parameter	0 mg/kg/day	4 mg/kg/day	10 mg/kg/day	25 mg/kg/day	Heparin
Fetuses/Litters	166/22	168/22	168/22	171/21	152/20
Free clotted blood in abdomen	0	0	0	1.2%/2	0

Visceral Examination of F_1 Fetuses. Data expressed as % Incidence/number of litters.

Parameter	0 mg/kg/day	4 mg/kg/day	10 mg/kg/day	25 mg/kg/day	Heparin
Fetuses/Litters	160/22	164/22	161/22	168/21	144/20
Head					
Blood on tongue/in mouth/nasal sinuses/nasopharynx	0	1.2%/2	0	1.2%/2	1.4%/2
Slightly hemorrhagic vitreous humor	0	0	0	0	0.7%/1
Bilateral severe microphthalmia	0	0	0	0	0.7%/1
Thorax and abdomen					
Blood filled thoracic lymph duct	0	0.6%/1	0	1.2%/2	1.4%/2
Innominate artery reduced in length/absent	0	0	0.6%/1	0.6%/1	1.4%/2
Retro-esophageal right subclavian artery	0	0		0.6%/1	0
Additional azygos vein on right side; heart rotated slightly to left	0	0	0	0	0.7%/1
Slightly increased amount of pericardial fluid	0	0.6%/1	0	2.4%/3	1.4%/2
Localized internal abdominal hemorrhage	0.6%/1	2.4%/4	0.6%/1	1.2%/2	3.5%/4
Hemorrhagic peritoneal fluid	0.6%/1	1.2%/1	0	0	1.4%/2
Intra-muscular left hind-limb	0	0	0	0	0.7%/1
Subcutaneous hemorrhage					
Lateral/ventral/dorsal thoracic	3.8%/6	6.1%/6	3.7%/5	7.1%/8	10.4%/9
Anal region	0	1.8%/2	3.1%/3	0	2.8%/3
Slightly subcutaneous edema-trunk generalized	5.0%/5	9.1%/9	6.8%/8	6.5%/7	15.3%/13

Skeletal Examination of F_1 Fetuses. Data expressed as % Incidence/number of litters.

Parameter	0 mg/kg/day	4 mg/kg/day	10 mg/kg/day	25 mg/kg/day	Heparin
Fetuses/Litters	166/22	168/22	168/22	171/21	152/20
Head					
Discrete unossified area in basiocciptial bone	0	0	0.6%/1	0.6%/1	0
Presphenoid bone incompletely ossified or unossified	0.6%/1	0.6%/1	1.2%/2	1.2%/2	0
Sternebrae and ribs					
Incomplete ossification of 1 sternebra	7.8%/9	17.9%/9	14.9%/11	19.9%/13	17.8%/11
13th rib or ribs reduced in length	2.4%/3	0.6%/1	6.0%/6	2.9%/4	5.3%/5
Wavy rib or rib(s)	0	0.6%/1	0	0	2.0%/2
Additional cervical rib or ribs	0	0	0	0	1.3%/2
Vertebrae					
Ossification of ventral arch of 1st cervical vertebra	4.8%/6	4.2%/5	4.8%/5	6.4%/6	10.5%/7
Ossification of the majority of cervical vertebral centra	0	0	0	0.6%/1	0
Limbs and Girdles					
Metacarpals/Metatarsals 4/4	20.5%/14	31.5%/14	31.5%16	42.7%/18	42.8%/5
≥ 1 phalangeal bones ossified	1.8%/1	0.6%/1	1.8%/2	3.5%/4	1.3%/2

Group Mean Litter Data for F_0 Dams Allowed to Deliver Their Offspring: Viability of F_1 pups at 10 and 25 mg/kg/day were both reduced to 91% by day 25 postpartum as compared to 97% for the control. Necropsy examination of F_0 dams found that incidences of hemorrhage, hemorrhagic areas, and hematomas at the injection site(s) were increased for treatment groups.

Group mean litter size (F_1 pups).

Parameter	0 mg/kg/day	4 mg/kg/day	10 mg/kg/day	25 mg/kg/day	Heparin
#Pregnant animals	12	12	12	12	10*
Implantation sites/dam	16.0	15.4	16.3	17.1	16.9
Total pups/dam on day1	14.2	14.1	14.4	14.5	13.7
Viable pups/dams on day 1	13.8	13.8	14.1	15.0 (13.7)**	13.6
Postimplantation survival index	88 (14.2/16.0)	91 (14.1/15.4)	88 (14.4/16.3)	85 (14.5/17.1)	81 (13.7/16.9)
Live birth index	97 (13.8/14.2)	98 (13.8/14.1)	98 (14.1/14.4)	95 (13.7/14.5)	99 (13.6/13.7)
Viability on day 25 postpartum	97 (13.3/13.8)	95 (13.1/13.8)	91 (12.8/14.1)	91 (12.5/13.7)	93 (12.7/13.6)

*Excludes Dam #1160 sacrificed on day 17 of gestation.

** Litter of F_0 Dam #1115 at 25 mg/kg/day lost by day 1 postpartum

Necropsy Examination of F_0 Dams Allowed to Litter

Parameter	0 mg/kg/day	4 mg/kg/day	10 mg/kg/day	25 mg/kg/day	Heparin
N =	12	12	12	12	10
Hemorrhage at ≥ injection sites	1	3	5	5	9
Hemorrhagic areas at ≥ injection sites	0	1	2	3	0
Hematoma at ≥ injection sites	0	0	0	1	4

Necropsy Examination of F_0 Male Rats.

Parameter	0 mg/kg/day	4 mg/kg/day	10 mg/kg/day	25 mg/kg/day	Heparin
N =	34	34	33	34	33
Hematoma/Hemorrhage at injection sites	0	3	6	6	14
Hemorrhagic areas at injection sites	0	1	2	2	6

F_2 Fetuses: External, internal, visceral, and skeletal examinations of F_2 fetuses found no treatment-related abnormalities.

F_2 pups: There were no treatment-related effects on viability, growth, or development observed up to termination following weaning and completion of vaginal opening.

Segment II: Intravenous Teratology Study with Tinzaparin in the Rat (LSR Report No.: 92/NLP138/0361).

Testing Laboratory: [redacted]

Date Started: August 28, 1991 (Animals received)

Date Completed: September 10, 1992

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

Animals: Pregnant female Sprague-Dawley rats (CD strain) were used in the present study. At the start of the treatment, pregnant female rats were 10-11 weeks of age and had a body weight range of 239-290 g.

Drug Batch: Tinzaparin, Lot No. LMW 9101 [redacted] anti-Factor Xa IU/mg).

Methods: In an intravenous Segment II teratology study, pregnant female Sprague-Dawley rats received tinzaparin at doses of 0, 5, 20, or 75 mg/kg/day (equivalent to 0, 400, 1700, and 6500 anti-Factor Xa IU/kg/day, respectively) from days 7 to 17 of gestation. Control animals received the vehicle, [redacted] solution. The sponsor's dose selection was based upon a preliminary intravenous Segment II dose range finding study in which pregnant female rats received tinzaparin at doses of 0, 25, 50, 75, or 100 mg/kg/day from days 7 to 17 of gestation (LSR Report No.: 91/NLP135/1160). Mortality occurred for 1 of 6 female rats at 75 mg/kg/day and 2 of 6 female rats at 100 mg/kg/day. At doses ≥50 mg/kg/day, observed effects included skin pallor and pale eyes. Body weight gain for dams at 50, 75, and 100 mg/kg/day from days 7 to 18 of gestation were reduced to 88.9, 88.5, and 33.9% of the control, respectively. Necropsy examinations of dams found that 2 of 4 animals at 100 mg/kg/day had enlarged spleens. Early resorptions/dam were increased to 6.75 at 100 mg/kg/day as compared to 1.33 for the control. Post-implantation loss was increased to 50% at 100 mg/kg/day as compared to 8.6% for the control. Viable pups/dam were decreased to 7.5 at 100 mg/kg/day as compared to 14.2 for the control. External examination of fetuses found that the incidence of white rim to placenta was increased to 23.3% (1 litter) as compared to no similar findings for the control. In the present study, there were 32 pregnant female rats/group. For the control, 5 mg/kg/day, and 20 mg/kg/day groups, 1, 2, and 3 animals, respectively, were not pregnant. Animals received the vehicle or drug solution by the intravenous route at a dose volume of 1 mL/kg. Animals were observed daily for clinical signs of toxicity. Animals found dead or sacrificed in a moribund condition were submitted to a complete gross examination. Body weights were measured on days 0, 3, 7-18 inclusive, and 20 of gestation. Food consumption was measured for the following intervals: days 0-2, 3-6, 7-10, 11-14, 15-17, and 18-19 of gestation. Approximately two-thirds (≤21 rats/group) of surviving F_0 female rats per group were sacrificed on day 20 of gestation and the reproductive tract complete with ovaries was examined for number corpora lutea in each ovary, number of implantation sites, number of resorption sites (classified as early or late), and number and distribution of fetuses in each uterine horn. Each fetus was weighed, sexed, and examined for external abnormalities. Approximately one-half of the fetuses of each litter were submitted to an internal examination of the neck and the thoracic and abdominal cavities followed by evisceration and fixation. Remaining fetuses were processed for visceral examinations. Eviscerated fetuses were processed for skeletal examinations. Remaining F_0 dams were allowed to deliver their offspring and rear them until day 25 postpartum. From day 20 of gestation, dams were observed 2 to 3 times per day for onset, progress, and completion of parturition. Individual gestational lengths were calculated. Body weights of F_0 dams were measured on days 1, 4, 7-11, 14, 18, 21, and 25 postpartum. Offspring were examined 24 hr after birth and the following parameters were determined: number born (live and dead), body weights, individual sexes, and external abnormalities. Litters were monitored daily for signs of ill-health or mortality. On day 4 postpartum, litters with >8 pups were reduced to 4 pups/sex/litter, if possible, by random culling. Culled offspring were discarded with further examination. Pups were sexed on days 1, 4 (before and after culling), 14, and 25 postpartum. Body weights for pups were measured on days 1, 4 (before culling), 7, 11, 14, 18, 21, and 25 postpartum. Physical development of pups was monitored for onset and completion of

the following parameters: pinna unfolding, hair growth, testis descent, tooth eruption, and eye opening. Due to a high mortality rate for dams at 75 mg/kg/day from days 8 to 18 of gestation, the sponsor decided not to continue the study into a further generation, and all surviving F_0 dams and F_1 pups were sacrificed on day 25 postpartum and submitted to complete gross examination.

Results:

1. **Observed Effects for F_0 Dams**: There were no reported observed effects for dams at doses ≤5 mg/kg/day.

2. **Mortality of F_0 dams**: Mortality occurred with doses of 20 and 75 mg/kg/day. At 20 mg/kg/day, four dams at 20 mg/kg/day were sacrificed in moribund condition (#1084 on day 13, #1086 on day 17, #1088 on day 16, and #1095 on day 15). All dams had evidence of evidence of hemorrhage into the urogenital area. At 75 mg/kg/day, 2 dams were found dead (#1104 on day 14 and #1121 on day 8) and 14 dams were sacrificed in a moribund condition (#1097 on day 18, #1099 on day 8, #1103 on day 16, #1107 on day 17, #1111 on day 17, #1115 on day 13, #1116 on day 17, #1119 on day 12, #1122 on day 13, #1123 on day 11, #1124 on day 11, #1126 on day 15, #1127 on day 15, and #1128 on day 15). Thirteen animals at 75 mg/kg/day had evidence of hemorrhage. For animals that died or were sacrificed in a moribund condition at 20 or 75 mg/kg/day, bruising of the peri-anal area, pallor, and pale eyes were observed in the majority of these animals. Internal examination of rats at 20 mg/kg/day found that 2 animals had a congested thymus and one animal had an enlarged spleen. Internal examination of rats at 75 mg/kg/day (i.e., died or moribund sacrifice) found that nine had a congested thymus and five had an enlarged spleen.

3. **Body Weight and Food Consumption for F_0 Dams**: There were no treatment-related effects on body weight gains or food consumption of F_0 dams from days 7 to 18 of gestation. There were no treatment-related effects on body weight gains of F_0 dams from days 1 to 25 postpartum. Body weights for female controls on days 7 and 18 of gestation were 314 and 407 g, respectively. Body weight gains for F_0 dams at 5, 20, and 75 mg/kg/day from days 7 to 18 were 101.6, 102.8 and 96.8% of the control, respectively. Body weights of control dams on days 1 and 25 postpartum were 341 and 377 g, respectively. Body weight gains of dams at 5, 20, and 75 mg/kg/day from days 1 to 25 postpartum were 80.8, 104.6, and 153.3% of the control, respectively.

4. **Litter Parameters for F_0 Dams Sacrificed on Day 20 of Gestation**: There were no treatment-related effects on numbers of corpora lutea/dam, implantations/dam, or viable fetuses/dam. The number of early resorptions was increased at 20 and 75 mg/kg/day. There were no treatment-related effects on pre-implantation loss, fetal body weight, or placental weight. Post-implantation loss was increased at 20 and 75 mg/kg/day. Necropsy examination of F_0 dams on day 20 found that 2 dams at 75 mg/kg/day had enlarged spleens.

Litter Parameters for F_0 Dams Sacrificed on Day 20 of Gestation

Parameter	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	75 mg/kg/day
Number of pregnant animals	20	19	18	13
Corpora lutea/dam	19.0	17.7	18.2	19.1
Implantations/dam	17.7	16.1	17.1	18.1
Viable fetuses/dam	17.0	15.2	15.8	16.5
Resorptions				
-early	0.60	0.89	1.22	1.62
-late	0.10	0	0	0
-total	0.70	0.89	1.22	1.62
Pre-implantation loss, %	6.6	9.5	7.3	5.2
Post-implantation loss, %	4.0	5.6	7.2	8.9
Fetal weight, g	3.65	3.65	3.65	3.62
Placental weight, g	0.52	0.53	0.54	0.54

5. External, Internal, Visceral, and Skeletal Examinations of F_1 Fetuses: External examination of fetuses at 75 mg/kg/day found an increased incidence of pale rimmed placenta and amniotic fluid that was tinged green. Visceral examination of fetuses at 75 mg/kg/day revealed increased incidences of blood-filled thoracic lymph ducts and inter-ventricular septal defects; although, significant maternal toxicity was associated with this dose. There were no treatment-related internal or skeletal abnormalities.

External Examination of F_1 Fetuses. Expressed as % Incidence/Number of Litters.

Parameters	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	75 mg/kg/day
Fetuses/Litters	340/20	288/19	285/18	214/13
Shiny Fetuses	0	0	0	0.5%/1
Small fetus (<2.80 g)	0.6%/2	0	1.1%/2	0.9%/1
Large Placenta (>0.70 g)	2.1%/3	3.5%/5	2.8%/4	3.7%/4
Pale rimmed placenta	**0**	**0**	**0**	**4.7%/1**
Amniotic fluid, tinged green	**0**	**0**	**0**	**6.5%/3**

Internal Examination of F_1 Fetuses. Expressed as % Incidence/Number of Litters.

Parameters	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	75 mg/kg/day
Fetuses/Litters	174/20	149/19	148/18	110/13
Bilaternal hydronephrosis	0	0	0.7%/1	0
Unilateral hydronephrosis	0	1.3%/2	0	0
Bilateral hydroureter	0	0	0.7%/1	0

Visceral Examination of F_1 Fetuses. Expressed as % Incidence/Number of Litters.

Parameters	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	75 mg/kg/day
Fetuses/Litters	166/20	139/19	137/18	104/13
Head				
Blood on tongue/in mouth/nasopharynx	0	0	1.5%/2	1.0%/1
Hemorrhage between ≥ 1area(s) of the brain and pia mater	0.6%/1	1.4%/2	0	1.0%/1
Thorax and abdomen				
Blood in trachea	0.6%/1	0	0	1.0%/1
Blood-filled thoracic lymph duct	**0**	**0**	**0**	**1.9%/2**
Inter-ventrcular septal	**0**	**0**	**0**	**1.0%/1**

Defects (M)				
Hepatic hemorrhages	4.2%/6	6.5%/5	6.6%/5	6.7%/5
Small additional liver lobes	18.7%/16	18.0%/13	17.5%/13	25.0%/11
Hemorrhagic abdomen	0	0	0.7%/1	0
Bilateral, slight hydroureter	1.2%/2	1.4%/2	0	2.9%/3
Limbs and others				
Intra-muscular hemorrhage-hind-limb	0	0.7%/1	5.8%/3	1.8%/1
Subcutaneous hemorrhages				
Cranial	0.6%/1	0	3.6%/4	0
Cervical	0.6%/1	0.7%/1	1.5%/2	0

Skeletal Examination of F_1 Fetuses. Expressed as % Incidence/Number of Litters.

Parameters	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	75 mg/kg/day
Fetuses/Litters	166/20	139/19	137/18	104/13
Head				
Small anterior fontanelle	0	0.7%/1	1.4%/2	0
Incomplete ossification of squamosal bone	0.6%/1	1.3%/1	1.4%/1	0
Incomplete ossification of palatine bones associated with cleft palate	0	0	0	1.9%/1
Some additional plaque of bone between interparietal and parietal bones	0.6%/1	0	0	1.8%/2
Hyoid bone unossified	10.3%/10	12.8%/9	12.8%/11	18.2%/7
Sternebrae and ribs				
Incomplete ossification of 3 sternebrae	13.8%/12	12.1%/10	10.8%/9	19.1%/8
Incomplete ossification of 5 sternebrae	0.6%/1	0	0	0.9%/1
≥1 sternebrae offset	0	0.7%/1	1.4%/2	0
Vertebrae				
Incomplete ossification of cervical vertebral arches	0	0	0.7%/1	0.9%/1
1st thoracic vertebral centrum unossified	0	0.7%/1	0.7%/1	1.8%/2
One thoracic vertebral centrum bipartite	1.1%/2	0.7%/1	0	1.8%/2
Incomplete ossification of 2nd sacral vertebral arch	0	0	0.7%/1	0
Incomplete ossification of caudal vertebral arches	8.6%/8	14.8%/8	13.5%/11	15.5%/8
Limbs and girdles				
≥ phalangeal bones ossified	1.1%/2	1.3%/1	3.4%/2	2.7%/2
One or both ischial bones incompletely ossified	0	1.3%/2	0.7%/1	0

6. **Litter Parameters for F_0 Dams Allowed to Deliver Their Offspring**: Due to a high mortality rate at 75 mg/kg/day, only 3 dams at this dose were available for this phase of the study. There were no treatment-related effects on length of gestation. The gestation index was reduced at a dose of 75 mg/kg/day, because 1 of the 3 dams failed to deliver.

Gestation length and Index.

Parameters	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	75 mg/kg/day
Number of pregnant animals	11	11	9	3*
Number of live litters born	11	11	9	2
Gestation Index, %	100	100	100	67

*Percent calculated from 2 animals – one pregnant female failed to litter. Excludes 7 pregnant female rats killed in extremis during gestation.

7. **Viability, Growth, and Development of F_1 Pups**: Post-implantation survival and the birth index were reduced for F_1 pups at 75 mg/kg/day; although, significant maternal toxicity was evident at this dose. There were no treatment-related effects on pup survival from days 1 to 25 postpartum. There were no treatment-related effects on growth or development (i.e., pinna unfolding, hair growth, testes descent, tooth eruption, and eye opening) of F_1 pups. The number of pups at 75 mg/kg/day was extremely low due to high mortality rates for dams at this dose (i.e., 28 pups were available for examination at 75 mg/kg/day as compared to 172 pups for the control). Necropsy examinations of pups that died prior to scheduled termination or pups that were sacrificed at scheduled termination revealed no treatment-related findings.

F_1 Offspring Survival Indices and Body Weight.

Parameter	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	75 mg/kg/day
Implantation sites/dam	17.5	16.8	17.8	17.0
Total pups/dam on day 1	15.6	15.4	15.9	14.0
Viable pups/dam on day 1	15.6	15.4	15.7	14.0
Post-implantation survival index, %	89 (15.6/17.5)	91 (15.4/16.8)	89 (15.9/17.8)	78 (14.0/17.0)
Birth Index, %	89 (15.6/17.5)	91 (15.4/16.8)	89 (15.7/17.8)	78 (14.0/17.0)
Live Birth Index, %	100 (15.6/15.6)	100 (15.4/15.4)	88 (15.7/15.9)	100 (14.0/14.0)
Viability Index, % day 4 postpartum	97 (15.1/15.6)	99 (15.2/15.4)	98 (15.3/15.7)	96 (13.5/14.0)
Lactation Index, % on day 25 postpartum	99 (7.9/8.0)	93 (7.5/8.0)	96 (7.7/8.0)	94 (7.5/8.0)
Body weights, g (M/F)				
-day 1	6.8/6.4	6.9/6.5	6.5/6.1	7.3/6.6
-day 4 before cull	8.7/8.1	8.7/8.2	8.5/7.9	9.7/8.7
-day 4 after cull	8.8/8.3	8.9/8.4	8.7/8.1	10.1/8.7
-day 25 after cull	75.8/72.2	72.4/68.3	74.1/67.1	83.8/75.8
Male/Female Pups (Ratio)				
-Total on day 1 postpartum	82/90	90/79	68/75	21/7
-# Alive on day 1 postpartum	82/90	90/79	66/75	21/7
-# Alive on day 4 before cull	79/87	89/78	65/73	20/7
-# Alive on day 4 after cull	43/45	44/44	36/36	9/7
-# Alive on day 25 after cull	42/45	42/40	35/34	9/6

Necropsy Findings for F_1 Offspring at Termination (Day 25 postpartum). Expressed as % Incidence/Number of Litters.

Parameter	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	75 mg/kg/day
Offspring/Litters	87/11	82/11	69/9	15/2
Male: Female Offspring	42:45	42:40	35:34	9:6
Unilateral hydronephrosis	2.3%/1	6.1%/3	5.8%/3	6.7%/1
Bilateral hydronephrosis	0	0	1.4%/1	0
Bilateral hydroureter	0	0	1.4%/1	0

In an intravenous Segment II teratology study, pregnant female Sprague-Dawley rats received tinzaparin at doses of 0, 5, 20, or 75 mg/kg/day from days 7 to 17 of gestation. Mortality occurred for F_0 dams at 20 and 75 mg/kg/day during the treatment period. The mortality rate for F_0 dams at 75 mg/kg/day was 50%. Tinzaparin was not teratogenic at intravenous doses ≤75 mg/kg/day. The number of pups at 75 mg/kg/day available for teratological examinations was reduced to 214 as compared to 340 for the control group. For F_0 dams allowed to deliver their pups, only 3 dams were available at 75 mg/kg/day as compared to 11 for the control group. Consequently, only 28 pups at 75 mg/kg/day were available for examination from days 1 to 25 postpartum as compared to 172 pups for the control group.

Segment II: Intravenous Teratology Study with Tinzaparin in the Rat (LSR Report No.: 92/NLP150/0828).

Testing Laboratory: [redacted]

Date Started: January 2, 1992

Date Completed: December 22, 1992

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

Animals: Pregnant female Sprague-Dawley rats (CD strain) were used in the present study. At the start of the treatment, pregnant female rats were 10-11 weeks of age and had a body weight range of 216-268 g.

Drug Batch: Tinzaparin, Lot No. LMW 9101 ([redacted] anti-Factor Xa IU/mg).

Methods: In an intravenous Segment II teratology study, pregnant female Sprague-Dawley rats received tinzaparin at doses of 0, 5, 10, or 25 mg/kg/day (equivalent to 0, 400, 900, and 2200 anti-Factor Xa/kg/day, respectively) from days 7 to 17 of gestation. Control animals received the vehicle [redacted] solution. The sponsor's dose selection was based on the intravenous Segment II teratology study, described above, in which rats received doses of 0, 5, 20, and 75 mg/kg/day (LSR Report No.: 92/NLP138/0361). In the control, 5 mg/kg/day, and 10 mg/kg/day groups, there were 32 pregnant female rats/group. In the 25 mg/kg/day group, there were 40 pregnant female rats/group. Vehicle or drug solution was administered by the intravenous route using an intravenous dose volume of 1 mL/kg. Animals were monitored daily for clinical signs of toxicity. Animals sacrificed in a moribund condition during the treatment period were necropsied and any abnormal tissues were retained. Body weights of F_0 dams were measured on days 0, 3, 7 to 18, and 20 of gestation. Food consumption of F_0 dams was measured on days 0-2, 3-6, 7-10, 11-14, 15-17, and 18-19 of gestation. For the

teratology phase of the study, 21 pregnant F_0 female rats from the control, 5 mg/kg/day, and 10 mg/kg/day groups, and 27 pregnant female rats from the 25 mg/kg/day group were randomly selected for examination of their uterine contents. On day 20 of gestation, these pregnant female rats were sacrificed, necropsied, and the reproductive tract complete with ovaries was examined for number of corpora lutea in each ovary, number of implantation sites, number of resorption sites (classified as early or late), and number and distribution of fetuses in each uterine horn. Each fetus was weighed, sexed, and examined for external abnormalities. Individual placental weights and placental abnormalities were noted. Approximately one-half of the fetuses of each litter were submitted to an internal examination of the neck and the thoracic and abdominal cavities followed by evisceration and fixation. Remaining fetuses in each litter were processed and examined for visceral abnormalities. Eviscerated fetuses were processed and examined for skeletal abnormalities. Remaining F_0 dams in each group were allowed to deliver their offspring and rear them until day 25 postpartum. From day 20 of gestation, pregnant F_0 dams were observed two to three times per day for onset, progress, and completion of parturition. Gestation lengths were determined for each F_0 dam. The body weight of each F_0 dam was measured on days 1, 4, 7, 11, 14, 18, 21, and 25 postpartum. F_1 offspring were examined at 24 hr after birth and the following were recorded for each litter: number born (live and dead), individual body weights of live offspring, individual sexes, and observation on individual offspring. Litters were monitored daily for potential illness, clinical signs of toxicity, and mortality. On day 4 postpartum, litters were reduced to 4 F_1 pups/sex/litter when possible. Culled F_1 offspring were discarded without further examination. F_1 pups were sexed on days 1, 4 (before and after culling), 14, and 25 postpartum. Body weights of individual F_1 pups were determined on days 1, 4 (before culling), 7, 11, 14, 18, 21, and 25 postpartum, and weekly thereafter. Physical development of F_1 pups was assessed on a whole litter basis by recording the days of onset and completion of the following parameters: pinna unfolding, hair growth, testis descent, tooth eruption, eye opening, and vaginal opening. F_0 dams, allowed to deliver and rear their offspring, were sacrificed after day 25 postpartum and examined for external and internal macroscopic abnormalities as well as number of implantation sites. On day 25 postpartum, auditory and visual responses of F_1 pups were determined. Locomotor activity of male and female F_1 pups was determined for a 12 hr over the night of day 26/27. A water-filled Y-maze was used to evaluate the learning ability of F_1 pups on day 27 postpartum. Neuromuscular function (i.e., traversing flat and round rods, rotarod treadmill, mid-air righting reflex, fore- and hind-limb wire-hanging, and grid-gripping ability) of F_1 pups was assessed from days 28 to 30 postpartum. At 5 weeks postpartum, 20 F_1 pups/sex/group were randomly selected to form the F_1 generation (2 F_1 pups/sex/litter when possible). These animals were used for assessment of physical and sexual maturation and reproductive performance. F_1 pups not selected were sacrificed at 8 weeks of age and examined for macroscopic abnormalities. Body weights for F_1 male rats were measured weekly until termination. Body weights for F_1 female rats were measured weekly until detection of mating and on days 0, 6, 13, and 20 of gestation. At 9 to 10 weeks of age, F_1 male and female rats were paired on a one to one basis within treatment groups. The time

elapsing between initial pairing and detection of mating was noted. Pregnant F_1 dams were sacrificed on day 20 of gestation for examination of their uterine contents as described earlier for F_0 dams. F_2 fetuses were examined for external abnormalities and discarded. F1 male rats were sacrificed and examined for external and internal gross abnormalities.

Results:

1. **Observed Effects for F_0 Dams:** One dam at 10 mg/kg/day and 2 dams at 25 mg/kg/day displayed signs of pallor during the treatment period.

2. **Mortality of F_0 Dams:** One female rat at 25 mg/kg/day was sacrificed on day 16 of gestation. Observed effects included pallor, pale eyes, and bleeding from the vagina. External observations included fur matted and stained red around peri-genital area, and red staining on nose, ears, limbs, and tail. Internal examination revealed 14 fetuses *in utero* and 2 early resorptions.

3. **Body Weight and Food Consumption for F_0 Dams:** There were no treatment-related effects on body weight gain or food consumption from days 7 to 18 of gestation, or on body weight gain from days 1 to 25 postpartum. Body weights for female controls on days 7 and 18 were 291 and 397 g, respectively. Body weight gains for dams at 5, 10, and 25 mg/kg/day from days 7 to 18 of gestation were 103, 103.9, and 99.4% of the control, respectively. Body weights of female controls on days 1 and 25 postpartum were 323 and 336 g, respectively. Body weight gains for dams at 5, 10, and 25 mg/kg/day from days 1 to 25 postpartum were 82.6, 76.9, and 98.2% of the control, respectively.

4. **Litter Parameters for F_0 Dams Sacrificed on Day 20 of Gestation:** There were no treatment-related effects on numbers of corpora lutea/dam, implantations/dam, or viable fetuses/dam. There were no treatment-related effects on pre-implantation loss, fetal weight, or placental weight. Early resorptions and post-implantation loss were increased at 25 mg/kg/day. The sponsor attributed this change to a single litter with 10 early resorptions.

Litter Parameters for F_0 Dams Sacrificed on Day 20 of Gestation

Parameter	0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	25 mg/kg/day
Number of pregnant animals	21	21	21	26
Corpora lutea/dam	16.9	17.3	17.7	17.8
Implantations/dam	16.2	16.2	16.5	16.9
Viable fetuses/dam	15.5	15.4	15.6	15.3
Resorptions -early -late -total	 0.67 0.10 0.76	 0.76 0 0.76	 0.71 0.24 0.95	 1.54 0.04 1.58
Pre-implantation loss, %	5.5	6.6	6.5	5.2
Post-implantation loss, %	4.7	4.7	5.8	9.3
Fetal weight, g	3.79	3.80	3.76	3.80
Placental weight, g	0.52	0.53	0.53	0.55

5. External, Internal, Visceral, and Skeletal Examinations of F_1 Fetuses: External examination of F_1 fetuses found that the incidences of amniotic sac tinged green and green rim to placenta were increased at 25 mg/kg/day. There were no treatment-related internal, visceral, or skeletal abnormalities.

External Examination of F_1 Fetuses. Expressed as % Incidence/Number of Litters.

Parameters	0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	25 mg/kg/day
Fetuses/Litters	325/21	324/21	327/21	398/26
Amniotic sac tinged green	0	0	0	3.3%/2
Large placenta (>0.70 g)	1.8%/4	2.8%/5	3.4%/8	4.5%/6
Small placenta (<0.35 g)	0	0.3%/1	0.3%/1	0.5%/1
Green rim to placenta	0	0	0	2.3%/1
Clotted blood around placenta	0	0	1.2%/1	0

Internal Examination of F_1 Fetuses. Expressed as % Incidence/Number of Litters.

Parameters	0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	25 mg/kg/day
Fetuses/Litters	169/21	167/21	168/21	205/26
Unilateral hydronephrosis	0	0.6/1	1.2/2	0.5/1
Both kidneys reduced in size	0	0	0.6/1	0

Visceral Examination of F_1 Fetuses. Expressed as % Incidence/Number of Litters.

Parameters	0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	25 mg/kg/day
Fetuses/Litters	156/21	157/21	159/21	193/26
Head				
Hemorrhage between dorsal brain/superior colliculi and pia mater	0.6%/1	0.6%/1	0.6%/1	1.0%/2
Thorax and abdomen				
Lobe of thyroid gland slightly reduced in size	0	0.6%/1	0.6%/1	0.5%/1
Blood-filled thoracic lymph duct	0	0	0	0.5%/1
Hemorrhage within sheath of umbilical vein	0	0	0	0.5%/1
Hemorrhagic abdomen	0	0.6%/1	0.6%/1	0
Bilateral hydroyreter	0.6%/1	4.5%/5	1.3%/2	2.1%/4

Skeletal Examination of F_1 Fetuses. Expressed as % Incidence/Number of Litters.

Parameters	0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	25 mg/kg/day
Fetuses/Litters	169/21	167/21	168/21	205/26
Head				
Incomplete ossification of palatine bones	0	0	0	0.5%/1
Incomplete ossification of jugal bone	0	0	0	0.5%/1
≥ 1 incisors ossified	1.2%/3	2.4%/3	3.6%/5	3.9%/3
Sternebrae and ribs				
Incomplete ossification of 3 sternebrae	4.7%/7	12.0%/12	11.9%/11	14.1%/12
≥ 1 sternebrae offset	0	0.6%/1	0.6%/1	1.5%/3
14th rib enlarged	0	0	0.6%/1	1.0%/1
Vertebrae				
Incomplete ossification of 2nd sacral vertebral arch	0.6%/1	0	0.6%/1	0.5%/1
Limbs and girdles				
Metacarpals and/or metatarsals incompletely ossified or unossified	0	0.6%/1	1.8%/3	2.4%/4
One ischial bone incompletely ossified	0	0	0	0.5%/1

6. **Litter Parameters for F_0 Dams Allowed to Deliver Their Offspring**: There were no treatment-related effects on length of gestation or gestation index. Necropsy examinations of F_0 dams allowed to deliver their offspring and sacrificed after day 25 postpartum found no treatment-related effects.

Gestation length and Index.

Parameters	0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	25 mg/kg/day
Number of pregnant animals	11	11	11	13
Number of live litters born	11	11	11	13
Gestation Index, %	100	100	100	100

7. **Viability, Growth, and Development of F_1 Pups**: There were no treatment-related effects on implantation sites/dam, the post-implantation survival index, the birth index, the live birth index, the viability index on day 4 postpartum, or the lactation index on day 25 postpartum. There were no treatment-related effects on body weight or the male to female pup ratio through day 25 postpartum. There were no treatment-related effects on offspring development (i.e., pinna unfolding, hair growth, testes descent, tooth eruption, eye opening, or vaginal opening). There were no treatment-related effects on auditory and responses (i.e., normal auditory startle response, normal visual placing response, normal pupil closure response). Locomotor activity and performance in the water maze test were unaffected by treatment. Neuromuscular function, as assessed with stationary rods, rotarod treadmill, grid gripping, wire hanging, and mid-air righting reflex, were unaffected by treatment. Body weights of F_1 offspring from weeks 5 to 8 were unaffected by treatment. Necropsy examinations of F_1 offspring that died before termination or were sacrificed at scheduled termination after week 8 found no treatment-related effects.

F_1 Offspring Survival Indices and Body Weight.

Parameter	0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	25 mg/kg/day
Implantation sites/dam	16.4	16.9	15.1	16.8
Total pups/dam on day 1	14.9	15.3	14.0	14.8
Viable pups/dam on day 1	14.9	15.3	13.8	14.8
Post-implantation survival index, %	91 (14.9/16.4)	90 (15.3/16.9)	93 (14.0/15.1)	89 (14.8/16.8)
Birth Index, %	91 (14.9/16.4)	90 (15.3/16.9)	92 (13.8/15.1)	89 (14.8/16.8)
Live Birth Index, %	100 (14.9/14.9)	100 (15.3/15.3)	99 (13.8/14.0)	100 (14.8/14.8)
Viability Index, % day 4 postpartum	98 (14.5/14.9)	100 (15.3/15.3)	100 (13.8/13.8)	99 (14.7/14.8)
Lactation Index, % on day 25 postpartum	99 (7.9/8.0)	100 (8.0/8.0)	100 (7.8/7.8)	100 (8.0/8.0)
Body weights, g (M/F)				
-day 1	6.7/6.3	6.8/6.4	6.7/6.3	6.9/6.5
-day 4 before cull	9.4/8.8	9.8/9.1	9.8/9.4	9.6/9.3
-day 4 after cull	9.6/9.1	10.0/9.3	9.8/9.5	9.8/9.3
-day 25 after cull	78.5/73.7	81.0/75.7	80.8/76.5	79.8/75.5
Male/Female Pups (Ratio)				
-Total on day 1 postpartum	86/78	92/76	73/81	94/99
-# Alive on day 1 postpartum	86/78	92/76	71/81	94/99
-# Alive on day 4 before cull	85/75	92/76	71/81	92/97
-# Alive on day 4 after cull	44/44	44/44	42/44	51/53
-# Alive on day 25 after cull	43/44	44/44	42/44	51/53

Necropsy Findings for F_1 Offspring Sacrificed at Scheduled Termination after Week 8. Expressed as % Incidence/Number of Litters.

Parameter	0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	25 mg/kg/day
Offspring/Litters	47/11	48/11	46/11	64/13
Pale or raised white areas on surface of spleen	2.1%/1	8.3%/3	0	4.7%/2
Clotted blood in abdomen	0	0	0	1.6%/1

8.- **F_1 Generation Selected for Assessment of Sexual Maturity, Fertility, and Reproductive Performance**: Body weights for F_1 male and female offspring from weeks 5 to 13 were unaffected by treatment of F_0 dams. Duration of the mating periods for F_1 offspring were unaffected by treatment of F_0 dams. Fertility and mating performance in the F_1 generation were unaffected by treatment of F_0 females. The distribution of copulatory plugs in treatment groups was different from that observed in the control group; however, estimated sperm counts from vaginal smears at mating were not different between control and treatment groups. Body weight gains of F_1 female rats during gestation were unaffected by treatment of F_0 female rats. Necropsy examination of F_1 dams on day 20 of gestation revealed no macroscopic abnormalities. There were no treatment-related effects on numbers of corpora lutea/dam, or resorptions/dam. Implantations/dam and viable fetuses/dam were slightly decreased at 25 mg/kg/day. There were no treatment-related effects on pre-implantation loss, post-implantation loss, fetal weight, or placental weight. External examination of F_2 fetuses found no treatment-related effects. Necropsy examination of F_1 male rats found no treatment-related changes.

Mating Performance and Fertility of the F_1 Generation

Parameter	0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	25 mg/kg/day
# Males/Females paired	20/20	20/20	20/20	20/20
#Males mating	19	20	20	20
#Females mating	20	20	20	20
#Males producing pregnancy	19	19	20	20
#Females achieving pregnancy	20	19	20	20
% Males Mating	95	100	100	100
% Females Mating	100	100	100	100
Males-Conception Rate, %	100	95	100	100
Females-Conception Rate, %	100	95	100	100
Males-Fertility Rate, %	95	95	100	100
Females-Fertility Rate, %	100	95	100	100

Group mean litter data for F_1 pregnant female rats sacrificed on day 20 of gestation.

Parameter	0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	25 mg/kg/day
Number of pregnant animals	20	19	20	20
Corpora lutea/dam	18.7	17.3	18.1	17.8
Implantations/dam	17.1	16.0	16.4	15.6
Viable fetuses/dam	16.0	15.3	15.5	14.6
Resorptions				
-early	1.00	0.68	0.90	1.0
-late	0.05	0.05	0.00	0
-total	1.05	0.74	0.90	1.0
Pre-implantation loss, %	9.3	7.9	10.1	12.6
Post-implantation loss, %	6.2	4.6	5,5	6.4
Fetal weight, g	3.55	3.64	3.52	3.75
Placental weight, g	0.48	0.50	0.50	0.53

External Examination of F_2 Fetuses. Data expressed as %Incidence/number of litters.

Parameter	0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	25 mg/kg/day
Fetuses/Litters	320/20	290/19	310/20	291/20
Large fetus (>4.10 g)	3.8%/5	4.8%/5	3.9%/4	12.4%/11
Clotted blood around placenta	0	2.1%/2	0	1.4%/1
Green/black edge to placenta	0	0	1.3%/1	0
Conjoined placenta	0	0.3%/1	0.6%/1	0

Necropsy examination of F_1 male rats following sacrifice of F_1 female rats on day 20 of gestation (n = 20/group).

Parameter	0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	25 mg/kg/day
Unilateral hydronephrosis	1	1	2	3

In an intravenous Segment II teratology study, pregnant female Sprague-Dawley rats received tinzaparin at doses of 0, 5, 10, or 25 mg/kg/day from days 7 to 17 of gestation. Tinzaparin at intravenous doses ≤25 mg/kg/day was not teratogenic in rats. One pregnant F_0 female rat at 25 mg/kg/day was sacrificed in moribund condition during the treatment period. External examination of F_1 fetuses found that the incidences of amniotic sac tinged green and green rim to placenta were increased at 25 mg/kg/day. There were no treatment-related internal, visceral, or skeletal abnormalities found with F_1 fetuses. Viability, growth, and development of the F_1 generation was unaffected by treatment of F_0 dams.

Segment II: Subcutaneous Teratology Study in the Rat (LSR Report #88/NLP025/100).

Conducting Lab: [redacted]

Dates of Conduct: Initiated 4/12/87, completed 9/20/87.

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

Chemical: LHN-1, lot #s BN 100486, 120487, and Heparin lot # 150487.

Animals: Pregnant Rats, Sprague-Dawley, [redacted], 32/dose group.

Doses: 0 (control vehicle), 4, 10, and 25 mg/kg/day LHN-I, and Heparin at 12.5 mg/kg/d, given subcutaneously in 0.5 mL/kg volume, at different sites, once daily on days 6 through 17 of gestation. Doses were selected from the 87/NLP022/460 study the results of which were not provided.

Methods: The test agent or control vehicle was administered to the dams once daily on days 6 through 17 of gestation as mentioned above. Dams were observed daily for changes in behavior and any clinical abnormal findings. Body weights were recorded on days 0, 3, 6-18, and 20 of gestation until autopsied on day 20 and their uteri examined. Food consumption was recorded on days 3, 6, 10, 13, 17, and 20 of gestation. Following C-section of the 20 dams/dose group (control, LHN treatment) and 21 dams in the Heparin group, individual fetal weights and sex were recorded, and so were the implantation sites in uteri, and gross, skeletal, and visceral malformations/ variations. For skeletal and visceral malformations, 50% of each litter was examined for skeletal (by Alizarin technique) and 50% for visceral anomalies by Bouin technique. The remaining dams in each group (11-10/group) were allowed to deliver naturally and allowed to rear their young until day 25 postpartum. The fetuses (F_1) were weighed, sexed, and their development recorded including behavioral, auditory, visual, neuromuscular and reproductive function by a standard battery of tests mentioned in the Segment I study above.

Results: During the study there were no drug-related deaths. The most common finding was hematomas at injection sites in 4 of 32 high dose LHN-1, and 6 of 32 Heparin group dams. One to two rats in the mid and high dose had to be sacrificed due to hematomas.

The body weight gain of the treated dams throughout gestation was not affected adversely when compared to controls. Food consumption in the Heparin group and not in the LHN-1 groups, was slightly but significantly reduced.

The fetal weights decreased at 4 mg/kg/d but not at the higher doses.

There were no changes in the following reproductive parameters: number of corpora lutea, implantations, litter size, resorptions, pregnancy rate, sex ratio, and # of live/dead fetuses.

The incidence of incomplete ossification of the supra-occipital bones and 3/4 metacarpals/metatarsals in the 4 mg/kg/d group was higher when compared to the historical controls, but not when compared to the concurrent controls. No such increases occurred with higher doses. The incidence of other skeletal or visceral abnormalities/malformations in the treated rats was not significantly higher than in the controls. In the postnatal period, both the physical development and reproductive performance of F_1 offspring were normal.

In summary, the treatment with LHN-1 at doses of up to 25 mg/kg/d and Heparin at 12.5 mg/kg/d did not produce any teratogenic effects in the rat. The other effects such as incomplete ossification or decreased fetal weights in the lowest dose but not in the higher doses were not of any significance. The no-toxic (teratogenic) dose was 25 mg/kg/d for the rat.

Addendum:

Testing Laboratory: [redacted]

Drug Batch: Tinzaparin bulk drug batch F668A ([redacted] anti-Factor Xa IU/mg) was supplied as solutions in vials that contained sodium metabisulfite. The following lot numbers were used: BN100487, BN110487, and BN120487.

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

Dose Range Finding Study (LSR Report No.: 87/NLP022/460): Tinzaparin was administered by the subcutaneous route to pregnant female rats at doses of 0, 10, 50 or 100 mg/kg/day from days 6 to 17 of gestation. A positive control group received heparin by the subcutaneous route at 50 mg/kg/day. Dams were sacrificed on day 20 of gestation for examination of their uterine contents. For tinzaparin-treated groups, mortality occurred for 1 of 6 dams at 50 mg/kg/day and 4 of 6 dams at 100 mg/kg/day. For the heparin treatment group, 4 of 6 dams died. The incidence of hematomas and hemorrhagic areas at injection site(s) was increased in all treatment groups. For tinzaparin treatment groups, there were no effects on the number of corpora lutea/dam, number of implantations/dam, number of viable fetuses/dam, number of resorptions/dam, pre-implantation loss, post-implantation loss, fetal body weight, or placental weight; however, due to the high mortality rate at 100 mg/kg/day, only 28 fetuses were obtained as compared to 89 for the control.

Mortality: One female at 4 mg/kg/day was sacrificed on day 17 of gestation due to a gastrointestinal abnormality (i.e., esophagus swollen with food and fur at the level of the thoracic cavity) considered to be unrelated to treatment. One heparin-treated female rat was sacrificed on day 17 of gestation due to severe bleeding from the injection site.

Litter Data for F_0 Female Rats Sacrificed on Day 20 of Gestation: Pre-implantation loss at 25 mg/kg/day was increased to 16.1% as compared to 9.6% of the control. The incidence of subcutaneous hemorrhage, hematomas, and hemorrhagic areas at ≥1 injection site(s) was increased in all treatment groups.

Group mean litter data for F_0 pregnant dams killed on day 20 of gestation.

Parameter	0 mg/kg/day	4 mg/kg/day	10 mg/kg/day	25 mg/kg/day	Heparin
# pregnant rats	21	21	21	21	20
Corpora lutea/dam	16.3	16.1	16.9	17.2	16.6
Implantations/dam	14.8	14.6	15.1	14.4	15.6
Viable fetuses/dam	13.9	13.3	14.3	13.5	14.5
Resorptions					
-early	0.67	1.14	0.67	0.67	0.95
-late	0.29	0.19	0.10	0.29	0.20
-total	0.95	1.33	0.76	0.95	1.15
Pre-implantation loss, %	9.6 (1.5/16.3)	9.4 (1.5/16.1)	10.5 (1.8/16.9)	16.1* (2.8/17.2)	6.0 (1/16.6)
Post-implantation loss, %	6.4	9.1	5.0	6.6	7.4
Fetal Body Weight, g	3.37	3.24	3.35	3.39	3.37
Placental Weight, g	0.52	0.54	0.52	0.57	0.53

Necropsy of F_0 dams on day 20 of gestation.

Finding	0 mg/kg/day	4 mg/kg/day	10 mg/kg/day	25 mg/kg/day	Heparin
N =	21	21	21	21	20
Subcutaneous hemorrhage, hematoma, or hemorrhagic area at ≥ 1 injection sites	0	8	12	15	19

External, Internal, Visceral, and Skeletal Examination of F_1 Fetuses: There were no treatment-related external, internal, visceral, or skeletal abnormalities in F_1 fetuses.

External Examination of F_1 Fetuses. Data expressed as % Incidence/Number of Litters.

Parameter	0 mg/kg/day	4 mg/kg/day	10 mg/kg/day	25 mg/kg/day	Heparin
Fetuses/Litters	291/21	279/21	301/21	283/21	289/20
Large Placenta (>0.70 g)	0.7%/2	5.4%/6	1.3%/4	2.8%/4	2.1%/4

Visceral Examination of F_1 Fetuses. Data expressed as % Incidence/Number of Litters.

Parameter	0 mg/kg/day	4 mg/kg/day	10 mg/kg/day	25 mg/kg/day	Heparin
Fetuses/Litters	142/21	138/21	149/21	143/21	141/20
Head					
Hemorrhage within meninges in region of superior colliculi	0	0	0	0.7%/1	0
Increased dilatation of lateral ventricles	0	0	2.0%/3	0.7%/1	0
Unilateral slightly folded retina; slight internal hydrocephaly; lungs appear slightly immature	0	0	0	0.7%/1	0
Thorax					
Right lobe of thyroid gland very reduced in size/absent	0	0	0	0.7%/1	0
Blood-filled thoracic lymph duct	0	0.7%/1	1.3%/2	1.4%/2	0
No innominate artery	0	0	0	0.7%/1	0
Abdomen					
Hemorrhagic peritoneal fluid	2.1%/3	0.7%/1	0.7%/1	3.5%/2	0.7%/1
Hemorrhagic abdomen	1.4%/1	0.7%/1	0.7%/1	2.8%/4	0.7%/1
Papilla of left kidney misshapen	0	0	0	0.7%/1	0
Bilateral hydronephrosis	0	1.4%/2	0	0.7%/1	0
Bilateral hydroureter	1.4%/1	4.3%/3	2%/3	4.2%/5	0.7%/1
Blood in anus	0	1.4%/2	0.7%/1	0.7%/1	0.7%/1

Skeletal Examination of F_1 Fetuses. Data expressed as % Incidence/Number of Litters.

Parameter	0 mg/kg/day	4 mg/kg/day	10 mg/kg/day	25 mg/kg/day	Heparin
Head					
Small anterior fontanelle	0.7%/1	0	0	4.3%/4	0.7%/1
Incomplete ossification of parietal bone	0.7%/1	1.4%/1	3.3%/3	2.1%/3	2.7%/3
Incomplete ossification of squamosal bone	0.7%/1	3.5%/3	2.0%/2	2.9%/4	1.4%/2
Fronto-nasal suture enlarged	0	0.7%/1	1.3%/2	2.1%/3	0.7%/1
Sternebrae and Ribs					
Ribs 14/14	0	2.1%/2	1.3%/2	0	0
Vertebrae, Limbs, and Girdles					
≥ 1 Phalangeal bones ossified	0.7%/1	0	1.3%/2	2.1%/2	2.0%/3
Pubic bones incompletely ossified or unossified	4.0%/6	7.8%/8	8.6%/8	11.4%/7	3.4%/5
Incomplete ossification of one or both ischial bones	0.7%/1	2.1%/2	2.0%/2	1.4%/2	0.7%/1

Litter Data for F_0 Female Rats Allowed to Deliver Their Offspring: For F_0 dams allowed to deliver their offspring, there were no treatment-related effects on gestation length or index. There were no treatment-related effects on pup viability.

Group mean litter data for F_0 pregnant dams allowed to deliver their offspring.

Parameter	0 mg/kg/day	4 mg/kg/day	10 mg/kg/day	25 mg/kg/day	Heparin
Implantation sites/dam	14.7	15.6	15.6	15.5	15.0
Total fetuses/dam, day 1	14.1	14.4	14.4	14.8	14.1
Viable fetuses/dam, day 1	13.8	14.3	14.2	14.7	13.2
Post-implantation Survival Index, %	95 (14.1/14.7)	92 (14.4/15.6)	92 (14.4/15.6)	87 (14.8/15.5)	94 (14.1/15.0)
Live Birth Index, %	98 (13.8/14.1)	99 (14.3/14.4)	99 (14.2/14.4)	99 (14.7/14.8)	94 (13.2/14.1)
Viability Index Day 4, %	93 (12.9/13.8)	92 (13.2/14.3)	88 (12.5/14.2)	92 (13.5/14.7)	90 (11.8/13.2)
Lactation Index on Day 25 Postpartum, %	97 (7.6/7.9)	95 (7.6/8.0)	83 (7.3/8.0)	96 (7.7/8.0)	94 (7.3/7.7)

Necropsy examination of F_0 Dams Allowed to Deliver Their Offspring.

Parameter	0 mg/kg/day	4 mg/kg/day	10 mg/kg/day	25 mg/kg/day	Heparin
N =	11	10	11	11	11
Subcutaneous hemorrhage/hematoma/ hemorrhagic area(s) at ≥ 1 injection sites	0	0	0	3	6

F_1 Offspring Growth and Development: There were no treatment-related effects on F_1 offspring body weight gain, development, auditory and visual responses, locomotor activity, learning ability, or neuromuscular function. There were no treatment-related effects on fertility or reproductive function in F_1 offspring. For F_1 dams at 25 mg/kg/day, sacrificed on day 20 of gestation, implantation sites/dam and viable fetuses/dam were decreased, and pre-implantation loss was increased. No changes in litter data were evident for tinzaparin-treated groups at 4 or 10 mg/kg/day and the heparin-treated group. There were no treatment-related findings in necropsy examinations of F_1 male and female offspring. External examination of F_2 fetuses found no treatment-related findings.

Group Mean Litter Data for F_1 Female Dams Killed on Day 20 of Gestation.

Parameter	0 mg/kg/day	4 mg/kg/day	10 mg/kg/day	25 mg/kg/day	Heparin
#Pregnant animals	18	19	20	19	20
Corpora lutea/dam	15.8	15.7	16.3	15.0	15.7
Implantations/dam	14.7	15.1	15.0	13.1	14.5
Viable fetuses/dam	13.9	14.3	14.2	12.4	13.9
Resorptions -early -late -total	 0.72 0.06 0.78	 0.68 0.05 0.74	 0.65 0.10 0.75	 0.53 0.16 0.68	 0.50 0.05 0.55
Pre-implantation loss, %	6.7	4.7	9.1	12.6	8.3
Post-implantation loss, %	5.3	4.9	5.0	5.2	3.8
Fetal body weight, g	3.22	3.33	3.27	3.33	3.30
Placental weight, g	0.51	0.51	0.52	0.55	0.51

External Examination of F_2 Fetuses. Data expressed as %Incidence/Number of Litters.

Parameter	0 mg/kg/day	4 mg/kg/day	10 mg/kg/day	25 mg/kg/day	Heparin
Fetuses/Litters	251/18	272/19	284/20	236/19	278/20
Thread-like tail	0	0	0.4%/1	0.4%/1	0
Large placenta (>0.70 g)	0.8%/2	0.7%/2	0	5.5%/4	0.7%/1
Conjoined placenta (1 viable fetus, 1 late resorption)	0	0	0	0.4%/1	0
Thickened placenta	0	0	0	1.3%/1	0

Rabbit

Segment II: Intravenous Teratology Study in the Rabbit (LSR Report No.: 92/NLP140/0183).

Testing Laboratory: [redacted]

Date Started: August 28, 1991

Date Completed: July 30, 1992

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

Animals: Pregnant female New Zealand White rabbits were used in this study. At the start of the study, animals were approximately 19-26 weeks of age and had a body weight range of 3.42-4.76 kg.

Drug Batch: Tinzaparin, Lot No. LMW 9101 ([redacted] anti-Factor Xa IU/mg).

Methods: In a Segment II intravenous teratology study, pregnant female rabbits received tinzaparin at doses of 5, 20, or 75 mg/kg/day (0, 400, 1700, and 6500 anti-Factor Xa IU/kg/day, respectively) from days 6 to 18 of gestation. Control animals received the vehicle, [redacted] The sponsor's dose selection was based upon a preliminary Segment II dose range finding study in which pregnant female rabbits received tinzaparin at doses of 0, 25, 50, 75, or 100 mg/kg/day from days 6 to 18 of gestation. On day 29 of gestation, animals were sacrificed and their content examined. One female at 100 mg/kg/day aborted on day 19 of gestation. In the present study, there were 15 pregnant rabbits/group. Vehicle or drug solution was administered by the intravenous route at a dose volume of 1 mL/kg. Animals were weighted and monitored for clinical signs of toxicity daily. Food consumption was measured during the following intervals: days 1-5, days 6-12, days 13-18, days 19-23, and days 24-28. On day 29 of gestation, animals were sacrificed and their uterine contents were examined as follows: number of corpora lutea in each ovary, number of implantation sites, number of resorption sites, and number and distribution of live and dead fetuses in each uterine horn. Fetuses were weighed, examined for external abnormalities, and sacrificed. Placental weights were also measured. The neck and thoracic and abdominal cavities of all fetuses from each litter were dissected and the contents were examined using a fresh microdissection technique. Sex of each fetus was determined. All fetuses in each litter were eviscerated and the heads of one-third of the fetuses in each litter were processed for examination following serial sectioning. Torsos and remaining intact fetuses were fixed. Eviscerated fetuses were processed for skeletal examination.

Results:

1. **Observed Effects**: Two pregnant female rabbits at 75 mg/kg/day (#911429 and #911469), spontaneously aborted on days 26 and 19, respectively. Examination of female #911429 found that this animal had 5 corpora lutea and 3 implantation sites. Examination of female #911469 found that this animal had 12 corpora lutea and 6 implantation sites. Fecal output was reduced for 5 rabbits at 20 mg/kg/day and 6 rabbits at 75 mg/kg/day during the treatment period.

2. **Mortality**: None.

3. **Body Weight and Food Consumption**: Body weight gains and food consumption were slightly reduced for rabbits at 20 and 75 mg/kg/day from days 6 to 18. The biological significance of these changes is questionable. Body weights of female controls on days 6 and 18 were 4.01 and 4.24 kg, respectively, yielding a 5.7% increase of body weight at day 6. For female rabbits at 5, 20, and 75 mg/kg/day, body weights on day 18 were increased by 5.4, 4.4, and 4.0%, respectively, of body weights on day 6. Food consumption for female rabbits at 5, 20, and 75 mg/kg/day from days 6 to 12 were decreased to 93.6, 89.4, and 90.4% of the control (188 g/rabbit/day), respectively. Food consumption for female rabbits at 20 and 75 mg/kg/day from days 13 to 18 were decreased to 92.1 and 87.6% of the control (178 g/rabbit/day), respectively.

4. **Litter Data for Pregnant Female Rabbits Sacrificed on Day 29 of Gestation**: There were no treatment-related effects on number of corpora lutea/dam, number of implantation sites/dam, number of viable fetuses/dam, number of resorptions dam, pre-implantation loss, post-implantation loss, fetal body weight, or placental weight.

Group mean litter data for pregnant female rabbits sacrificed on day 29 of gestation following treatment with tinzaparin by the intravenous route at doses of 0, 5, 20, and 75 mg/kg/day from days 6 to 18 of gestation.

Parameter	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	75 mg/kg/day
#Pregnant animals	14	14	14	15
% Abortions	0	0	0	13.3
Corpora lutea/dam	10.8	9.0	9.7	10.1
Implantation sites/dam	9.1	7.6	7.6	8.7
Viable fetuses/dam	8.0	7.3	7.1	8.4
Resorptions/dam				
-early	0.6	0.4	0.4	0.1
-late	0.5	0	0.1	0.2
-total	1.1	0.4	0.5	0.3
Pre-implantation loss, %	15.2	15.1	21.9	14.4
Post-implantation loss, %	12.5	4.7	6.5	3.5
Fetal body weight, g	41.9	44.4	44.4	41.3
Placenta weight, g	5.7	5.8	6.2	6.1

5. **External and Skeletal Examinations of Fetuses and Visceral Examinations of Fetal Heads**: The incidences of small fetuses were increased at 20 and 75 mg/kg/day. There were no treatment-related findings for skeletal examinations of fetuses and visceral examinations of fetal heads. A complete visceral examination of the contents of neck and thoracic and abdominal cavities was conducted using a fresh microdissection technique.

External/Visceral Examination of Fetuses. Data Expressed as % Incidence/Number of Litters.

Parameter	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	75 mg/kg/day
Fetuses/Litters	112/14	102/14	100/14	109/13
Pale area on liver	0	0	0	0.9%/1
Small fetus (<32.0 g)	4.5%/5	5.9%/2	13.0%/4	16.5%/5
Amniotic sac tinged yellow	0	0	0	2.8%/1

Skeletal Examination of Fetuses. Data Expressed as % Incidence/Number of Litters.

Parameter	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	75 mg/kg/day
Head				
Fetuses/Litters	78/14	70/14	70/14	77/13
Extra small anterior fontanelle, negligible size of suture lines only	0	0	1.4%/1	0
Medium anterior fontanelle	21.8%/6	24.3%/8	25.7%/9	41.6%/10
Posterior fontanelle enlarged	3.8%/3	7.1%/3	8.6%/3	19.5%/6
Incomplete ossification of interparietal bone	0	0	1.4%/1	0
Additional suture in parietal bone	0	0	0	1.3%/1
Irregular ossification of frontal suture	7.7%/4	5.7%/4	7.1%/2	16.9%/8
Additional plaque of bone in frontal suture at fronto-nasal junction	0	1.4%/1	0	1.3%/1
Additional plaque of bone in nasal suture	0	0	1.4%/1	1.3%/1
Incomplete ossification of 1st cervical vertebral centrum	0	0	1.4%/1	3.9%/2
Sternebrae and Ribs				
Fetuses/Litters	112/14	102/14	100/14	109/13
Incomplete ossification of 2 sternebrae	2.7%/3	2.9%/2	2.0%/2	4.6%/4
Incomplete ossification of 3 sternebrae	0	0	1.0%/1	0
One or more sternebrae offset	6.3%/6	2.0%/2	5.0%/3	9.2%/6
Rudimentary 13th rib	1.8%/2	1.0%/1	0	2.8%/2
Vertebrae, Limbs, and Girdles				
Fetuses/Litters	112/14	102/14	100/14	109/13
27 pre-sacral vertebrae	8.0%/6	16.7%/7	11.0%/7	21.1%/9
Incomplete ossification of heads of limb long-bones	42.9%/13	41.2%/11	40.0%/10	57.8%/12
Centrales incompletely ossified	0.9%/1	1.0%/1	3.0%/3	3.7%/3
Double association pelvis, ilial bones associated with both sacral vertebrae	0.9%/1	1.0%/1	0	1.8%/2

Examination of Fetal Heads. Data Expressed as % Incidence/Number of Litters.

Parameter	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	75 mg/kg/day
Fetuses/Litters	34/14	32/14	30/14	32/14
Unilateral slightly folded retina	0	3.1%/1	6.7%/2	9.4%/3
Blood on tongue/in mouth/nasal sinus/nasopharynx	2.9%/1	0	6.7%/2	6.3%/2

In an intravenous Segment II teratology study, pregnant female rabbits received tinzaparin at doses of 5, 20, or 75 mg/kg/day from days 6 to 18 of gestation. Two pregnant female rabbits at 75 mg/kg/day spontaneously aborted on days 26 and 19, respectively. Tinzaparin at intravenous doses ≤75 mg/kg/day was not teratogenic in rabbits.

Segment II: (First) Teratology Study in the Rabbit (LSR Report # 88/NLP063/360).

Conducting Lab: [redacted]

Dates of Conduct: Initiated 1/13/88, completed 4/20/88.

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

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

Animals: Pregnant Rabbits, New Zealand white rabbits; 15/dose group for LHN-1 and 16/dose for Heparin.

Doses: 0 (control vehicle), 4, 10, and 25 mg/kg/day LHN-1, and Heparin at 12.5 mg/kg/d, given subcutaneously in 0.5 mL/kg volume, at different sites, once daily on days 6 through 19 of gestation. These doses were selected from the Exploratory Teratology Study in the Rabbit (89-NLP082/063) using 6 pregnant rabbits/dose given subcutaneous LHN-1 at 25 mg/kg/d (lot # 120487 [batch # F668A], another group of 6 rabbits also given 25 mg/kg/d (lot 825731 [batch F682X], and 50 mg/kg/d (lot 825732 [batch # F682X]), and Heparin 25 mg/kg/d (lot 825733) on days 6-19 of gestation. There was some maternal body weight loss during gestation, and some fetal weight loss at 25 and 50 mg/kg/d of LHN-1, but not significant from the controls. Similar changes in maternal and fetal weights, and increased pre- & post-implantation loss were seen with Heparin. There were acute inflammatory cells in the placental myometrium of 50 mg/kg/d LHN-1 and in the Heparin group rabbits. No such effects were seen in the 25 mg/kg/d group. There were no malformations at any dose. Therefore, 25 mg/kg/d was selected as the highest dose for the main teratology study.

Methods: The dams were given the vehicle or the test agent once daily on days 6 through 19 of gestation. The dams were observed for morbidity and mortality (and standard observations of general appearance, body weights, food intake and abortions) daily throughout the pregnancy until day 29 when they were killed and their uteri examined for implantation sites. The blood samples were collected 24-hr post-dosing on days 6, 19 and 28 for determining packed cell volume, Hb concentration, and erythrocyte counts. The following parameters were recorded: pregnancy rates, number

of animals aborted, implantation sites, number and distribution of live/dead fetuses, intrauterine live/dead embryos, corpora lutea and ovarian weight. Fetal examinations consisted of fetal/placental weights and sex ratio, abnormal litter ratio, malformation rate, and ossification variance. One-third of the fetuses from each litter were examined for visceral and 1/3 for skeletal anomalies (by Bouin and Alizarin's techniques). The remainder 1/3 fetuses' heads were examined for abnormalities by serial sectioning. Detailed histopathology of the rabbits that died during the study was also performed. The mean fetal weight, placental weight, post-implantation loss, and sex ratios were analyzed on the fetus basis.

Results: There were 4 deaths during the study: one rabbit in each group, low, mid LHN-1 group, and Heparin group died or were killed during the study. The main findings in these animals were: weight loss, prostration, and hemorrhage at various sites including the injection sites.

Maternal body weight gain of the high dose LHN-1 and Heparin group rabbits was somewhat less than the controls throughout the study including in the post-dosing period. However, it was stated that the controls also gained weight somewhat less than the historical controls. Food intake paralleled the body weight gain throughout gestation.

Findings at Autopsy: Significant finding at necropsy was increased incidence of abortions, pre-term delivery and total litter loss. The number of resorptions were: 2, 1, 0, 1, 0; premature births were: 0, 1, 1, 1, and I in the control, 4, 10, and 25 mg/kg/d LHN-I and Heparin groups, respectively. In addition, there were 5 abortions in the high dose LHN-1 group. Thus, the incidence of abortion/premature delivery in the high dose group was about 43%, much higher than seen before ($p \leq 0.05$), the background control being 2.2%

The incidence of malformations and skeletal variations was not significantly higher in any group when compared to controls, even though there was some delayed ossification noticed in the 25 mg/kg/d group, but that was due to one large litter with small fetuses (possibly a fetal immaturity).

The other parameters like the number of pre- and post-implantation losses, mean placental weights, fetal weights etc. were not significantly affected by the treatment with LHN-1 or Heparin.

In summary, the treatment of rabbits with LHN-1 at doses of 4, 10, and 25 mg/kg/d LHN-1 and Heparin at 12.5 mg/kg/d produced significant maternal toxicity (maternal weight loss, and high incidence of abortions/premature delivery at high dose) and fetal toxicity (some decreased fetal weight). However, the treatment with LHN-1 did not produce any teratogenic effects in the rabbits. Nevertheless, the sponsor conducted another teratology study with the rabbit to determine if LHN-1 had any teratogenic potential.

Addendum:

Testing Laboratory: [redacted]

Drug Batch: Tinzaparin bulk drug batch F668A ([redacted] anti-Factor Xa IU/mg) was supplied as solutions containing sodium metabisulfite. The following lot numbers were used: 100487, 110487, and 120487.

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

Dose Selection: The sponsor's dose selection was based upon a preliminary Segment II dose range finding study in which pregnant female rabbits received tinzaparin by the subcutaneous route at doses of 0, 10, or 25 mg/kg/day from days 6 to 19 of gestation (LSR Report No.: 87/NLP028/932). A positive control group received heparin at 12.5 mg/kg/day. One female receiving tinzaparin at 10 mg/kg/day and one female receiving heparin at 12.5 mg/kg/day aborted during the study. There were no effects on number of corpora lutea, number of implantation sites, number of viable fetuses/dam, number of resorptions, pre-implantation loss, post-implantation loss, fetal body weight, or placental weight. External examination of fetuses found that the incidence of small fetuses (<32.0 g) was increased for tinzaparin at 10 and 25 mg/kg/day and heparin.

Mortality: One female (#1041) at 4 mg/kg/day was sacrificed in a moribund condition on day 24 of gestation. Necropsy examination revealed free blood in the uterus. One female (#1135) at 10 mg/kg/day was sacrificed in a moribund condition on day 28 of gestation. This animal was observed with hemorrhage into the anterior chamber of the left eye. Necropsy examination found subcutaneous hemorrhages underlying the treatment site. One female (#1073) that received heparin was found dead on day 11 of gestation. Hemorrhage was associated with injection sites. Pallor and hemorrhage were observed in abdominal organs.

Disposition of Animals: For tinzaparin at 25 mg/kg/day, five female rabbits (#895, day 23; #1006, day 22; #1040, day 29; #1047, day 28; #1092, day 21) were observed with spontaneous abortions and one female rabbit was observed with a premature delivery (#1102, Aborted/Premature delivery).

Disposition of female rabbits treated with tinzaparin or the positive control, heparin.

Parameter	0 mg/kg/day	4 mg/kg/day	10 mg/kg/day	25 mg/kg/day	Heparin
#Animals	15	15	15	15	16
Mortality	0	1	1	0	1
Not Pregnant	2	0	2	1	1
Total litter loss	2	1	0	1	0
Abortion	0	0	0	5	0
Premature delivery	0	1	1	1	1
Pregnant to term with viable young	11	12	11	7	13

Body Weight Gain and Food Consumption: Body weight gain was suppressed in all tinzaparin groups as well as the control group during and after the treatment period. Food consumption was decreased in tinzaparin-treated groups; although, it displayed no relationship to dose. Body weights for female controls on days 6 and 20 were 4.70 and 4.67 kg, respectively, yielding a 0.6% decrease in body weight from day 6. For tinzaparin-treated groups at 4, 10, and 25 mg/kg/day, body weights on day 20 were decreased by 2, 1.8, and 0.9%, respectively, of body weights on day 6. From days 20 to 28 of gestation, the mean control body weight increased by 0.08 kg. For tinzaparin-treated groups at 4, 10, and 25 mg/kg/day, body weights from days 20 to 28 decreased by –0.02, -0.01, and –0.20 kg, respectively.

Litter Data for Female Rabbits Sacrificed on Day 29 of Gestation: There were no effects on number of corpora lutea, number of implantation sites, number of viable fetuses/dam, number of resorptions, pre-implantation loss, post-implantation loss, fetal body weight, or placental weight.

Litter Data for Pregnant Rabbits Sacrificed on Day 29 of Gestation.

Parameters	0 mg/kg/day	4 mg/kg/day	10 mg/kg/day	25 mg/kg/day	Heparin
#Pregnant Animals	13	14	12	14	14
% Abortions and Total Litter Loss	15.4	14.3	8.3	50	7.1
Corpora Lutea/Dam	11.3	10.4	10.8	11.7	11.3
Implantation Sites/Dam	7.8	8.8	7.9	7.9	9.5
Viable Fetuses/Dam	6.6	7.3	7.5	7.0	8.3
Resorptions -early -late -total	 0.3 0.9 1.2	 0.5 1.0 1.5	 0.2 0.3 0.5	 0.6 0.3 0.9	 0.5 0.8 1.3
Pre-implantation loss, %	30.6	16.7	26.9	32.9	15.6
Post-implantation loss, %	15.1	17.1	5.7	10.9	12.9
Fetal body weight, g	40.2	34.4	34.3	32.9	36.1
Placental weight, g	5.5	4.8	5.3	5.6	5.4

External and Skeletal Examinations of Fetuses and Visceral Examinations of Fetal Heads: External examination found that the incidences of small fetuses (<32.0 g) were increased for tinzaparin treatment groups. Skeletal examination found increased incidences of incompletely ossified/unossified for bones in the head, sternebrae and ribs, and vertebrae, limbs, and girdles for tinzaparin treatment groups. Examination of fetal heads found that tinzaparin at all dose levels increased the incidence of blood in cochlea(s). A complete visceral examination of the contents of neck and thoracic and abdominal cavities using a fresh microdissection technique was conducted.