sacrificed at 9 weeks of age; however, no historical data was included to support this claim. Changes in the heart were not observed for other studies with hirulog in rats; however, the sponsor neglected to include a positive control group receiving hirulog to allow an assessment of the importance of these results.

Hirulog: 28 Day Intravenous Toxicity Study in the Rat (Biogen Study No. P90-021).

Testing Laboratory: [redacted]

Study Started: March 29, 1990

Study Completed: October 10, 1990

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

Animals: Sprague Dawley rats (Crl:CD(SD)BR (VAF Plus)) were used in this study. At the start of treatment, animals were 5-6 weeks old, and body weight ranges were 160-212 g for male rats and 119-[illegible] g for female rats.

Drug Batch: Hirulog, Lot No. 67W04T (solid phase peptide method).

Methods: Rats received hirulog by the intravenous route of administration at doses of 0, 4, 12, and 36 mg/kg/day for 28 days. Dose selection was based upon an intravenous dose range finding study, in which rats received hirulog at doses of 0, 12, or 36 mg/kg/day for 7 days (Biogen Study No. P90-009; solid phase peptide method). Body weight gains for the male 12 and 36 mg/kg/day groups and the female 36 mg/kg/day groups were impaired by >10%. There were no significant findings at necropsy for any treatment group. In the present study, there were 10 rats/sex/group. Hirulog was administered by intravenous bolus injection. Control animals received the buffered vehicle. The dose volume was 2.5 mL/kg. Animals were observed for mortality twice daily. Animals were observed for clinical signs of toxicity daily, with particular attention toward the eyes and discoloration of the urine and feces. Body weight was measured on the first day of dosing and weekly thereafter. Food consumption was measured weekly. Ophthalmic examinations were performed for all treatment groups prior to start of treatment and for the control and 36 mg/kg/day groups during

week 4. Blood samples for hematological determinations were collected at days 7/8 and week 4. Blood samples for biochemistry determinations were collected at week 4. Urine samples for analysis were collected during weeks 1 and 4 under food and water deprivation. Fecal samples for determination of occult blood were collected prior to treatment and during weeks 1 and 4 when animals were separated for urine collection. At the end of treatment, animals were sacrificed and subjected to gross examination. Absolute and relative organ weights were determined for the adrenal glands, kidneys, ovaries, testes, brain, liver, pituitary, thymus, heart, lungs, spleen, and thyroids with parathyroids. Organs were collected and preserved for histopathological analysis as follows: adrenal glands, caecum, epididymis, heart, jejunum, lungs including bronchi, esophagus, pituitary gland, salivary glands, skeletal muscle, spleen, testes, trachea, aorta, colon, eyes with optic nerves, ileum, kidneys, mammary gland, ovaries, prostate, seminal vesicles, skin, stomach, thymus, urinary bladder, brain, duodenum, femur including marrow, injection site, liver, mesenteric lymph nodes, pancreas, rectum, sciatic nerve, spinal cord, submandibular lymph nodes, thyroids with parathyroids, and uterus.

Results: There were no clinical signs of toxicity or mortality. There were no treatment-related effects on body weight gain, hematological or clinical chemistry parameters, blood coagulation, or organ weights. There were no treatment-related urinalysis changes, ophthalmic effects, gross pathological changes, or histopathological changes.

Rats received hirulog by the intravenous route of administration at doses of 0, 4, 12, and 36 mg/kg/day for 28 days. The no effect level was 36 mg/kg/day. A target organ of toxicity was not identified.

A 28-Day Intravenous Infusion Toxicity Study of Hirulog in the Sprague Dawley Rat with a 14-Day Recovery Period (Biogen Study No. 88967-94-02).

Testing Laboratory: [redacted]

Study Started: June 22, 1994

Study Completed: June 28, 1996

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

Animals: Sprague Dawley rats (Crl:CD^{R}(SD)BR) were used in this study. At the start of treatment, rats were 10-14 weeks old with a weight range of 270-369 g and 218-267 g for females.

Drug Batch: Hirulog, Lot No. 67A03Q (modified homogenous phase commercial scale).

Methods: Rats received hirulog by continuous intravenous infusion at doses of 0, 25, 75, and 250 mg/kg/day for 28 days using a permanent catheter was placed in the right femoral vein. There were 15 rats/sex/group in the control and 250 mg/kg/day groups and 10 rats/sex/group in the 25 and 75 mg/kg/day groups. Following the 28 day treatment period, 5 rats/sex/group from the control and 250 mg/kg/day groups entered a 14 day recovery period. The vehicle was 0.9% NaCl for injection. Animals were observed once daily during a two week period prior to the start of treatment and the during the recovery period. During the treatment period, animals were observed for clinical signs of toxicity and mortality twice daily during the treatment period. Body weights were measured weekly during the pretreatment, treatment, and recovery periods. A final fasted body weight was taken prior to sacrifice. Food consumption was measured weekly during the pretreatment, treatment, and recovery periods. Ophthalmic examinations were performed prior to the start of treatment, at the end of the treatment period, and during the second week of the recovery period. Blood for determination of hematological and clinical chemistry parameters was collected at the end of the treatment period following overnight deprivation of food. For urine collection, each animal was placed in a metabolic cage and urine was collected during a fasting period in which animals were deprived of water. Blood for determination of plasma hirulog levels was collected prior to the start of treatment, immediately prior to the end of the infusion on day 29, and prior to sacrifice of recovery animals. Plasma hirulog levels were measured by an enzyme-linked immunoassay (ELISA) that detects the peptide in samples through its ability to inhibit the binding of a specific monoclonal antibody to a bovine serum albumin (BSA)-peptide conjugate bound to the solid phase. The BSA-peptide conjugate is a hirulog analog, H133, that is covalently coupled to BSA using a heterobifunctional reagent. Blood for determination of serum antibody levels was collected prior to the start of treatment and at necropsy following the end of the treatment and recovery periods. Animals sacrificed at the end of the treatment and recovery periods were subjected to a gross examination. Absolute

and relative weights were determined for the adrenal glands, brain, heart, kidneys, liver, lungs, ovaries, pituitary gland, prostate, salivary glands, seminal vesicles, spleen, thymus, testes, thyroid and parathyroid, and uterus. Organs and tissues for histopathological analysis were retained as follows: abnormal tissues (plus gross lesions), adrenal glands, aorta (thoracic), bone marrow (femur), bone (sternum), brain (cerebral cortex, midbrain, cerebellum, and medulla), colon, cecum, duodenum, epididymides, esophagus, eyes, heart, ileum, jejunum, infusion site including catheter tip, kidney, liver, lungs and bronchi, lymph nodes (mandibular and mesenteric), mammary gland (inguinal), optic nerves, ovaries, pancreas, pituitary gland, prostate, salivary gland (submandibular), sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord (cervical, thoracic, and lumbar), spleen, stomach, testes, thymus, thyroid lobes and parathyroid, tongue, trachea, bladder, uterus (horns and body), and vagina. A section of liver was collected and frozen for possible evaluation of cytochrome P450 metabolism.

Results:

1. **Observed Effects**: There were no treatment-related observed effects.

2. **Mortalities**: From the 250 mg/kg/day group, the deaths of 1 male (#4071) and 1 female (#4571) were considered treatment-related. One male rat from each of the control (#1041) and 250 mg/kg/day (#4082) groups were removed from the study due to blocked catheters on days 25 and 20, respectively. One male (#2072) from the 25 mg/kg/day group was sacrificed due to a broken catheter on day 29. One female (#1541) died during blood sampling on day 29. Male #4071 of the 250 mg/kg/day group was found dead on day 21. Gross pathological findings included dark fluid in the abdominal cavity, a mass at the infusion site, and enlarged fluid. Histopathological examination found necrosis of the liver, peritonitis of the intestinal tract, extramedullary hematopoiesis for the spleen, lung histiocytosis, lymph node hyperplasia, lymphoid hemorrhage, pancreatitis, and periphlebitis at the infusion site. Female #4571 of the 250 mg/kg/day group died on day 16. Gross pathological findings included a mass at the infusion site. Histopathological examination found necrosis of the liver and thymus, myocardial degeneration and/or necrosis, myeloid hypocellularity, increased erythropoiesis, lymphoid hyperplasia at the mandibular lymph node and periphlebitis of the infusion site.

3. **Body Weight and Food Consumption**: Body weight gains of the female 25, 75, and 250 mg/kg/day groups were impaired; although, there was not a dose response relationship. Body weights for the male control group on days -1, 28, and 43 were 312.4, 434.5, and 483.0 g, respectively. Body weight gains during the 28 day treatment period for the male 25, 75, and 250 mg/kg/day groups were 95.1, 96.1, and 101.8% of the control, respectively. Body weight gain during the 14 day recovery period for the male 250 mg/kg/day group was 102.2% of the control. Body weights for the female control group on days -1, 28, and 43 were 243.5, 283, and 309.2 g, respectively. Body weight gains during the 28 day treatment period for the female 25, 75, and 250 mg/kg/day groups were 74.3, 87.7, and 66.1% of the control, respectively. Body weight gain during the 14 day recovery period for the female 250 mg/kg/day group was 12.7% of the control. During the 28 day treatment period there was no significant effect (<5%) on food consumption for any treatment group. During the recovery period, food consumption for the female 250 mg/kg/day group was reduced to 89.0% of the control.

4. **Hematology and Blood Coagulation**: There were no significant treatment-related changes of hematological parameters during either the treatment or recovery periods. The APTT on day 29 prior to the end of the hirulog infusion for male 25, 75, and 250 mg/kg/day groups were increased to 201, 226.7, and 394.4% of the control (19.5 sec), respectively; however, by day 30, APTT values for male treatment groups had returned to the control level (19.3 sec). There were no differences in APTT values between the male control and 250 mg/kg/day groups at the end of the recovery period. The APTT on day 29 prior to the end of the hirulog infusion for female 25, 75, and 250 mg/kg/day groups were increased to 187.5, 231.8, and 384.4% of the control (19.2 sec), respectively; however, by day 30, APTT values for female treatment groups had returned to the control level (17.9 sec). There were no differences in APTT values between the female control and 250 mg/kg/day groups at the end of the recovery period.

5. **Blood Biochemistry and Urinalysis**:

Blood Biochemistry Changes at the End of the Treatment Period: Glucose levels for the male and female 250 mg/kg/day groups were decreased to 68.6 and 88.3% of respective control values (129.1 and 128.2 mg/dL). Aspartate transaminase activity for the male 250 mg/kg/day group was increased to 133% of the control (164.3 U/L); although, this difference was not significant. Triglyceride levels for the male 250 mg/kg/day group were elevated to 175.1% of the control (31.3 mg/dL). Cholesterol levels for female 25, 75, and 250 mg/kg/day groups were elevated to 125.5, 126.5, and 126.1% of

the control (52.9 mg/dL), respectively. Globulin levels for the female 250 mg/kg/day group were increased to 113.3% of the control (3.0 g/dL). Beta-globulin levels for the male 250 mg/kg/day group were increased to 113.8% of the control (1.23 g/dL). Percent and absolute γ-globulin levels for the female 250 mg/kg/day group were increased to 147.5 and 159.5% of respective control values (5.9% and 0.37 g/dL).

Blood Biochemistry Changes at the End of the Recovery Period: Potassium levels for the male 250 mg/kg/day group were decreased to 90.1% of the control (4.19 mEq/L). Chloride levels for the female 250 mg/kg/day group were increased to 103% of the control (99.6 mEq/L). Beta-globulin levels for the male 250 mg/kg/day group were decreased to 88.5% of the control (1.30 g/dL). Percent albumin for the female 250 mg/kg/day group was decreased to 96.1% of the control (63.9%).

Urinalysis: At the end of the treatment period, urinary ketone levels for male 250 mg/kg/day group (4 at 1.5 mmol and 5 at ±) were increased as compared to the control (1 at 1.5 mmol). At the end of the recovery period, ketone levels for the male 250 mg/kg/day group (2 at ±) were increased as compared to the control (0). At the end of treatment period, urinary potassium levels for the female 25, 75, and 250 mg/kg/day groups were decreased to 64.1, 61.6, and 66.8% of the control (292.4 mEq/L), respectively; however, no differences were found between the control and 250 mg/kg/day groups at the end of the recovery period. At the end of the recovery period, chloride levels for the female 250 mg/kg/day group were increased to 145.5% of the control 71.6 mEq/L).

6. **Ophthalmic Examination**: No treatment-related effects were found with the ophthalmic examination.

7. **Organ Weights**: At the end of the treatment period, relative liver weight for the male 250 mg/kg/day group was increased to 112.6% of the control (2.729%). At the end of the treatment period, relative kidney weights for the male 75 and 250 mg/kg/day groups were increased to 111 and 110% of the control (0.675%), respectively. There were no significant differences in absolute and relative organ weights between the control and 250 mg/kg/day groups at the end of the recovery period.

8. **Gross Pathology**: Prominent gross pathological changes were observed for the thymus and lung, which appear to correspond with hemorrhage. Spleen enlargement was observed at doses of 75 and 250 mg/kg/day, which appeared to correlate with increased extramedullary hematopoiesis. Masses were observed at the infusion site in hirulog-treated rats.

Gross pathological changes following the 28 day treatment period for rats that received hirulog by continuous intravenous infusion at doses of 0, 25, 75, and 250 mg/kg/day.

Organ/Tissue	0		25		75		250	
	M	F	M	F	M	F	M	F
Thymus								
-area dark	0	1	0	3	0	3	3	2
-small	0	0	0	0	1	0	0	2
Spleen								
-enlargement	0	0	0	0	2	0	2	1
-area pale	0	0	0	0	1	1	0	0
Lung								
-area dark	0	0	1	0	2	0	2	0
Lymph node								
-enlargement	1	0	1	0	3	0	2	0
-area dark	0	0	1	1	1	0	1	0
Infusion Site								
-mass	0	0	0	0	1	0	1	0
-thickening	1	1	0	0	1	0	1	0

Gross pathological changes following the 14 day recovery period for rats that received hirulog by continuous intravenous infusion at doses of 0 and 250 mg/kg/day.

Organ/Tissue	0 mg/kg/day		250 mg/kg/day	
	M	F	M	F
Lung				
-adhesion	0	0	0	1
Spleen				
-area pale	0	0	0	1

9. **Histopathology**: The target organ of toxicity was the liver. The applicant attributed sinusoidal histiocytosis in the liver and pancreatitis as extension of injury from the drug infusion site. Hemorrhage was observed in the thymus and optic nerve at all dose levels. Hemorrhage was also found in the lung at 250 mg/kg/day group. It should be emphasized that the severity of hemorrhage appeared to be low as there were no changes of red blood cell counts, hemoglobin levels, or hematocrit at any dose level. It is possible that blood collection obscured any changes in red blood cell counts, hemoglobin levels, or hematocrit. The severity of phlebitis at the infusion site was increased in treatment groups in

a dose-related manner. Increased extramedullary hematopoiesis in the spleen and increased myelopoiesis in the bone marrow were attributed by the sponsor to the influx of inflammatory cells at the infusion site; however, these changes may have also been related to hemorrhage present in several organs. With the exception of periphlebitis, no treatment-related changes were observed at the end of the recovery period.

Histopathological changes following the 28 day treatment period for rats that received hirulog by continuous intravenous infusion at doses of 0, 25, 75, and 250 mg/kg/day.

Organ/Tissue	0		25		75		250	
	M	F	M	F	M	F	M	F
Thymus								
-hemorrhage	0	1	2	3	3	1	3	2
Optic nerve								
-hemorrhage	0	0	0	2	2	1	1	3
Lung								
-hemorrhage	2	0	0	0	0	0	4	0
-histiocytosis	1	1	2	0	0	0	3	3
Liver								
-histiocytosis, sinusoidal	0	0	0	0	3	1	3	2
-centrilobular and midzonal necrosis	0	0	0	0	0	0	2	0
Pancreas								
-pancreatitis	0	2	2	0	3	0	6	0
Lymph node (Not identified)								
-lymphoid hyperplasia	1	0	0	0	2	0	2	0
-hemorrhage	0	0	1	1	1	1	1	0
Mandibular lymph node								
-lymphoid hyperplasia	2	1	1	4	3	1	3	3
-hemorrhage	0	1	1	1	2	2	1	3
Spleen								
-increased extramedullary hematopoiesis	0	0	0	0	2	0	1	0
-lymphoid hyperplasia	0	0	0	0	2	0	1	0
Bone marrow								
-increased myelopoiesis	0	0	0	0	1	0	1	0
Infusion Site								
-thrombosis	5	5	6	6	1	4	3	9
-phlebitis	2	1	1	5	4	2	6	7
-periphlebitis	1	1	0	2	3	2	2	0

Histopathological changes following the 14 day recovery period for rats that received hirulog by continuous intravenous infusion at doses of 0 and 250 mg/kg/day.

Organ/Tissue	0 mg/kg/day		250 mg/kg/day	
	M	F	M	F
Infusion Site				
-thrombosis	1	2	2	0
-periphlebitis	0	0	1	0

10. **Plasma Drug Levels**: Plasma hirulog levels were determined from blood samples collected prior to the start of the infusion and immediately prior to the end of the infusion. Hirulog levels were measured using an enzyme-linked immunoassay. On day 28, plasma hirulog levels were proportional to dose. There was no difference in plasma levels between male and female rats.

Plasma hirulog levels (ng/mL) on day 28 for rats that received hirulog by continuous intravenous infusion at doses of 25, 75, and 250 mg/kg/day.

Parameter	25 mg/kg/day	75 mg/kg/day	250 mg/kg/day
C_{ss} male+female	2020 ± 1309	7474 ± 3342	20690 ± 10891
C_{ss} male	2699	6909.6	25121
C_{ss} female	1355.2	8039.1	18052.3
Normalized C_{ss} (M+F)/Dose	80.8	99.7	82.8

Rats received hirulog by continuous intravenous infusion at doses of 0, 25, 75, and 250 mg/kg/day for 28 days. Rats from the control and 250 mg/kg/day groups entered a 14 day recovery following the treatment period. The no effect level was 25 mg/kg/day. Treatment-related mortality occurred at 250 mg/kg/day. The target organ of toxicity was the liver. Sinusoidal histiocytosis and centrilobular and midzonal necrosis were observed for the liver at doses of 75 and 250 mg/ kg/day. An increased incidence of pancreatitis was found at 250 mg/kg/day; although, it was not test article-specific. Hemorrhage was observed in the thymus and optic nerve at all dose levels. Hemorrhage was also found in the lung at 250 mg/ kg/day group. It should be emphasized that the severity of hemorrhage appeared to be low as there were no changes of red blood cell counts, hemoglobin levels, or hematocrit at any dose level. The severity of phlebitis at the infusion site was increased in

treatment groups in a dose-related manner. Increased extramedullary hematopoiesis in the spleen and increased myelopoiesis in the bone marrow were found and attributed by the sponsor to an influx of inflammatory cells at the infusion site. With the exception of periphlebitis, no treatment-related changes were observed at the end of the recovery period.

Monkeys

Seven Day Repeated Dose Pilot Toxicity Study of Hirulog Administered Intravenous to Cynomolgus Monkeys (Biogen Study No. P90-007).

Testing Laboratory: [redacted]

Study Started: March 5, 1990

Study Completed: September 6, 1990

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

Animals: Cynomolgus monkeys (Macaca fascicularis) were used in this study. At the start of treatment, mean body weight ranges were 3.7 to 4.1 kg for males and 3.0 to 3.2 kg for females. These animals were wild-caught and ages were unknown.

Drug Patch: Hirulog, Lot No. 67W03T (solid phase peptide method).

Methods: Monkeys received hirulog by intravenous bolus administration at doses of 0, 12, and 36 mg/kg/day for 7 days. This was only study included in the NDA submission that examined hirulog toxicity in monkeys when given by intravenous bolus administration. Each group consisted of 1 monkey/sex. Body weights were recorded prior to treatment (day -1) and immediately prior to necropsy (day 8). Animals were observed daily for clinical signs of toxicity. Ophthalmic examinations were performed prior to treatment (day -1) and immediately prior to necropsy (day 8). Blood for determination of hematology, clinical chemistry, coagulation profiles, and serum antibodies directed against hirulog was collected on day 8 prior to necropsy. Urine specimens were collected from the cage pan or cystocentesis prior to treatment (day -1) and immediately prior to necropsy (day 8). Fecal samples

for determination of occult blood were collected prior to treatment (day -1) and immediately prior to necropsy (day 8). Animals were sacrificed on day 8 and subjected to a gross examination. Absolute and relative organ weights were determined for the adrenal glands, brain, liver (with gallbladder), kidney (right), spleen, pituitary gland, ovaries, testis (right), and thyroid with parathyroid. Histopathological examination was performed on tissues from all monkeys.

1. **Observed Effects**: Doses at 12 and 36 mg/kg/day were associated with prolonged bleeding from the IV catheter site. Following removal of the percutaneous catheter for the 12 and 36 mg/kg/day groups, prolonged pressure at the entry site was required to prevent hematoma formation. There was significant bruising at administration sites for both control and treatment groups.

2. **Mortalities**: None.

3. **Body Weight**: There were no significant treatment-related effects on body weight gain.

4. **Hematology**: No changes in PT, PTT, or fibrinogen levels were evident in treatment groups. White blood cell counts for the male and female of the 12 mg/kg/day group were decreased to 72.9% of the pretreatment value (15 x 10^3 cells/mm^3). White blood cell counts for the female of the 36 mg/kg/day group were decreased to 63.7% of the pretreatment value (9.1 x 10^3 cells/mm^3). Absolute neutrophil counts for the male and female of the 12 mg/kg/day groups were decreased to 59.7% of the control (9.92 x 10^3 cells/mm^3). Absolute neutrophil counts for the female of the 36 mg/kg/day groups were decreased to 40.3% of the control (4.73 x 10^3 cells/mm^3).

5. **Blood Chemistry and Urinalysis**: No treatment-related effects were identified clinical chemistry analysis or urinalysis.

6. **Physical and Ophthalmic examinations**: There were no treatment-related effects on respiratory rate, heart rate, or body temperature. There were no treatment-related ophthalmic effects.

7. **Organ Weights**: Organ weight differences may be related to differences in ages, which were unknown. Further, organ weights appear to have little correlation to histopathological changes. Absolute ovary weight for females of the 12 and 36 mg/kg/day groups were increased to 128.7 and 348.3% of the control (0.3925 g), respectively. Relative ovary weights for females of the 12 and 36 mg/kg/day groups were increased to 128.2 and 348% of the control (1.31, OW/BW x 10^{-4}), respectively. Absolute spleen weights for females of the 12 and 36 mg/kg/day groups were decreased to 81.8

and 56.4% of the control (12.696 g), respectively. Relative spleen weights for females of the 12 and 36 mg/kg/day groups were decreased to 81.8 and 56.5% of the control (4.23, SW/BW x 10^3), respectively. Absolute thyroid weights for males of the 12 and 36 mg/kg/day groups were increased to 163.6 and 295% of the control (0.3798 g), respectively. Relative thyroid weights for the males of the 12 and 36 mg/kg/day groups were increased to 163 and 294.5% of the control (1.27, TW/BW x 10^4), respectively. Adrenal gland weights for males of the 12 and 36 mg/kg/day groups were increased to 132.6 and 142.9% of the control (0.5124 g), respectively. Absolute weight of the right testis for the males of the 12 and 36 mg/kg/day groups were increased to 170.3 and 292% of the control (3.9562 g), respectively. Relative weight of the right testis for the males of the 12 and 36 mg/kg/day groups were increased to 170 and 291.6% of the control (1.07, RTW/BW x 10^3), respectively.

8. **Gross Pathology**: A liver foci was observed for 1 male of 12 mg/kg/day group and 1 female of the 36 mg/kg/day group. Ovaries cysts were observed in the female of the 36 mg/kg/day group. Hemorrhage at the injection site had a higher incidence in the control group.

9. **Histopathology**: Skeletal muscle inflammation was observed for 1 female of the 12 mg/kg/day group and both the male and female of the 36 mg/kg/day group. Inflammation was characterized by infiltration of lymphocytes, some neutrophils, and macrophages.

10. **Serum Antibodies Directed Against Hirulog**: Serum samples were recovered after dosing and tested for the presence of antibodies to hirulog by enzyme-linked immunosorbent assay. There was no evidence for the development of anti-hirulog cross-reacting antibodies in Cynomolgus monkeys that received hirulog at 12 or 36 mg/kg/day for 7 days.

Monkeys received hirulog by intravenous bolus administration at doses of 0, 12, and 36 mg/kg/day for 7 days. The maximum tolerated dose was 36 mg/kg/day. Skeletal muscle inflammation was observed in both the 12 and 36 mg/kg/day groups.

Twenty-Eight Day Repeated Dose Toxicity Study of Hirulog Administered Subcutaneously to Cynomolgus Monkeys (Biogen Study No. P90-008).

Testing Laboratory: [redacted]

Study Started: June 19, 1990

Study Completed: October 23, 1990

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

Animals: Cynomolgus monkeys (Macaca fascicularis) were used in this study. At the start of treatment, mean body weight ranges were 4.20 to 4.43 kg for males and 3.40 to 3.70 kg for females. The animals' ages were unknown.

Drug Batch: Hirulog, Lot No. 67W06T (solid phase peptide method).

Methods: Monkeys received hirulog by the subcutaneous route of administration at doses of 0, 4, 12, and 36 mg/kg/day for 28 days. There were 3 animals/sex/group. Control animals received the vehicle. The dose volume was 1.44 mL/kg. Animals were observed daily for clinical signs of toxicity and mortality. Body weights were measured on days 1, 8, 15, 22, and 29. Ophthalmic and physical (respiratory rate, heart rate, and body temperature) examinations were performed on animals prior to the start of treatment and on days 15 and 29. Bleeding times were determined for each animal prior to the initiation of treatment and on days 15 and 29. Blood for determination of hematological, clinical chemistry, and coagulation parameters and the titer of anti-hirulog antibodies was collected prior to treatment and on days 15 and 29. Urine specimens were collected from the cage pan or by cystocentesis prior to the initiation of treatment and on days 15 and 29. Fecal specimens for determination of occult blood were collected from each animal prior to the initiation of treatment and on days 15 and 29. Prior to sacrifice, animals were fasted overnight. Animals were sacrificed on day 29 and subjected to a gross examination. Absolute and relative organ weights were determined for the adrenal glands, brain, liver with gallbladder, kidney, spleen, pituitary gland, ovaries, testis, and thyroid with parathyroid. Tissue evaluated by histopathological analysis were as follows: heart, aorta, vena cava, mandibular salivary gland, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, pancreas, liver, gall bladder, trachea, lungs, adrenal glands, thyroid glands, parathyroid glands, testes, epididymides, prostate gland, seminal vesicles, kidneys, urinary bladder, cerebrum, thalamus, midbrain, cerebellum, thoracolumbar spinal cord, sciatic nerve, eyes, costochrondral junction, skeletal muscle (caudal and thigh), skin with mammary gland, administration/injection site, gross lesions, thymus, spleen, bronchial lymph node, mandibular lymph node, mesenteric lymph node, bone marrow, ovaries, and uterus.

1. **Observed Effects**: Bruising was observed at sites of drug administration. The severity of bruising was proportional to the hirulog dose.

Hirulog mg/kg/day	Bruising	
	Male	Female
0	0	1
4	1	3
12	1	3
36	3	3

2. **Mortality**: None.

3. **Body Weight**: There were no significant treatment-related effects on body weight gain. Body weights for male controls on days 1 and 29 were 4.43 and 4.60 kg, respectively, yielding a 3.84% increase of initial body weight. Body weights for the male 4 and 12 mg/kg/day groups were increased by 1.63 and 0.71% of initial weights on day 1, respectively. Body weight for the male 36 mg/kg/day group was decreased by -0.71% of initial body weight on day 1. Body weights for the female controls on days 1 and 29 were 3.70 and 3.63 kg, respectively, yielding a decrease of -1.89% of initial body weight. Body weights for the female 4 and 36 mg/kg/day groups were decreased by -2.72 and -2.80% of initial body weight on day 1, respectively. Body weight for the female 12 mg/kg/day group was unchanged during the treatment period.

4. **Hematology and Blood Coagulation**:

Hematology: Reticulocyte counts for the male and female 12 and 36 mg/kg/day groups were increased on days 15 and 29.

Males: On day 15, the reticulocyte percentage for the male 12 and 36 mg/kg/day were elevated to 0.9 and 1.1%, respectively, as compared to the control value of 0.43%. On day 15, absolute reticulocyte counts for the male 12 and 36 mg/kg/day groups were elevated to 0.05×10^6 and 0.07×10^6 cells, respectively, as compared to a control value of 0.023×10^6 cells. On day 15, the neutrophil percentage and count for the male 36 mg/kg/day group were elevated to 61% and 5.79×10^3 cells as compared to control values (39.3% and 3.55×10^3 cells), respectively. On day 29, the reticulocyte percentage for the male 12 and 36 mg/kg/day group were elevated to 0.80 and 1.07%, respectively, as compared to a control

value of 0.53%. On day 29, the reticulocyte counts for the male 12 and 36 mg/kg/day groups were elevated to 0.047 x 10^6 and 0.06 x 10^6 cells, respectively, as compared to a control value of 0.03 x 10^6 cells. On day 29, white blood cell counts for the male 36 mg/kg/day group were decreased to 7.60 x 10^3 cells as compared to a control value of 10.4 x 10^3 cells.

Females: On day 15, the neutrophil percentage and count for female 36 mg/kg/day group were decreased to 32% and 2.1 x 10^3 cells, respectively, as compared to control values (47% and 3.97 x 10^3 cells). On day 15, the lymphocyte percentage for the female 36 mg/kg/day group was increased to 63.7% as compared to a control value of 49%. On day 29, the reticulocyte percentage for female 12 and 36 mg/kg/day group were increased to 0.70 and 1.47%, respectively, as compared to a control value of 0.4%. On day 29, the reticulocyte counts for the female 12 and 36 mg/kg/day groups were increased to 0.043 x 10^6 and 0.077 x 10^6, respectively, as compared to a control value of 0.023 x 10^6. On day 29, the neutrophil count for the female 36 mg/kg/day group was decreased to 2.52 x 10^3 as compared to a control value of 4.7 x 10^3. On day 29, the lymphocyte percentage for the female 36 mg/kg/day group was increased to 59.6% as compared to a control value of 43.7%.

Blood Coagulation: There were no changes of PT, APTT, fibrinogen, or bleeding time in either male or female treatment groups. On day 15, the prothrombin time and APTT for 1 male of the 36 mg/kg/day group were increased to 105 and 92.3 sec, respectively, while the fibrinogen level was decreased to 149 mg/dL. Tests for fecal occult blood were negative.

5. **Blood Chemistry and Urinalysis:**

Blood Chemistry: On day 15, blood urea nitrogen (BUN) levels for the male 36 mg/kg/day groups were elevated to 127.8% of the control (13.3 mg/dL). On day 29, BUN levels for the male 12 and 36 mg/kg/day groups were elevated to 124.1 and 131.3% of the control (9.67 mg/dL), respectively. On day 15, aspartate transaminase activities for the male 12 and 36 mg/kg/day groups were increased to 147.5 and 166% of the control (30.3 U/L), respectively.

Urinalysis: For 2 females of the 36 mg/kg/day group, Red blood cell/HPF and Epithelial cell/HPF were detected as compared to no significant findings for controls.

6. **Physical and Ophthalmic Examinations:** There were no treatment changes of respiratory rate, heart rate, or body temperature on days 15 or 29. No treatment-related ophthalmic effects were found.

7. **Organ Weights**: The absolute right kidney weight for the male 36 mg/kg/day group was decreased to 84.9% of the control (8.795 g). Absolute brain weight for the male 36 mg/kg/day group was decreased to 90.7% of the control (56.877 g). Absolute lung weight for the male 36 mg/kg/day group was decreased to 81.1% of the control (25.17 g).

8. **Gross Pathology**: Gross pathological examination identified minor changes in the lungs, spleen, and body cavities.

Gross pathological changes for monkeys that received hirulog by the subcutaneous route of administration at doses of 0, 4, 12, and 36 mg/kg/day for 28 days.

Organ/Tissue	0		4		12		36	
	M	F	M	F	M	F	M	F
Lungs -foci	0	0	0	0	0	0	0	1
Spleen -foci	0	0	0	0	0	0	0	2
Body cavities -adhesions	0	0	0	0	0	1	1	0

9. **Histopathology**: An increased incidence of histopathological changes were identified for the parathyroid glands, sciatic nerve, stomach, and bronchial lymph nodes; however, these changes generally did not occur in a dose response related manner and were not test article specific.

Histopathological changes for monkeys that received hirulog by the subcutaneous route of administration at doses of 0, 4, 12, and 36 mg/kg/day for 28 days.

Organ/Tissue	0		4		12		36	
	M	F	M	F	M	F	M	F
Parathyroid glands -cysts	0	0	0	1	2	0	1	1
Sciatic nerve -inflammation, perin.	1	0	0	1	1	0	2	0
Stomach -infiltrate, lymphocy	1	1	0	1	1	2	2	3
Bronchial lymph nodes -hyperplasia, lymph	1	1	1	0	2	0	3	0

10. **Anti-Hirulog Levels**: There was no evidence of anti-hirulog antibodies in monkeys treated with hirulog by the subcutaneous route of administration at doses of 0, 4, 12, or 36 mg/kg/day for 28 days. Sampling times were day 1 prior to the start of treatment and on days 15 and 29.

Monkeys received hirulog by the subcutaneous route of administration at doses of 0, 4, 12, and 36 mg/kg/day for 28 days. The maximum tolerated dose was 36 mg/kg/day. There was no target organ of toxicity. Bruising was observed at sites of drug administration. The severity of bruising was proportional to the hirulog dose. An increased incidence of histopathological changes were identified for the parathyroid glands, sciatic nerve, stomach, and bronchial lymph nodes; however, these changes generally did not occur in a dose response related manner and were not test article specific.

A 28-Day Intravenous Infusion Toxicity Study of Hirulog in the Cynomolgus Monkey with a 14-Day Recovery Period (Biogen Study No. P8967-94-01).

Testing Laboratory: [redacted]

Study Started: June 15, 1994

Study Completed: June 3, 1997

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

Animals: Cynomolgus monkeys (Macaca fascicularis) were used in this study. At the start of treatment, body weight ranges were 2.8 to 3.9 kg for males and 2.6 to 3.0 kg for females. The animals' ages were not specified.

Drug Batch: Hirulog, Lot No. 67AO2Q (modified homogenous phase commercial scale).

Methods: Cynomolgus monkeys received hirulog by continuous intravenous infusion at doses of 0, 15, 45, and 150 mg/kg/day for 28 days. The control and 150 mg/kg/day groups were composed of 5 animals/sex/group. The 15 and 45 mg/kg/day groups were composed of

3 animals/sex/group. Two animals/sex/group from the control and 150 mg/kg/day groups entered a 14 day recovery period following the treatment period. Dose selection was based upon a 14 day dose range finding study in which cynomolgus monkeys received hirulog by continuous intravenous infusion (Biogen Study No. P8967-93-01). This range finding toxicity study examined the commercial chemistry lyophilized formulation (CCLF) of hirulog at 40 mg/kg/day (Lot No. 67Z17S) and the pilot chemistry frozen clinical formulation (PCF) of hirulog at 40 and 150 mg/kg/day (Lot No. 67Z10S). The CCLF is intended to be representative of the final manufacturing chemistry material. Mortality occurred for 1 animal in each of the control and 150 mg/kg/day groups due to bacterial infection. Red blood cell counts, hemoglobin levels, and hematocrits declined in both control and treatment groups due to blood collection procedures, but were most pronounced in the 150 mg/kg/day group due to both blood collection as well as hemorrhage at several sites (i.e., rectal serosa, gallbladder and lumenal contents, ventral abdominal and right thigh skeletal muscles, lungs, mesentry, and lesser omentum). White blood cell and neutrophils counts were increased in both control and treatment groups due to neutrophil influxes into the catheter infusion site. Myeloid hyperplasia of the bone marrow was found in both control and treatment groups. Liver Kupffer cell hypertrophy was observed for the 150 mg/kg/day group due to engulfment of erythroid elements. Elevations of the APTT were similar for the CCLF and PCF of hirulog at 40 mg/kg/day. In the present study, catheters were inserted into the right femoral vein and the tip was placed in the vena cava at the approximate level of the kidneys. The infusion rate was 1 mL/kg/hr. The dose rate for the 15, 45, and 150 mg/kg/day groups were 0.625, 1.875, and 6.25 mg/kg/hr, respectively. All animals were observed daily during the 3 weeks of the pretreatment period and during the recovery period. During the treatment period, animals were examined twice daily for clinical signs of toxicity. A mortality check was performed at least once daily throughout the study. During the pretreatment, treatment, and recovery periods, animals were weighed weekly. A fasted body weight was measured prior to sacrifice. Food consumption was estimated daily during the pretreatment, treatment, and recovery periods. Ophthalmic examinations were performed prior to treatment, during week 4 of the treatment period, and during week 2 of the recovery period. Electrocardiograms and blood pressure (indirect systolic) were measured prior to treatment, on day 27 during the treatment period, and on day 43 during the recovery period. Recordings were made using limb leads (I, II, III, aVR, aVL, and aVF). Physical examinations to assess health status, respiratory rate, and body temperature were performed twice prior to treatment, on days 15 and 26/27 during the treatment period, and on day 43 during the

recovery period. Once prior to the start of treatment, on day 30 at the end of the treatment period, and on day 44 at the end of the recovery period, blood for determination of hematology and clinical parameters and urine for analysis were collected. For each sacrificed animal, three femoral bone marrow were prepared, one of which was stained with May-Grunwald-Giemsa. All smears were retained for possible analysis. Blood for determination of plasma hirulog levels were collected prior to treatment on day -1, immediately prior to the end of infusion on days 2 (first treatment) and 29 were collected. At the end of the infusion on day 29, blood for determination of plasma hirulog levels were collected at 5, 10, 20, 30, and 45 min, and 1, 1.5, 2, 4, and 6 hr from all non-recovery animals. An additional blood sample for determination of plasma hirulog levels was collected prior to sacrifice of recovery animals. Blood for determination of anti-hirulog antibodies were collected prior to treatment (day -1) and on day 30 prior to sacrifice of non-recovery animals. Animals were sacrificed on day 30 after the treatment period and on day 44 after the recovery period and subjected to a gross examination. Absolute and relative organ weights were determined for the adrenal glands, brain, heart, kidneys, liver, lungs, ovaries, pituitary gland, prostate, salivary gland, seminal vesicles, spleen, testes, thyroid lobes and parathyroids, thymus, and uterus. Organs and tissues for histopathological analysis were collected as follows: abnormal tissues, adrenal glands, aorta (thoracic) infusion site(s) including catheter tip(s), jejunum, salivary glands, sciatic nerves, seminal vesicles, skeletal muscles, bone and marrow (femur and sternum), brain (cerebral cortex, midbrain, cerebellum, and medulla), bronchi, cecum, colon, duodenum, epididymides, esophagus, eyes, gall bladder, heart, ileum, kidneys, liver (sample of 2 lobes), lungs (sample of 2 lobes), lymph nodes (mandibular and mesenteric), mammary gland (thoracic), optic nerves, ovaries, pancreas, pituitary gland, prostate, skin (thoracic), spinal cord (cervical, thoracic, and lumbar), spleen, stomach, testes, thymus, thyroid lobes and parathyroids, tongue, trachea, urinary bladder, uterus, and vagina. A section of liver was obtained for possible evaluation of P450 metabolism and frozen.

Results:

1. **Observed Effects**: There were no significant treatment-related observed effects. Two of 5 male in the 150 mg/kg/day group were observed infrequently to have reduced use of one or both hindlimbs; although, this effect was not observed in females and was attributed to physical trauma associated with experimental procedures.

2. **Mortality**: None.

3. **Body Weight and Food Consumption**: Body weight gain was slightly impaired for all male treatment groups and the female 45 and 150 mg/kg/day groups. Food consumption was unaffected. Body weights for male controls on days -1, 28 and 42 were 3.02, 3.30, and 3.50 kg, respectively. Percentage increases of initial body weight for the male 0, 15, 45, and 150 mg/kg/day groups during the treatment period were 9.3, 5, 5.6, and 5.8%, respectively. Percentage increases of initial body weight for the male 0 and 150 mg/kg/day groups during the recovery period were 6.1 and 12.1%, respectively. Body weights for female controls on days -1, 28 and 42 were 2.80, 3.02, and 3.00 kg, respectively. Percentage increases of initial body weight for the female 0, 15, 45, and 150 mg/kg/day groups during the treatment period were 7.9, 10.7, 5.6, and 5.0%, respectively. Percentage increases of initial body weight for the female 0 and 150 mg/kg/day groups during the recovery period were -0.7 and 7.1%, respectively. Food consumption was unaffected during the treatment and recovery periods.

4. **Hematology and Blood Coagulation**: There minimal to moderate changes of hematological parameters between the control and treatment groups during the treatment and recovery periods; although, statistical significance was not achieved. White blood cell and neutrophil counts increased for treatment groups, which may have been related to neutrophil influxes into the infusion site. APTT values were elevated for male and female treatment groups during the treatment period.

Hematology: White blood cell counts for male and female 150 mg/kg/day groups at the end of the recovery period were elevated to 130.9 and 136.1% of the control (8.4×10^3 and 7.2×10^3 cells/mm^3), respectively. The percentage of segmented neutrophils for the male and female 150 mg/kg/day groups at the end of the recovery period were elevated to 179.2% of the control (24.0%), while the percentage of lymphocytes for the male 150 mg/kg/day group were reduced to 75.2% of the control (70.5%). The segmented neutrophil count for the male 150 mg/kg/day group at the end of the recovery period was elevated to 256.5% of the control (1927.5 cells). The percentage of segmented neutrophils for the female 15, 45, and 150 mg/kg/day groups were elevated to 135.4, 133, and 132% of the control (28.8%); although, there was no evidence of a dose response relationship. The percentage of segmented neutrophils for the female 150 mg/kg/day group at the end of the recovery period was elevated to 125% of the control (30%). The segmented neutrophil count for the female 150 mg/kg/day group at the end of the recovery period was elevated to 158% of the control (2232).

The percentage of monocytes for the female 150 mg/kg/day group at the end of the recovery period were elevated to 300% of the control (1.5%). Monocyte counts for the female 150 mg/kg/day group at the end of the recovery period were increased to 339.3 of the control (126). Lymphocyte counts for the female 15, 45, and 150 mg/kg/day groups at the end of the treatment period were decreased to 75.2, 82.1, and 61.4% of the control (7878), respectively; although, there was no evidence of a dose response.

APTT: On day 2, the APTT values for the male 15, 45, and 150 mg/kg/day groups were elevated to 223.1, 287.4, and 473.4% of the control (19.9 sec), respectively. On day 29, the APTT values for the male 15, 45, and 150 mg/kg/day groups were elevated to 144.7, 279.8, and 297.6% of the control (18.8 sec), respectively. On day 2, the APTT values for the female 15, 45, and 150 mg/kg/day groups were increased to 179.3, 343.5, and 564.8% of the control (19.3 sec), respectively; although, only the value for the 150 mg/kg/day group was significantly increased. On day 29, the APTT values for the female 15, 45, and 150 mg/kg/day groups were elevated to 154,4, 379.7, and 508.8% of the control (18.2 sec), respectively. By day 30, the APTT values for the male and female 15, 45, and 150 mg/kg/day groups had returned to the control level.

5. **Blood Chemistry and Urinalysis:** There minimal to moderate changes of blood chemistry and urinalysis parameters between the control and treatment groups during the treatment and recovery periods; although, statistical significance was not achieved.

Blood Chemistry: Glucose levels for the male 15, 45, and 150 mg/kg/day at the end of the treatment period were reduced to 71.9, 63.7, and 75.5% of the control (68.6 mg/dL), respectively. Alkaline phosphatase activity for the female 150 mg/kg/day group at the end of the recovery period was increased to 141.1% of the control (447.5 U/L).

Urinalysis: Urine sodium levels for the male 150 mg/kg/day group at the end of the recovery period were elevated to 288.4% of the control (27.5 mEq/L). Urine chloride levels for the male 150 mg/kg/day group at the end of the recovery period were elevated to 136% of the control (37.8 mEq/L). Urine calcium levels for the male 150 mg/kg/day group at the end of the recovery period were decreased to 22.5% of the control (25.3 mg/dL). Tests for nitrite were positive for 1 of 3 males at 15 mg/kg/day, 2 of 3 males at 45 mg/kg/day, and 4 of 5 males at 150 mg/kg/day as compared to 0 for the control.

6. **Physical and Ophthalmic Examinations**: There were no treatment-related effects found with electrocardiograms or ophthalmic examinations during the treatment or recovery periods. Heart rate and systolic blood pressure were unaffected during the treatment and recovery periods.

7. **Organ Weights**:

Males: Variations in absolute and relative gonad, seminal gland, and prostate weights were observed due to the sexual immaturity of the animals. Liver weight for male 150 mg/kg/day group at the end of the treatment period was decreased to 87% of the control (72.525 g). Absolute and relative thyroid/parathyroid weight for the male 150 mg/kg/day group at the end of the recovery period were decreased to 61.6 and 58.7% of the control (0.349 g and 0.109%), respectively. Absolute and relative thymus weight for the male 150 mg/kg/day group at the end of the recovery period were increased to 184.7 and 178.7% of the control (3.11 g and 0.959%), respectively. Relative salivary gland weight for the male 150 mg/kg/day group at the end of the recovery period was decreased to 80% of the control (0.732%).

Females: Variations in absolute and relative gonad weights were observed due to the sexual immaturity of the animals. Thyroid/Parathyroid weight for female 15, 45, and 150 mg/kg/day group at the end of the treatment period were decreased to 74, 62.8, and 61% of the control (0.439 g). Absolute and relative thyroid/parathyroid weights for female 150 mg/kg/day group at the end of the recovery period was decreased to 55.5 and 55.0% of the control (0.454 g and 0.160%).

8. **Gross Pathology**: There no prominent gross pathological findings; although, hemorrhage was evident in the thymus.

Pathological changes in monkeys that received hirulog by continuous intravenous infusion at doses of 0, 15, 45, and 150 mg/kg/day for 28 days.

Organ/Tissue	0		15		45		150	
	M	F	M	F	M	F	M	F
Thymus								
-small	0	0	0	0	1	0	1	0
-foci dark	0	0	0	0	1	0	0	1
-area dark	0	0	0	0	0	2	2	1
Lymph node								
-discoloration	0	0	0	1	1	1	0	1
Lung								
-area dark	0	0	0	0	0	0	1	0
Kidney								
-area pale	0	0	0	0	0	0	0	1
Urinary bladder								
-thickening	0	0	0	0	0	0	1	1

Pathological changes in monkeys following a 14 day recovery after treatment with hirulog by continuous intravenous infusion at doses of 0 or 150 mg/kg/day for 28 days.

Organ/Tissue	0		150	
	M	F	M	F
Lung				
-adhesion	0	0	0	1
-area dark	0	0	0	1
Thyroid				
-small	0	0	1	1
Liver				
-area pale	0	0	0	1
Colon				
-nodule	0	0	1	0

9. **Histopathology**: Target organs of toxicity included the heart, skeletal muscle, and thymus. For the 150 mg/kg/day group, 1 male and 1 female were observed with myocardial degeneration/necrosis and hemorrhage. The myocardial lesion for the male was focal with evidence of fibrin accumulation at the site, while for the female, the lesion was multifocal. In both animals, the myocardial lesion was subacute (i.e., within the last 7-10 days) and associated with

slight to mild hemorrhage. Skeletal muscle degeneration and/or necrosis was observed for 2 females of the 150 mg/kg/day group, which the sponsor attributed to experimental procedures; however, these lesions were not observed in the control group. The incidence of thymic hemorrhage was increased in the 45 and 150 mg/kg/day groups; although, there was not a dose response relationship and the effect was not test article selective. At the infusion site, thrombosis, phlebitis, periphlebitis, and intimal proliferation were observed from the control and treatment groups.

Histopathological changes in monkeys that received hirulog by continuous intravenous infusion at doses of 0, 15, 45, and 150 mg/kg/day for 28 days (n = 3 animals/sex/group).

Organ/Tissue	0		15		45		150	
	M	F	M	F	M	F	M	F
Heart								
-myocardial degeneration and/or necrosis	0	0	0	0	0	0	1	1
-hemorrhage	0	0	0	0	0	0	1	1
Skeletal muscle								
-degeneration and/or necrosis	0	0	0	0	0	0	0	2
Thymus								
-hemorrhage	0	1	0	0	1	2	2	2
-lymphoid atrophy	0	0	1	1	1	0	1	0
Kidney								
-interstitial nephritis	0	0	0	0	0	0	1	0

Histopathological changes in monkeys following a 14 day recovery after treatment with hirulog by continuous intravenous infusion at doses of 0 or 150 mg/kg/day for 28 days.

Organ/Tissue	0		150	
	M	F	M	F
Heart				
-myocardial fibrosis	0	1	0	0
Lung				
-hemorrhage	0	0	0	1
-pleural fibrosis	0	0	0	1
-interstitial pneumonia	0	1	1	0
Kidney				
-glomerular lipidosis	0	0	1	0
Infusion Site				
-thrombosis	1	2	1	1
-intimal proliferation	0	0	1	1

10. **Plasma Drug Levels**: Plasma hirulog levels were determined on day 29 at the termination of the infusion. Concentration values at steady state increased with dose; although, the value at 15 mg/kg/day was lower than expected based upon values at 45 and 150 mg/kg/day. The sponsor attributed lower than expected values at 15 mg/kg/day to sampling problems. It is possible that there could be a saturation of metabolism at 45 and 150 mg/kg/day. There were no differences in C_{ss}, C_{max}, or AUC between male and female monkeys.

Pharmacokinetic parameters for plasma hirulog on day 29 in cynomolgus monkeys that received the drug by continuous intravenous infusion at doses of 15, 45, and 150 mg/kg/day for 28 days.

Parameter	15 mg/kg/day	45 mg/kg/day	150 mg/kg/day
C_{ss}, µg/mL	0.76	4.39	13.38
$T_{1/2}$(eff), hr	0.103	0.074	0.314
Dose adjusted C_{ss}	15.0	28.7	29.3
C_{max}, µg/mL	0.698	3.378	10.85
T_{max}, hr	0.083	0.111	0.097
AUC_{inf}, µg-hr/mL	0.218	1.275	6.76

Cynomolgus monkeys received hirulog by continuous intravenous infusion at doses of 0, 15, 45, and 150 mg/kg/day for 28 days. Animals from the control and 150 mg/kg/day groups entered a 14 day recovery period following the treatment period. The no effect dose was 45 mg/kg/day. APTT values were increased at all dose levels during the treatment period. Target organs of toxicity included the heart, skeletal muscle, and thymus. For the 150 mg/kg/day group, 1 male and 1 female were observed with myocardial degeneration/necrosis and hemorrhage. Skeletal muscle degeneration and/or necrosis was observed for 2 females of the 150 mg/kg/day group. The incidence of thymic hemorrhage was increased in the 45 and 150 mg/kg/day groups; although, there was not a dose response relationship and the effect was not test article selective.

Reproductive Toxicity

Rats

Segment I Study of the Toxicity of Hirulog on Fertility and Reproductive Performance in Crl:CD®BR VAF/Plus® Rats When Administered by the Subcutaneous Route (Biogen Study No. P8967-93-16).

Testing Laboratory: [redacted]

Study Started: March 1, 1994

Study Completed: September 29, 1995

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

Animals: Male and Female Sprague Dawley rats were used in this study. Male rats were 72 days old at arrival and had a weight range of 313-381 g at study assignment. Female rats were 66 days old at arrival and had a weight range of 223-295 g at study assignment.

Drug Batch: Hirulog, Lot No.67Z02Z, 67Z05Z, 67Z06Z, 67Z07Z, 67Z08Z, and 67Z01Z (homogenous phase pilot scale).

Methods: In this specialized Segment I study, the effects of hirulog on fertility and reproductive performance were evaluated in male and female rats. Male and female rats received hirulog by the subcutaneous route of administration at doses of 0, 50, 150, and 500 mg/kg/day. Dose volumes for the doses of 0, 50, 150, and 500 mg/kg/day were 20, 2, 6, and 20 mL/kg, respectively. The vehicle was 0.9% NaCl. Multiple injections were made if necessary as injections were administered in 2 mL increments. Male rats received hirulog for 4 weeks prior to mating and through the mating period. Female rats received hirulog for 2 weeks (15 days) prior to mating, through the mating period, and from days 0 to 17 of gestation. Dose selection was based was based upon a range finding study in which male and female rats received hirulog by the subcutaneous route at doses of 0, 50, 150, and 500 mg/kg/day [Biogen Study No. P8967-93-06 (homogeneous phase pilot scale)]. In this range finding study, male and female rats received hirulog for

2 weeks prior mating, through the mating period, and from days 0 to 7 of gestation. At a dose of 500 mg/kg/day, the deaths 1 male (day 28) and 1 female (day 8 of gestation) appeared to be treatment-related. Body weight gain was impaired by >10% at 500 mg/kg/day for male rats following 28 days of treatments and for female rats from days 0 to 10 of gestation. The mating index, fertility index, and number of implantation sites/dam were similar at all dose levels. Spleen enlargement was observed at necropsy for several male and female rats at 500 mg/kg/day. Masses were observed on the backs of male and female rats treated with a dose of 500 mg/kg/day. In the present study, there were 25 rats/sex/group. Estrous cycling was evaluated for 14 days prior to the start of treatment and for another 14 days after the start of treatment. Rats were observed for signs of clinical toxicity and mortality at least twice a day. Observations for general health were performed twice during the acclimation period, twice prior to the start of treatment, daily during the treatment period, following the completion of treatment (female rats), and at the day of necropsy. Body weight were measured twice during the acclimation period, on a weekly basis prior to the start of treatment, daily during the treatment period, daily following the completion of treatment (female rats), and on the day of necropsy. Food consumption was measured weekly during the treatment period for male rats except during the mating period. Food consumption for female rats was measured weekly prior to mating and daily during the gestation period. Mating performance was evaluated daily during the period of cohabitation and confirmed by the observation of pregnancy (implantation sites present in utero on day 20 of presumed gestation). Male rats were sacrificed after the mating period and verification of pregnancy in all mated female rats. The testes and epididymides were excised and individually weighed. Evaluation of sperm number and viability was not performed. Testicular toxicity was not evident in any intravenous toxicity studies. The testes, epididymides, and any gross lesion were retained for possible histopathological analysis. Female rats were sacrificed on day 20 of gestation. The number of corpora lutea in each ovary and the number and distribution of implantation sites, early and late resorptions, and live and dead fetuses were noted. Gross lesions in female rats were retained for possible histopathological analysis. Each fetus was removed, individually weighed, and examined for sex and any gross external observations. Approximately one-half of the fetuses were examined for soft tissue alterations. The remaining fetuses were examined for skeletal alterations.

Results:

1. **Observed Effects**: Observations at injection sites on the backs of male and female rats that received hirulog at doses of 150 and 500 mg/kg/day included subcutaneous masses, areas of edema, generalized thickening of the skin, and hemorrhage starting on days 17 and 3, respectively, and persisting until the day of necropsy. A red perivaginal substance was observed for 1 female of the 150 mg/kg/day group (resorption of the entire litter) and 3 females of the 500 mg/kg/day group (partial resorption of the litter for 2 of 3 females). Paleness, possibly related to related to hemorrhage, was observed at 500 mg/kg/day for 2 females during the period prior to mating and 4 females during the gestation period.

2. **Mortality**: For the 500 mg/kg/day group, hemorrhage occurred at various sites and 2 male and 5 female rats died or were sacrificed in a moribund condition. Male rat #19081 was found dead on day 2 of the study. A necropsy examination revealed areas of subcutaneous hemorrhage and edema at three sites on the back. Further, congestion of the lungs and adrenal glands was found. Male rat #19094 was found dead on day 28 of the study. Necropsy revealed a solid red substances filling the abdominal cavity, which was considered secondary to intestinal and/or abdominal hemorrhage. Female rat #19178 was found dead after the 16th injection. This rat had a mass on its back from days 11 to 16. Necropsy revealed a dark red fluid and a semi-solid red substance filling the abdominal cavity, presumably due to hemorrhage. Female rat #19183 was sacrificed in a moribund condition (i.e., pale, cold to touch, and decreased motor activity) of day 6 of gestation. This rat had a mass on its back from days 12 to 17 that persisted to day 6 of gestation. Necropsy revealed a red substance, presumably hemorrhage, in the abdominal cavity. Its litter consisted of 15 embryos. Female rat #19187 was found dead on day 8 of gestation. This rat had a subcutaneous hemorrhagic mass on its back from days 4 to 17 that persisted to day 8 of gestation. Additional hemorrhage consisted of splenic enlargement and a small amount of red material in the stomach. Its litter consisted of 16 embryos. Female rat #19194 was found dead on day 4 of gestation. There was a mass present on the right side of its back on days 4 to 15 and persisted through days 0 to 3 of gestation. Necropsy found that the mass consisted of thickened, dark red skin. Its spleen was enlarged and pregnancy status was unknown as implantation had not yet occurred. Female rat #19199 was found dead on day 17 of gestation. This rat had a mass on its back from days 11 to 15 that persisted until day 3 of gestation. A mass consisting of a generalized thickening of the skin was found on day 16 of gestation. Necropsy revealed two masses on the back and

subcutaneous hemorrhagic areas. This animal also had a red perivaginal substance, an enlarged spleen, and numerous red spots on the surface of the thymus. Its litter consisted of 15 fetuses that appeared normal.

3. **Body Weight and Food Consumption**: Body weight gain and food consumption were decreased for the male 500 mg/kg/day group during the 28 day treatment period prior to mating. Body weight gains and food consumption for female treatment groups during the 2 week period prior to mating were unaffected; however, body weight gain and food consumption during gestation were decreased for the female 500 mg/kg/day group. Body weight gains during the 28 day treatment period prior to mating for the male 50, 150, and 500 mg/kg/day groups were 101.2, 98.3, and 67.4% of the control. Food consumption for male 500 mg/kg/day group during the 28 day treatment period prior to mating was decreased to 89.8% of the control (26.5 g/day). Body weight gains during days 0 to 20 of gestation for the female 50, 150, and 500 mg/kg/day groups were 109.1, 101.6 and 73.4% of the control, respectively. Food consumption for male 500 mg/kg/day group during the 28 day treatment period prior to mating was decreased to 89.7% of the control (26.5 g/day).

4. **Fertility and General Reproductive Performance**: At 500 mg/kg/day, the mating and fertility indexes were slightly reduced. The sponsor attributed these changes to altered mating behavior due to large masses on the backs of male and female rats, rather than systemic effects of hirulog. At 500 mg/kg/day, the female estrous cycle was unaffected during the 14 day period prior to mating. Further, the number of days until mating was not different between the control and 500 mg/kg/day groups. At 500 mg/kg/day, the number of corpora lutea, implantations, litter sizes, and live fetuses were reduced. Fetal body weight was slightly lower at 500 mg/kg/day. The number of late resorptions were increased at 500 mg/kg/day.

5. **Gross Pathology**: Gross pathological changes were primarily observed at 500 mg/kg/day. Many of the changes were related to hemorrhage induced by the pharmacological effects of hirulog.

Gross pathological changes observed for male and female rats (F_0 generation) that received hirulog by the subcutaneous route at doses of 0, 50, 150, and 500 mg/kg/day during a combined Segment I/ Segment II study.

Gross pathological change	0		50		150		500	
	M	F	M	F	M	F	M	F
Back: Masses and/or hemorrhage and/or skin, generalized thickening	0	0	0	0	8	17	21	24
Epididymides: left, yellow mass present on cauda and or caput	0	-	0	-	1	-	1	-
Red perivaginal substance/red substance in cage pan	-	0	-	0	-	0	-	1
Enlarged Spleen	0	0	0	0	C	0	0	6
Abdominal Cavity: red substance and/or dark red fluid	0	0	0	0	C	0	1	2
Stomach/Intestines: small amount of red material	0	0	0	0	C	0	1	1
Thymus: numerous red spots	0	0	0	0	C	0	0	1
Lungs: congested	0	0	0	0	C	0	1	0
Adrenal glands: congested	0	0	0	0	C	0	1	0

6. **Organ Weights**: The absolute right epididymis weight for the male 500 mg/kg/day group was decreased to 94.8% of the control (0.58 g). Testicular weight for males at 500 mg/kg/day was not significantly affected.

7. **Evaluation of Fetuses**: There were no gross external alterations for control or treatment groups. There were no dose-related soft tissue or skeletal malformations. There was an increased incidence of skeletal variation at 500 mg/kg/day that are generally associated with maternal toxicity. Fetal skeletal variations observed at 500 mg/kg/day included an increased incidence of right cervical rib at the 7th cervical vertebra and delayed sternal ossification. Delayed sternal ossification is generally reversible and interrelated to reduced fetal body weight. There was an increased incidence of wavy ribs, a skeletal variation, at 150 mg/kg/day; however, this change was not dose-dependent.

Fertility and general reproductive performance for male and female rats (F_0 generation) that received hirulog by the subcutaneous route at doses of 0, 50, 150, and 500 mg/kg/day during a combined Segment I/Segment II study.

Parameter	0	50	150	500
Female Estrous stages/14 days	3.2	3.2	3.6*	3.2
Mating Index	100% (25/25)	96% (24/25)	92% (23/25)	91.7% (22/24)
Fertility Index	100% (25/25)	95.8% (23/24)	91.3% (21/23)	85.7% (19/21)
Rats pregnant/rats in cohabitation	100% (25/25)	95.8% (23/24)	91.3% (21/23)	89.5% (18/21)
Days in cohabitation	2.2	2.3	2.8	2.4
No. female rats at day 20 caesarean section	25	23	21	15
Corpora lutea/dam	18.8	18.6	18.5	14.8*
Implantations/dam	16.2	15.9	16.2	12.0*
Litter size/dam	15.5	15.3	14.7	10.6*
Live fetuses/dam	15.5 (387/25)	15.3 (337/23)	14.7 (309/21)	10.6* (159/15)
Live male fetuses	209 (51.6%)	173 (51.3%)	156 (50.6%)	81 (50.3%)
Fetal body weight -male -female	 3.71 3.44	 3.62 3.40	 3.69 3.47	 3.54 3.12
Resorptions -total -early -late	 0.7 0.7 0	 0.6 0.6 0	 1.5 1.4 0	 1.4 0.7 0.7
± Resorbed conceptuses/litter	4.2 ± 5.8	3.9	3.7	15.1 ± 26.5
Dams with any resorptions	11 (44%)	9 (39.1%)	11 (52.4%)	8 (53.3%)
Dams with all conceptus resorbed	0	0	1 (4.8%)	1 (6.7%)
Dams with viable fetuses	25(100%)	23 (100%)	20 (95.2%)	14 (93.3%)

* $p < 0.05$

Skeletal alterations in F_1 fetuses derived male and female rats (F_0 generation) that received hirulog by the subcutaneous route at doses of 0, 50, 150, and 500 mg/kg/day during a combined Segment I/ Segment II study.

Parameter	0	50	150	500
Fetuses/Litters	200/25	181/23	158/20	84/14
Ribs				
Wavy ribs Fetuses/Litters	1 (0.5%) /1 (4.0%)	0	5 (5.2%) /3 (15%)*	0
Cervical rib at 7th Cervical Vertebra Fetuses/Litters	0	0	1 (0.6%) /1 (5%)	6 (7.1%)* /3 (21.4%)*
Sternal Centra Summarization (incomplete + not ossified) Fetuses/Litters	3 (1.5%) /2 (8.0%)	1 (0.6%) /1 (4.3%)	5 (3.2%) /4 (2.0%)	7 (8.3%)* /3 (21.4%)
Sternal Centra, not ossified Fetuses/Litters	0	1 (0.6%) /1 (4.3%)	1 (0.6%) /1 (5%)	4 (4.8%)* /2 (14.3%)

* $p < 0.05$

In a Segment I study, the effects of hirulog on fertility and reproductive performance were evaluated in male and female rats. Male and female rats received hirulog by the subcutaneous route of administration at doses of 0, 50, 150, and 500 mg/kg/day. Male rats received hirulog for 4 weeks prior to mating and through the mating period. Female rats received hirulog for 2 weeks (15 days) prior to mating, through the mating period, and from days 0 to 17 of gestation. Mating and general reproductive performance were unaffected at doses ≤150 mg/kg/day. At 500 mg/kg/day, the mating and fertility indexes were slightly reduced. Evaluation of sperm number and viability was not performed; although, testicular toxicity was not evident in any intravenous toxicity studies. At 500 mg/kg/day, the number of corpora lutea, implantations, litter sizes, and live fetuses were reduced. The number of late resorptions were increased at 500 mg/kg/day. There was an increased incidence of skeletal variation at 500 mg/kg/day that are generally associated with maternal toxicity. Fetal skeletal variations observed at 500 mg/kg/day included an increased incidence of right cervical rib at the 7th cervical vertebra and delayed sternal ossification.

Segment II Tetratogenicity Study in Rats (Biogen Study No. P8967-93-17).

Testing Laboratory: [redacted]

Study Started: December 13, 1993

Study Completed: October 3, 1995

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

Animals: Female Sprague Dawley rats were used in this study. Rats were 66 days old at arrival. At study assignment, the body weight range was 209 to 252 g.

Drug Batch: Hirulog, Lot No. 67Z01Z and 67Z02Z (homogenous phase pilot scale).

Methods: In a specialized Segment II toxicity study, potential teratogenic effects of hirulog were evaluated using Sprague Dawley rats. Hirulog was administered to 35 female rats per group from days 7 to 17 of gestation using the subcutaneous route of administration at doses of 0, 10, 50, and 150 mg/kg/day. At doses of 0, 10, 50, and 150 mg/kg/day, the dose volumes were 6, 0.4, 2, and 6 mL/kg, respectively. Dose selection was based upon a dose range finding study, in which 8 female rats per group received hirulog from days 7 to 17 of gestation by the subcutaneous route at doses of 0, 50, 150, and 500 mg/kg/day [Biogen Study No. P8967-93-07 (homogenous phase pilot scale)]. Animals were scheduled for sacrifice on day 20 of gestation to determine the number of corpora lutea, implantations, live and dead fetuses, and early and late resorptions. Two pregnant female rats of the 500 mg/kg/day group died on days 10 and 18 of gestation, respectively. Necropsy examination found that one female had a red substance at the subcutaneous injection site as well as approximately 3 mL of a red substance in the abdominal cavity, while the other rat had a red perivaginal substance. Another pregnant female rat of the 500 mg/kg/day group delivered prematurely on day 20 of gestation. This dam appeared pale and had a red substance in the vagina on days 18 and 19. Nine pups were dead and 3 late resorptions were evident at delivery. The liver of this animal was pale and tan with red areas on the lateral and medial lobes. Body weight gain for the female 50, 150, and 500 mg/kg/day groups were 95, 85, and 56% of the

control, respectively. Food consumption for the female 500 mg/kg/day group was reduced to 88.1% of the control (23.6 g/day). The number of corpora lutea, implantations, litter sizes, and live fetuses per dam were reduced for the 500 mg/kg/day group. Early resorptions and the percent resorbed conceptuses/litter were increased for 500 mg/kg/day group. Fetal body weights were reduced for the 500 mg/kg/day group. The dose of 150 mg/kg/day was selected as the high dose for the main study. In the present study, F_0 female rats were observed for clinical signs of toxicity and mortality twice daily during the treatment period and once daily following the treatment period. Body weight and food consumption were measured on days 0 and 7 of gestation and daily through day 20 for female rats sacrificed on day 20, through day 25 for female rats that failed to deliver a litter naturally, or through day 21 postpartum for female rats that delivered a litter naturally. Food consumption was not measured after day 14 of lactation due to consumption by both the dam and pups. Before day 20 of gestation, 23 F_0 pregnant female rats per group were selected for sacrifice. Remaining F_0 pregnant female rats in each group were allowed to deliver naturally. Female rats allowed to deliver naturally were evaluated for duration of gestation, length of parturition, litter sizes (live and dead pups), and pup viability. Maternal behavior of the dams was evaluated daily during the 21 day postpartum period and recorded on days 1, 4, 7, 14, and 21 postpartum. During the 21 day postpartum period, pups were counted and physical signs including gross external physical anomalies were recorded daily. On days 1, 4, 7, 14, and 21 postpartum, pup weight and sex as well as nursing behavior were recorded. Reflex and physical development parameters (i.e., surface righting reflex, pinna unfolding, eye opening, acoustic startle, and air righting reflex) in F_1 pups were monitored during the 21-day postpartum period and recorded on days 1, 2, 12, 13, and 14 postpartum. Developmental parameters were monitored in each litter on a daily basis until all pups in the litter reached a specified criterion for each test. Pupil constriction was evaluated on day 21 postpartum. On day 7 postpartum, litters were culled to 8 F_1 pups (4 male and 4 female pups) each if possible. On day 21 postpartum, 24 F_1 rats/sex/group were selected for continued evaluation. Also on day 21, F_0 female rats that delivered naturally were sacrificed and examined for gross lesions. Postweaning observations began on day 22. F_1 rats were monitored for viability twice daily and for general health once daily. Sexual maturation in F_1 male rats was monitored daily to identify testicular descent and preputial separation beginning days 22 and 35 postpartum, respectively. For F_1 female rats, vaginal patency was monitored daily beginning on day 28 postpartum. Body weights and food consumption for F_1 male and female rats were measured weekly. Beginning on day 22

postpartum, F_1 male and female rats from each dosage group were tested for learning, short-term retention, long-term retention, and response inhibition in a passive avoidance paradigm. At day 70 of age, 1 F_1 rat/sex/litter from each dosage group was evaluated for overt coordination, swimming ability, learning and retention in a water filled stainless-steel modified M-maze. At 90 days of age, F_1 male and female rats of each dosage group entered a 21 day cohabitation period (1 to 1 ratio). Female rats that did not mate during first 14 days were assigned to an alternate male that had already mated. After cohabitation, F_1 male rats were sacrificed and the testes and epididymides were weighed and preserved along with any gross lesions. Body weights and food consumption were measured on days 0, 7, 14, and 20 of gestation for pregnant F_1 female rats. F_1 female rats were sacrificed on day 20 of gestation and the uteri contents were examined. F_2 fetuses were removed from uteri and individually weighed and examined for gross external alterations. F_2 fetuses were processed and preserved for possible soft tissue and skeletal evaluations. For the 23 F_0 female rats/group sacrificed on day 20 of gestation, the number of corpora lutea, implantations, live and dead fetuses, early and late resorptions were determined for each dam. Each fetus was removed from the uterus, weighed, and examined for sex and gross external alterations. Approximately one-half of the F_1 fetuses in each litter were examined for soft tissue alterations. Remaining F_1 fetuses were examined for skeletal alterations.

Results: Pregnant F_0 dams that received hirulog at doses of 50 and 150 mg/kg/day had local reactions at injection sites (i.e., masses on the back during gestation and lactation) in 1 and 21 animals, respectively. For 8 dams necropsied on day 20 of gestation, masses appeared firm and dark or dark red with firm, red cut surfaces. Body weight gains for pregnant F_0 dams were not affected by treatment. For F_0 pregnant females at doses ≤150 mg/kg/day and sacrificed on day 20 of gestation, there were no treatment-related alterations of litters parameters. Further, there were no treatment-related gross external, soft tissue, or skeletal malformations or variations of F_2 fetuses. For F_0 pregnant females at doses ≤150 mg/kg/day and allowed to deliver naturally, the duration of gestation, delivery period, implantations/dam, live pups/dam, male to female pup ratio, pup viability index at days 7 and 21, and pup weight on days 1, 7, and 21 were unaffected by treatment. The live birth index for F_1 pups from F_0 dams treated with a dose of 150 mg/kg/day was slightly reduced to 86.5% as compared to a control value of 92.0%. Body weight gains for F_0 dams during lactation was unaffected. Reflex and physical development and sexual maturation of F_1 rats were unaffected.

Learning, short- and long-term retention, and response inhibition in a passive avoidance paradigm for F_1 rats were unaffected. Overt coordination, swimming ability, learning and retention in a water filled stainless-steel modified M-maze for F_1 rats were unaffected. Fertility and reproductive performance for F_1 rats were unaffected. Body weight gain and food consumption for pregnant F_1 female rats during the period of gestation were unaffected. Litter parameters for F_1 female rats were unaffected. Body weights for F_2 fetuses were unaffected. F_2 fetuses had no treatment-related external alterations.

Litters for pregnant F_0 female rats that received hirulog by the subcutaneous route at dose of 0, 10, 50, and 150 mg/kg/day from days 7 to 17 of gestation F_0 female subjected to caesarean section on day 20).

Parameters	0	10	50	150
Pregnant rats on day 20 of gestation	22	23	21	21
Corpora lutea/dam	18.1	17.3	17.0	17.0
Implantations/dam	15.0	14.2	13.8	13.9
Litter size/dam	14.6	13.8	13.4	13.3
Live fetuses/dam	14.6 (321/22)	13.8 (317/23)	13.4 (281/21)	13.3 279/21)
Resorptions -total -early -late	 0.4 0.4 0	 0.4 0.4 0	 0.4 0.4 0	 0.6 0.6 0
Live male fetuses	55.5% (177)	51.6% (163)	48.5% (138)	49.8% (137)
Fetal body weight -male -female	 3.55 3.40	 3.60 3.44	 3.53 3.33	 3.58 3.45
% Dead or resorbed conceptuses/litter	2.5	2.7	3.2	4.5

Gross external alterations of F_1 fetuses derived from pregnant F_0 female rats that received hirulog by the subcutaneous route at dose of 0, 10, 50, and 150 mg/kg/day from days 7 to 17 of gestation (F_0 female subjected to caesarean section on day 20).

Parameter	0	10	50	150
Fetuses/Litters	321/22	317/23	281/21	279/21
Eye: Bulge, depressed Fetuses/Litters	0	0	1 (0.4%) /1 (4.8%)	0
Snout: Appears divided Fetuses/Litters	0	0	1 (0.4%) /1 (4.8%)	0
Ears: Low set Fetuses/Litters	0	0	1 (0.4%) /1 (4.8%)	0
Jaw: Agnathia Fetuses/Litters	0	0	1 (0.4%) /1 (4.8%)	0

Soft tissue alterations of F_1 fetuses derived from pregnant F_0 female rats that received hirulog by the subcutaneous route at dose of 0, 10, 50, and 150 mg/kg/day from days 7 to 17 of gestation (F_0 female subjected to caesarean section on day 20).

Parameter	0	10	50	150
Fetuses/Litters	158/22	153/23	136/21	135/21
Brain:				
Third ventricle, moderate dilation Fetuses/Litters	0	0	0	1 (0.7%) /1 (4.8%)
Lateral ventricles, marked dilation Fetuses/Litters	0	0	0	1 (0.7%) /1 (4.8%)
Diaphragm				
Diaphragmatic hernia Fetuses/Litters	0	0	0	1 (0.7%) /1 (4.8%)
Kidneys				
Pelvis, slight to moderate dilation Fetuses/Litters	0	0	1 (0.7%) /1 (4.8%)	1 (0.7%) /1 (4.8%)