following either intravenous or subcutaneous administration, which might be consistent with degradation of the peptide and reincorporation of amino acids into new proteins. However, total clearance of ^{3}H-radiolabel was high following either intravenous or subcutaneous administration, exceeding both hepatic blood flow (55.2 mL/min/kg) and renal blood flow (36.8 mL/min/kg), suggesting metabolic clearance. Mean residence time (MRT) of ^{14}C-radiolabel was relatively long in comparison with ^{3}H radiolabel, which, again, might be consistent with degradation of the peptide and reincorporation of amino acids into new proteins. Tissue levels of radioactivity were examined 24 hr after intravenous administration. ^{3}H was confined to kidney, while ^{14}C was widely distributed in several tissues. Distribution of radioactivity was similar following subcutaneous administration. The tissue to plasma ratio for ^{14}C-radiolabel was extremely high for the kidney following either intravenous or subcutaneous administration, suggesting concentrating of hirulog into this organ, which may include metabolism and excretion. Isoelectric focusing of urine from 1 rat suggested <u>in vivo</u> cleavage of the peptide into two or more metabolites as ^{14}C activity and ^{3}H activity were separated from one another.

Pharmacokinetic results for ^{14}C and ^{3}H concentrations in blood and plasma of rats given a 3 mg/kg intravenous dose of hirulog.

Parameter	Blood (^{14}C)	Plasma (^{14}C)	Blood (^{3}H)	Plasma (^{3}H)
C_{max}, cpm/mL	5757.6	7532.1	2618.5	3433.1
T_{max}, hr	0.08	0.08	0.08	0.08
$AUC_{0-\infty}$, cpm·hr/mL	16949	14216	1151	1404
$T_{1/2}$, hr	0.33	0.26	0.30	0.27
MRT, hr	11.0	5.6	0.26	0.25
Cl mL/min/kg	4.1	4.5	90.3	50.2
Vd_{ss}, L/kg	2.7	2.8	0.92	0.74
Cl mL/min/kg (?)	0.016(?)	0.019(?)	0.556(?)	0.451(?)

(?) It was difficult to read this section and understand what it represented.

Pharmacokinetic results for ^{14}C and ^{3}H concentrations in blood and plasma of rats given a 3 mg/kg subcutaneous dose of hirulog.

Parameter	Blood (^{14}C)	Plasma (^{14}C)	Blood (^{3}H)	Plasma (^{3}H)
C_{max}, cpm/mL	4764.7	5718.4	1943.9	2909.0
T_{max}, hr	0.25	0.29	0.29	0.29
AUC_{0-t}, cpm*hr/mL	122211	71739	3774	3836
$T_{\frac{1}{2}}$, hr	2.3	1.7	1.7	1.0
MRT, hr	34.5	26.3	2.73	0.92
Cl, mL/min/kg	0.97	1.62	35.3	31.0
Vd_{ss}, L/kg	2.00	2.56	3.36	1.67
Cl, mL/min/kg (A)	0.017 (A)	0.031(A)	1.175 (A)	1.071 (A)

(A) It was difficult to read this section and understand what it represented.

^{14}C and ^{3}H concentration (cpm/g) in tissues of rats 24 hr following administration of a 3 mg/kg intravenous or subcutaneous dose of hirulog. The percent of dose is represented in parentheses.

Tissue	Intravenous			Subcutaneous		
	^{14}C (cpm/g)	^{14}C Tissue: Plasma Concent-ration	^{3}H (cpm/g)	^{14}C (cpm/g)	^{14}C Tissue: Plasma Concent-ration	^{3}H (cpm/g)
Brain	147 (0.034)	0.43	0	262.1 (0.038)	0.79	0
Heart	537 (0.051)	1.56	0	1050.3 (0.056)	3.17	0
Kidney	4690.5 (0.864)	13.57	244.0 (0.045)	5069.0 (0.710)	15.18	219.4 (0.076)
Liver	1133.4 (0.685)	3.25	17.5 (0.011)	1281.6 (0.719)	3.85	0
Spleen	1493.9 (0.099)	4.35	0	1510.7 (0.065)	4.55	0
Lymph node	456.4 (0.002)	1.34	0	639.8 (0.005)	1.94	0
Lung	879.9 (0.123)	2.54	9.3 (0.001)	1433.9 (0.119)	4.35	0
Thymus	1264.9 (0.075)	3.66	0	1961.7 (0.046)	5.90	0
Pancreas	825.3 (0.040)	2.43	122.0 (0.006)	1042.5 (0.021)	3.14	0
Total	(1.972%)		(0.063)	(1.781%)		(0.076%)

Pharmacokinetic and Tissue Distribution Study of $^{3}H/^{14}C$-Labeled Hirulog Following a Single Intravenous Administration to Sprague Dawley Rats (Biogen Study No. P8967-93-13).

Methods: The pharmacokinetics and tissue distribution of a 1 to 1 mixture of ^{3}H-hirulog and ^{14}C-hirulog were examined in rats following a single bolus intravenous administration at 10 mg/kg. The ^{3}H label was located on the proline$_2$ amino acid, while the ^{14}C was incorporated into glycine residues contained within the terminal 18 amino acid portion. The structures of these radiolabeled molecules are shown at the beginning of the ADME section. The ^{3}H-labeled D-phe hirulog was tested to evaluate the distribution and excretion of the proximal (or amino terminus) D-phe-pro portion of the 20 amino acid peptide, because other studies suggested that this portion of the molecule may be cleaved following administration. The ^{14}C-labeled hirulog was tested to trace the remaining portion of the peptide. The study consisted of 5 groups of five male rats/group. The dosing volume was 2 mL/kg. Blood for analysis of plasma radiolabel were collected prior to treatment, and at 2, 5, 10, 30, and 40 min and 1, 2, 4, 6, 8, and 24 hr after dosing. Samples for tissue distribution of radioactivity were collected at 0.5, 6, and 24 hr after dosing. Tissues included the adrenal glands, bone (femur), bone marrow (femur), brain, esophagus, fat (mesenteric), heart, large intestine, small intestine, kidneys, liver, lungs, lymph nodes (mandibular and mesenteric), pancreas, skeletal muscle, skin, spleen, stomach, testes, thymus, and urinary bladder. Urine and fecal samples were collected prior to treatment and at intervals of 0-4 hr, 4-8 hr, and 8-24 hr after dosing (Reported in Excretion section).

Results: Plasma ^{3}H levels decreased steadily over the first 2 hr following treatment, and then fell below the limit of detection. Plasma ^{14}C levels paralleled the decrease in ^{3}H levels for the first 40 min following dosing and then remained at a constant level for remaining period of the 24 hr study. In the initial phase of elimination of radioactivity from the plasma, half lives for ^{3}H and ^{14}C radiolabel were similar at 0.21 and 0.19 hr, respectively. A slower secondary elimination phase was observed for ^{14}C radiolabel with a half life of 34.02 hr. This prolonged elimination phase is probably due to degradation of the peptide into amino acids and reincorporation into new proteins. ^{14}C radiolabel was widely distributed in tissue at significant levels, while the distribution of ^{3}H radiolabel was small in comparison. ^{3}H radiolabel was found in high levels in the kidneys. The volume of distribution for ^{14}C radiolabel (1864.9 mL/kg) suggests distribution beyond the blood volume (54 mL/kg) into tissues. Clearance for ^{14}C radiolabel (38.7 mL/hr/kg) was lower than glomerular filtration rate (181.65 mL plasma/hr/kg), hepatic plasma flow (1913.6 mL/hr/kg) or renal plasma flow (1275.7 mL/hr/kg), which may, again, be indicative of degradation of the peptide and subsequent reincorporation of amino acids into new proteins.

Pharmacokinetic parameters for ^{3}H-hirulog and ^{14}C-hirulog administered in a 1 to 1 mixture to male rats by a single bolus intravenous injection.

Parameter	^{3}H	^{14}C
AUC_{0-2h} (DPM*hr/mL)	150567	124591
AUC_{0-24h} (DPM*hr/mL)	-	764361
$T_{1/2\alpha}$, hr	0.21	0.19
$T_{1/2\beta}$, hr	-	34.02
Mean Residence Time (hr)	-	48.15
V_{ss} (mL/kg)	-	1864.9
Cl_{tot} (mL/hr/kg)	-	38.7

Predominant tissue distribution of ^{14}C and ^{3}H radiolabel at 0.5, 6, and 24 hr after administration of a 1 to 1 mixture of ^{3}H-hirulog and ^{14}C-hirulog to rats by a single bolus intravenous injection.

Tissue	0.5 hr		6 hr		24 hr	
	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H
Bone (femur)	6.63	1.04	5.37	0.13	4.91	0.05
Bone marrow	1.59	0.07	8.79	0.46	0.99	0.01
Kidneys	5.13	2.89	2.09	0.04	2.20	0.02
Liver	3.80	1.56	3.97	0.15	2.76	0.03
Skeletal muscle	9.28	2.54	16.65	1.76	16.33	0.86
Skin	5.98	1.81	9.09	0.62	8.39	0.14
Spleen	0.26	0.01	3.55	0.04	0.59	0.01

Pharmacokinetic and Tissue Distribution Study of a ^{3}H-Labeled Dipeptide Following a Single Intravenous Administration to Sprague Dawley Rats (Biogen Study No. P8967-94-10).

Methods: The pharmacokinetics and tissue distribution of [^{3}H-labeled D-Phenylalanine]-Proline Dipeptide ([^{3}H-D-Phe]-Pro) was evaluated in Sprague Dawley rats following a single bolus intravenous administration of 1 mg/kg. There were four groups of 5 male rats/group. The dose volume was 2.2 mL/kg. Blood for determination of plasma hirulog levels was collected at 2, 5, 10, 15, 20, 30, 40, and 60 min and at 2, 4, 6, and 12 hr after dosing.

Distribution of radioactivity into selected tissue (i.e., adrenal glands, bone (femur), bone marrow (femur), brain, esophagus, fat (mesenteric), heart, large intestine, small intestine, kidneys, liver, lungs, mandibular and mesenteric lymph nodes, pancreas, skeletal muscle, skin, spleen, stomach, testes, thymus, and urinary bladder) was evaluated at 0.5, 2, 6, and 12 hr after treatment. Radioactivity excreted into urine and feces was evaluated at the following intervals: 0-3, 3-6, and 6-12 hr after dosing.

Results: Following intravenous administration of [^{3}H-D-Phe]-Pro, the distribution α phase had a half life of 0.03 hr. The interim β phase had a half life of 0.44 hr. By 30 min after dosing, 90% of the radioactivity dose was cleared from blood, suggesting rapid clearance during the β phase. The terminal γ phase had a half life of 12 hr, during which the remaining 10% of radioactivity in the blood was cleared. Clearance was greater than the glomerular filtration rate (314 mL/hr/kg), but less than hepatic blood flow (3312 mL/hr/kg) or renal blood flow (2208 mL/hr/kg). The volume of distribution suggested distribution into tissues beyond the blood volume (54 mL/kg). Distribution of radioactivity was found in the bone marrow, small intestines, kidneys, skeletal muscle, and skin. Radioactivity was primarily excreted into the urine.

Pharmacokinetic parameters for [^{3}H-D-Phe]-Pro in Sprague Dawley rats following intravenous administration at 1 mg/kg.

Parameter.	Whole Blood
$T_{1/2\alpha}$, hr	0.03
$T_{1/2\beta}$, hr	0.44
$T_{1/2\gamma}$, hr	12.0
AUC, (DPM*hr/mL)	214111
V_{ss} (mL/kg)	6616
Cl_{obs} (mL/hr/kg)	788
MRT (hr)	8.4

Tissue distribution of radioactivity following treatment of rats with [^{3}H-D-Phe]-Pro by intravenous administration at 1 mg/kg expressed as percent of dose.

Tissue	0.5 hr	2 hr	6 hr
Bone marrow	0.61	3.06	0.34
Small Intestines	2.12	2.24	0.73
Kidneys	1.48	0.32	0.73
Skeletal muscle	11.39	3.81	1.48
Skin	5.76	1.87	0.88
Plasma	1.30	0.20	0.07
Blood	2.02	0.37	0.12

Excretion of radioactivity into feces and urine following treatment of rats with [^{3}H-D-Phe]-Pro by intravenous administration at 1 mg/kg expressed as cumulative percent of dose.

Time (hr)	Feces	Urine	Total (Feces+Urine)
1-3	0.01	33.72	33.73
3-6	0.10	75.93	76.03
6-12	6.85	124.08[A]	130.93[A]

A. Recovery values exceeding 100% are due to significant variations in recovery for animals used in this study (i.e., large experimental errors).

Metabolism:

In Vitro

Stability of Hirulog in Physiological Fluids (Study Report No. TR-10a).

Methods: The stability of ^{14}C-hirulog (or ^{3}H-hirulog in some cases) was evaluated in various physiological fluids [i.e., citrated human plasma, citrated cynomolgus monkey plasma, citrated rat plasma, citrated human whole blood, heparinized human whole blood, human urine, and phosphate-buffered saline (control)] to determine their contribution to proteolysis, which may account for hirulog catabolism. Hirulog (homogenous phase commercial scale) was incubated at a concentration of 0.015 mg/mL (this concentration has been measured in plasma during in vivo administration in patients undergoing coronary angioplasty) at 37°C and samples were collected at 0.5, 4, and 24 hr. Reverse-phase —— with a C_{18} column and radioisotope detection was used to analyze ex vivo degradation.

Results: Hirulog was resistant to degradation in phosphate-buffered saline, citrated plasma from human, cynomolgus monkey, and rat sources, and human whole blood treated with citrate or heparin. These results suggest that degradation of hirulog in the vascular compartment (i.e., blood) does not play a significant role in the clearance or metabolism of hirulog administered in vivo. Significant degradation of hirulog occurred in human urine with 18.63% of the total remaining after 24 hr.

Stability of Hirulog in Cell Culture Media for Mutagenicity Studies #P8967-94-03 and #P8967-94-04 (Report No. TR-10-b).

Methods: The stability of ^{14}C-hirulog was evaluated in various cell media [i.e., phosphate-buffered saline (control), serum-free HAM's medium, serum-free HAM's medium with S-9, RPMI medium with 15% fetal calf serum, and serum-free RPMI medium with S-9] used in mutagenicity studies. ^{14}C-Hirulog was incubated in medium at 37°C and aliquots were collected at 0, 0.5, 4, and 24 hr. Samples were analyzed by ——— with on-line radioisotope detection.

Results: Hirulog (homogenous phase commercial scale) was stable in phosphate-buffered saline, serum-free HAM's medium, and RPMI medium w/S-9. Significant degradation of hirulog occurred in serum-free HAM's w/S-9 as it was completely metabolized by 4 hr. By 24 hr, 4 degradation products were observed. Degradation of hirulog also occurred in RPMI medium with 15% fetal calf serum. HAM's medium was used for the CHO/HGRPT assay. RPMI medium w/15% fetal calf serum was used in the human lymphocyte cytogenetics assay. The differences in degradation of hirulog between serum-free HAM's w/S-9 and RPMI medium w/S-9 was not explained.

Rats

Preparation and Characterization of Rat Liver Microsomal Fractions Obtained Following a 28-Day Continuous IV Infusion in Rats with Hirulog (Biogen Study No. P8967-94-06).

Methods: The effects of repeated treatment with hirulog on cytochrome P450 enzymes CYP1A, CYP2B, CYP2E, CYP3A, and CYP4A were evaluated in male and female Sprague Dawley rats. Ten rats/sex/group were administered hirulog (homogenous phase commercial scale) by continuous intravenous infusion at doses of 0, 25, 75, and 250 mg/kg/day for 28 days. This study was part of a larger toxicology study conducted by the sponsor (Biogen Study No. P8967-94-02) and reviewed later in this document. Five rats/sex/group from the control and 250 mg/kg/day group entered a 14 day recovery

period. Liver sections were collected at scheduled terminations of the 28 day treatment period and the 14 day recovery period. The following hepatic microsomal parameters were quantified: protein content, total cytochrome P450 content, and the activities of 7-ethoxyresorudfin O-deethylase (CYP1A), testosterone 6β- and 16β-hydroxylase (CYP3A and CYP2B, respectively), chlorzoxazone 6-hydroxylase (CYP2E), lauric acid 11-hydroxylase (CYP2E), and lauric acid 12-hydroxylase (CYP4A). Liver samples from 9 animals in each dose group were divided into 3 groups (3 liver samples per group) and processed into microsomes to provide 3 pooled microsomal samples per sex per dose group. During the pooling process, liver samples from the 14-day recovery animals were inadvertently included in the control and 250 mg/kg/day groups. The ratio of recovery group rats to treatment group rats were as follows: male control group (2/9); female control group (1/9); male 250 mg/kg/day group (1/9); and female 250 mg/kg/day group (2/9).

Results: Protein and cytochrome P-450 content were unaffected by treatment with hirulog for 28 days. The activities of CYP1A, CYP3A, CYP2B, and CYP2E were also unaffected by treatment with hirulog for 28 days. The activity of lauric acid 12-hydroxylase (an index of CYP4A) for male rats that were treated with hirulog at 250 mg/kg/day for 28 days was elevated to 232.8% of the control (1.34 nmol metabolite/mg/min); however, activity for the female 250 mg/kg/day group was unaffected. Activation of CYP4A may potentially be related to increased peroxisomal proliferative activity. The transcription factor that activates the CYP4A genes is the peroxisome proliferator-activated receptor, a member of the steroid/thyroid/retinoid superfamily of nuclear receptors that regulates transcription of the genes for fatty acyl-CoA oxidase, bifunctional enzyme (enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase) and fatty acid binding protein (J. Biol. Chem. 267: 10951-10953, 1992; and Xenobiotica 24: 943-956, 1994). Histopathological findings for the liver of animals treated with hirulog at 250 mg/kg/day for 28 days included sinusoidal histiocytosis and necrosis. The results may be questionable due to mixing of samples from the treatment and recovery control and 250 mg/kg/day groups. No results were shown for recovery group animals.

Excretion:

Rats

Absorption, Distribution, Metabolism, and Excretion Study in Albino Rats Administered ^{14}C and ^{3}H Labeled Hirulog by Intravenous and Subcutaneous Injection (Biogen Study No. P90-035).

Methods: The absorption, distribution, metabolism, and excretion of dual labeled hirulog (^{3}H and ^{14}C; solid phase peptide method) was examined in rats following intravenous or subcutaneous administration of a dose at 3 mg/kg. Following intravenous administration, urine and feces were collected at 0-4 hr, 4-8 hr, 8-24 hr, and 0-24 hr. Following subcutaneous administration, urine and feces were collected at 0-4 hr, 4-8 hr, 8-24 hr, 0-24 hr, 24-48, and 48-72 hr.

Results: Less than 5 to 6% of the hirulog dose at 3 mg/kg following either intravenous or subcutaneous administration was eliminated in the urine and feces combined. This low recovery may be suggestive that the peptide was degraded into individual amino acids and used for new protein synthesis in various organs and tissues. It should be noted that these results are not in agreement with results of the next study (Biogen Study No. P8967-93-13), where ~100% of the ^{3}H radiolabeled intravenous dose and ~23.6 of ^{14}C radiolabeled intravenous dose were recovered in the urine. A dose of 10 mg/kg was used in the next study, whereas, in the present study, only 3 mg/kg was used.

^{14}C and ^{3}H recoveries expressed as % of dose in urine and feces of rats following administration of a 3 mg/kg intravenous dose of hirulog.

Interval	^{14}C Recovery	^{3}H-Recovery	Interval	^{14}C Recovery	^{3}H-Recovery
	Urine	Urine		Feces	Feces
0-4 hr	0	0	0-4 hr	0.002	0
4-8 hr	0	0	4-24 hr	0.036	0.259
8-24 hr	0.2762	0.911			
Total	0.3059	1.015	Total	0.038	0.259

^{14}C and ^{3}H recoveries (% Of Dose) in urine and feces of rats following administration of a 3 mg/kg subcutaneous dose of hirulog.

Interval	^{14}C Recovery		^{3}H-Recovery	
	Urine	Feces	Urine	Feces
0-4 hr	0	0.001	0	0.001
4-8 hr	0.328	0.006	0.779	0.010
8-24 hr	0.739	0.138	2.131	0.726
24-48 hr	0.391	0.508	0.283	1.356
48-72 hr	0.324	0.165	0.017	0.060
Total	1.782	0.817	3.210	2.153

Pharmacokinetic and Tissue Distribution Study of $^{3}H/^{14}C$-Labeled Hirulog Following a Single Intravenous Administration to Sprague Dawley Rats (Biogen Study No. P8967-93-13).

Methods: The pharmacokinetics and tissue distribution of a 1 to 1 mixture of ^{3}H-hirulog and ^{14}C-hirulog were examined in rats following a single bolus intravenous administration at 10 mg/kg. The ^{3}H label was located on the proline$_2$ amino acid, while the ^{14}C was incorporated into the terminal 18 residue peptide. Urine and fecal samples were collected prior to treatment and at intervals of 0-4 hr, 4-8 hr, and 8-24 hr after dosing.

Results: Almost all of the ^{3}H radiolabel was recovered in the urine. These results suggests that the two amino acid portion of the N-terminal end, phenylalanine-^{3}H-proline, was cleaved off the peptide and excreted into the urine. Only 21.9 to 24.9% of the remaining 18 amino acid portion containing the ^{14}C radiolabel was excreted into the urine and feces. These results appear to further strengthen the sponsor's contention that the remaining 18 amino acid portion of the peptide was degraded and utilized for new protein synthesis. As noted earlier, these results are not in agreement with the previous study where extremely low recoveries of the ^{3}H- and ^{14}C-radiolabeled dose were found in the urine and feces. The dose for the earlier study was 3 mg/kg, whereas, in the present study, a dose of 10 mg/kg was used.

Cumulative percent of dose excreted following treatment of rats with a 1 to 1 mixture of ^{3}H-hirulog and ^{14}C-hirulog administered in a single bolus intravenous injection.

Interval	^{14}C Activity			^{3}H Activity		
	Feces	Urine	Total	Feces	Urine	Total
0-4	0.0	21.9	21.9	0.1	97.3	97.4
4-8	0.1	22.6	22.7	0.2	100.6	100.8
8-24	1.3	23.6	24.9	4.3	104.1[A]	108.4[A]

A. Recovery values exceeding 100% are due to significant variations in recovery for animals used in this study (i.e., large experimental errors).

A Pharmacokinetics Study of ^{3}H-Radiolabeled Hirulog in Partially Nephrectomized Sprague Dawley Rats (Biogen Study No. P8967-92-03).

Methods: The pharmacokinetic profile of ^{3}H-Hirulog was evaluated in partially nephrectomized Sprague Dawley rats following intravenous or subcutaneous administration to determine the role of renal function in the disposition of hirulog. Male rats were divided into 4 groups of 7 to 8 animals each. Six to eight days prior to dosing, animals in groups 2 and 4 underwent partial nephrectomy (i.e., removal of the right kidney and induction of ischemia in approximately two-thirds of the left kidney), while animals in groups 1 and 3 underwent sham surgery. Blood for determination of blood urea nitrogen and creatinine was collected 4 days prior to surgery and 3 days after surgery. Groups 1 and 2 received ^{3}H-hirulog at 4 mg/kg by the intravenous route of administration, while groups 3 and 4 received ^{3}H-hirulog at 4 mg/kg by the subcutaneous route of administration. The dosing volume was 3.5 mL/kg. For animals that received hirulog by the intravenous route, blood for determination of plasma hirulog levels was collected prior to dosing and at 0.083, 0.25, 0.5, 1, 3, 5, 9, 24, and 48 hr after dosing. For animals that received hirulog by the subcutaneous route administration, blood for determination of plasma hirulog levels was collected prior to dosing and at 0.25, 0.5, 1, 2, 5, 8, 24, and 48 hr after dosing. Total voided urine was collected at intervals of 0-6, 6-12, 12-24, 24-36, and 36-48 hr after dosing. Selected tissues were analyzed for ^{3}H activity (i.e., brain, heart, kidneys, liver, lungs, mandibular lymph nodes, mesenteric lymph nodes, pancreas, pituitary gland, seminal vesicles, spleen, and thymus).

Results: For nephrectomized rats, blood urea nitrogen and creatinine levels were elevated to 38 and 1.05 mg/dL, respectively, indicative of nephrectomy as compared to control values (15.5 and 0.6 mg/dL). Plasma C_{max} values for hirulog were higher in nephrectomized rats than sham-treated rats; however, no conclusion could be drawn from AUC and clearance data. Following intravenous or subcutaneous administration of ^{3}H-Hirulog, the percent of the administered dose excreted into the urine from 0-24 hr was lower in nephrectomized rats than in sham-treated rats. However, for urine collected for the 0-48 hr interval following intravenous administration, there was no difference between excretion between sham-treated and nephrectomized rats. Following intravenous or subcutaneous administration of ^{3}H-Hirulog to sham-treated rats, measurable radioactivity was recovered only from the kidneys (0.17-0.19 and 0.13% of the administered dose at 24 and 48 hr after dosing, respectively). Radioactivity in other tissues was below the limit of detection. Following intravenous or subcutaneous administration of ^{3}H-Hirulog to nephrectomized rats, radioactivity levels in ischemic kidney tissue was 0.07-0.08 and 0.03-0.08% of the administered dose at 24 and 48 hr, respectively; however, radioactivity levels in healthy kidney tissue was similar to that observed in sham-treated animals.

Pharmacokinetics of plasma hirulog following a single intravenous or subcutaneous injection of Hirulog in rats having undergone partial nephrectomy or sham surgery.

Group	Route	Surgical Procedure	C_{max} ng/mL	T_{max}, min	AUC_{0-48hr} ng*hr/mL	Clearance mL/hr/kg
1	IV	Sham	14141	5	17506	228
2		Nephr-ectomy	25899	5	28711	139
3	SC	Sham	2129	30	18318	218
4		Nephr-ectomy	5228	15	19507	205

Urinary excretion of radiolabel expressed as percent of administered dose following intravenous or subcutaneous administration of ^{3}H-Hirulog to sham-treated or nephrectomized rats.

Interval, % cumulative excretion	Intravenous Route		Subcutaneous Route	
	Sham	Nephrectomy	Sham	Nephrectomy
0-24 hr	39.77	24.58	35.37	11.63
0-48 hr	35.87	35.58	29.68	12.95

Monkeys

Metabolism and Excretion Study of $^{3}H/^{14}C$-Labeled Hirulog Following a Single Bolus Intravenous Administration in Cynomolgus Monkeys (Biogen Study No. P8967-93-05).

Methods: The pharmacokinetics and excretion of 1 to 1 mixture of ^{3}H-labeled and ^{14}C-labeled Hirulog were evaluated in cynomolgus monkeys following bolus intravenous administration of a dose at 10 mg/kg. The ^{3}H label was located on the proline$_2$ amino acid, while the ^{14}C was incorporated into the terminal 18 residue peptide. The study consisted of one group of two male and two female monkeys. Following intravenous administration, blood for determination of radioactivity levels was collected prior to the start of treatment and at 2, 5, 10, 20, 30, 40, and 60 min and at 2, 4, 6, 8, and 24 hr following administration. Urine and feces were collected at intervals of 0-4, 4-8, and 8-24 hr after dosing. Animals were sacrificed at 24 hr without further examination.

Results: Clearance of ^{3}H radiolabel was biphasic (phases α and β), while clearance of ^{14}C radiolabel was triphasic (phases α, β, and γ). Plasma ^{3}H radiolabel was measurable for 4 to 6 hr, while ^{14}C radiolabel was measurable for the entire 24 hr study period. ^{3}H radiolabel distributes beyond the blood volume (54 mL/kg) into the tissues and its half life of elimination was 0.58-0.64 hr. Clearance of ^{3}H radiolabel was very similar to the glomerular filtration rate for the kidney (181.65 mL plasma/hr/kg); although, it is significantly less than renal plasma flow (1275.7 mL plasma/hr/kg) or hepatic plasma flow (1913.6 mL plasma/hr/kg). ^{14}C-radiolabel distributed more extensively than ^{3}H radiolabel. Distribution of ^{14}C radiolabel into the tissues (α phase) had a half life ranging from 0.045 to 0.0595 hr. Further, its terminal half life of elimination (γ phase) was significantly longer at 41.5 to 65.6 hr, and its clearance was much lower at 15.45-20.2 mL/hr/kg. The mean residence time for ^{14}C-radiolabel ranged from 57.4 to 90.35 hr. The extensive distribution and slow elimination of ^{14}C radiolabel appeared to be consistent with degradation of the peptide into amino acids and their subsequent reincorporation into new proteins. In contrast, it appears that ^{3}H radiolabel associated with the two amino acid portion of the N-terminal is cleaved off and rapidly eliminated by glomerular filtration. ^{3}H radiolabel is primarily eliminated in the urine. Elimination of ^{14}C radiolabel in either the feces or urine was very low.

Pharmacokinetic parameters for a 1 to 1 mixture of ^{3}H-labeled and ^{14}C-labeled Hirulog in cynomolgus monkeys following bolus intravenous administration of a dose at 10 mg/kg.

Parameter	^{3}H Radioactivity		^{14}C Radioactivity	
	Male	Female	Male	Female
$T_{1/2\alpha}$, hr	0.048	0.0565	0.045	0.0595
$T_{1/2\beta}$, hr	0.6375	0.5835	0.388	0.3365
$T_{1/2\gamma}$, hr	-	-	65.6	41.5
AUC (DPM x 10^{5}*hr/mL)	4.4	2.9	34.1	21.2
V_{ss} (mL/kg)	146.8	196.55	1131.7	1157.7
Cl_{tot} (mL/hr/kg)	183.65	275.7	15.45	20.2
MRT (hr)	0.8	0.7	90.35	57.4

Fecal and urinary excretion of ^{14}C and ^{3}H activity by monkeys that received a 1 to 1 mixture of ^{3}H-labeled and ^{14}C-labeled Hirulog in cynomolgus monkeys by intravenous administration at a dose of 10 mg/kg.

Activity	Time Interval (hr)	Sex	Feces	Urine	Total
^{14}C	0-4	Male	0.0020	0.9395	0.9416
		Female	0.0004	0.5757	0.5761
	4-8	Male	0.0050	1.6683	1.6732
		Female	0.0004	5.8002	5.8007
	8-24	Male	0.1972	2.2404	2.4376
		Female	0.3470	7.4843	7.8313
^{3}H	0-4	Male	0.0021	12.3468	12.3489
		Female	0.0062	6.0097	6.0159
	4-8	Male	0.1057	19.5363	19.6420
		Female	0.0062	39.5097	39.5159
	8-24	Male	0.2627	22.4057	22.6684
		Female	0.2634	51.6631	51.9265

The absorption, distribution, metabolism, and excretion of hirulog were examined in rats, rabbits, baboons, and monkeys. Pharmacokinetic parameters for rats, rabbits, baboons, and monkeys are presented in the table below and compared with human data. Plasma drug parameters (i.e., C_{max}, AUC) in animal studies were equal to or exceeded concentrations found in humans. The volume of distribution of hirulog in rats following intravenous or subcutaneous administration exceeded blood volume suggesting distribution into tissues. Clearance of hirulog in rats exceeded the glomerular filtration rate; however, it was smaller than renal or liver plasma flow. The distribution and metabolism of ^{3}H and ^{14}C radiolabeled hirulog was examined in rats following intravenous or subcutaneous administration. In separate radiolabeled preparations, ^{3}H was located on proline in position 2, while ^{14}C was located on glycine in position(s) 5, 6, 7, 8, and/or 10. Blood and plasma concentrations of ^{3}H and ^{14}C radiolabel initially paralleled one another; however, ^{3}H activity disappeared between 1 and 4 hr, while ^{14}C activity maintained a constant level for between 24 and 72 hr after dosing. Clearance of ^{14}C-radiolabel was negligible, which appeared to be consistent with degradation of the peptide into individual amino acids and their reincorporation into new protein. In contrast, clearance of ^{3}H-radiolabel exceeded both hepatic and renal blood flow, suggestive of metabolic clearance. Examination of tissue radioactivity levels at 24 hr after dosing found that ^{3}H was confined to kidney, while ^{14}C was widely distributed in several tissues. Isoelectric focusing of rat urine suggested <u>in vivo</u> cleavage of the peptide into two or more metabolites as ^{14}C- and ^{3}H-radioactivity were separated from one another. Results indicated that the two amino acid portion of the N-terminal end, phenylalanine-^{3}H-proline, was cleaved off the peptide and excreted into the urine. Less than 25% of the remaining 18 amino acid portion containing the ^{14}C radiolabel was excreted into the urine and feces, which appeared to further strengthen the sponsor's contention that the remaining 18 amino acid portion was degraded and utilized for new protein synthesis. Excretion studies in cynomolgus monkeys yielded similar results as described for rats. Following continuous intravenous infusion of hirulog at 250 mg/kg/day for 28 days to male rats, the activity of CYP4A was elevated, while no change was found for corresponding female rats. Activation of CYP4A may potentially be related to increased peroxisomal proliferative activity.

Pharmacokinetic parameters for hirulog in rats, rabbits, baboons, monkeys, and humans. Three methods were used for preparation of hirulog as follows: a solid phase peptide method (SPPM), a homogenous phase pilot scale (HPPS), and a modified homogenous phase commercial scale (HPCS). The modified HPCS is intended for marketing.

Species	Route/ Form	Dose	C_{max}, ng/mL	T_{max}, hr	AUC ng*hr/mL	$T_{1/2}$, hr	F, %[C]
Rat	IV Bolus /HPPS	50 mg/kg	381,500	0[A]	-[F]	-	NA[E]
Rat	SC /HPPS	50 mg/kg 250	37,738 81,750	0.5 hr 0.5	- -	- -	-
Rat	4 hr IV infusion /HPCS	1.04 mg/kg/hr 3.12 10.42	2468 4525 19484	1 hr 1 1	6970[B] 11772[B] 60341[B]	2.2 - 0.4	NA NA NA
Rat	28 day IV infusion /SPPM	25 mg/kg/day 75 250	2020[C] 7474[C] 20690[C]	- - -	- - -	- - -	NA NA NA
Rabbit	IV Bolus /HPPS	50 mg/kg	635,478	0.03	-	-	NA
Rabbit	SC /HPPS	50 mg/kg 250	20,400 110,398	0.58 0.75	- -	- -	- -
Baboons	IV Bolus /SPPM	1 mg/kg	12,200	0.03	3083	0.18	NA
Baboons	SC /SPPM	1 mg/kg	970	0.25	3000	3.67	97.3
Monkeys	4 hr IV infusion /HPCS	1.7 mg/kg/hr 6.25 17	3792 22387.5 58221.5	3.23 3.63 3.96	12054 71056 197689	0.48 0.48 0.45	NA NA NA
Monkeys	28 day IV infusion /HPCS	15 mg/kg/day 45 150	698[D] 3378[D] 10850[D]	0.083 0.111 0.097	218[D] 1275[D] 6760[D]	0.08 0.11 0.10	NA NA NA
Human[E]	IV Bolus	0.3 mg/kg	1975	0.03	524	0.03	NA
Human[E]	15 min IV infusion	0.3 mg/kg 0.6	1440 4309	0.27 0.27	839 1681	0.15 0.17	NA NA
Human[E]	SC	0.3 mg/kg 0.6	191 377	2 2	552 1500	1.60 2.85	67 91
Human	4 hr IV infusion /HPCS	0.5 mg/kg/hr	1683.04	3.20	6721	1.05	NA
Human	4 hr IV infusion /SPPM	0.5 mg/kg/hr	1474.14	3.36	5915.5	0.61	NA

A. Immediately post-dose; B. AUC 0-4.33 hr; C. Steady-state plasma concentrations on day 28; D. Plasma drug concentrations on days 28 to 29; E. These studies were performed in healthy human volunteers, who most likely received the SPPM form; F. Dash lines throughout the table indicate that data was not provided; G. Percent bioavailability is represented as F; H. NA represents not applicable.

TOXICOLOGY:

Acute Toxicity in Mice, Rats, and Monkeys

Intravenous Acute Toxicity Studies in Mice, Rats, and Monkeys and Subcutaneous Acute Toxicity Studies in Mice and Rats

Testing Laboratory: [redacted]

Study Number, Start and Completion Dates, and Drug Batch:

Biogen Study No.	Study Started	Study Completed	Drug Batch
[illegible]	09-20-89	11-07-89	
P90-002		08-06-90	SPPM
P8967-93-10	12-07-93	03-04-94	HPCS
P90-004	02-06-90	10-03-90	SPPM
P90-003	02-06-90	09-11-90	SPPM
P90-025	04-06-90	05-03-90	
P8967-93-11	12-07-93	03-04-94	HPCS
P90-005	02-06-90	10-03-90	SPPM
P90-006	03-05-90	09-06-90	SPPM

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

Animals: Male and female CD-1 mice had a weigh range of 20-37 g and were generally 6-8 weeks of age. Male and female Swiss Webster mice Tac:(SW)FBR had a weight range of 23-32 g and were 5 weeks of age. Male and female Sprague Dawley rats (CD SD BR (VAF+)) had an average weight of 100-254 g and were 4 to 6 weeks of age Adult cynomolgus monkeys (Macaca fascicularis) had a weight range of 3.2 to 4.2 kg.

Methods: The acute toxicity of hirulog was examined in mice and rats following a single administration by either the intravenous or subcutaneous route. The acute intravenous toxicity of hirulog was examined in cynomolgus monkeys using a dose escalation design with 2 male and 2 female monkeys. Hirulog was administered by the intravenous route at doses of 5, 15, 50, and 100 mg/kg on days 1, 5, 9, and 13, respectively. There was a 3 day observation period at each dose level.

Results:

Acute toxicity of hirulog in mice and rats following intravenous or subcutaneous administration.

Species	Route	Dose, mg/kg	Dose volume & vehicle	Maximum nonlethal dose, mg/kg	Minimum lethal dose, mg/kg	Time to Death
CD-1 mice,	IV	0.2 (1 mouse/sex)[a] 1.0 (1 mouse/sex)[a] 5.0 (1 mouse/sex)[a] 12.5 (1 mouse/sex)[b] 25 (1 mouse/sex)[b] 50 (1 mouse/sex)[b] 100 (1 male)[a] 200 (5 mice/sex)[b]	a. 2 mL/kg in PBS b. 20 mL/kg in saline	N.D.	N.D.	N.D.
Swiss Webster mice	IV	0 (5 mice/sex) 200 (5 mice/sex)	10 mL/kg in saline	N.D.	N.D.	N.D.
Mice	SC	12.5 (1 mouse/sex) 25 (1 mouse/sex) 50 (1 mouse/sex) 100 (1 mouse/sex) 200 (5 mice/sex)	20 mL/kg in saline	N.D.	N.D.	N.D.
Rats	IV	12.5 (1 rat/sex) 25 (1 rat/sex) 50 (1 rat/sex) 100 (1 rat/sex) 200 (5 rats/sex)	10 mL/kg in saline	25[a]	50[a]	≥ 1.5 min
Rats	IV	0 (5 rats/sex) 100 (5rats/ex)	2 mL/kg in saline	N.D.	N.D.	N.D.
Rats	SC	12.5 (1 rat/sex) 25 (1 rat/sex) 50 (1 rat/sex) 100 (1 rat/sex) 200 (5 rat/sex)	10 mL/kg in saline	N.D.	N.D.	N.D.
Monkey	IV	5 (2 monkeys/sex) 15 (2 monkeys/sex) 50 (2 monkeys/sex) 100 (2 monkeys/sex)	0.22 0.64 2.15 4.30 mL/kg	N.D.	N.D.	N.D.

N.D. = not determined
a. See discussion.

The acute toxicity of hirulog was examined in mice and rats following administration by either the intravenous or subcutaneous route. Hirulog administered by either the intravenous or subcutaneous route to mice at doses between 0 and 200 mg/kg did not produce any mortality or signs of toxicity. When hirulog was administered to rats in saline at a dose volume of 10 mL/kg, respiratory distress rapidly ensued. The maximum nonlethal and minimum lethal intravenous doses were 25 and 50 mg/kg, respectively. Hirulog administered by the subcutaneous route to rats at doses between 12.5 and 200 mg/kg produced no mortality or clinical signs of toxicity. Hirulog produced pulmonary distress when administered by the intravenous route at high dose volumes in saline and possibly dextrose, but not in vehicle (composition was not clearly specified). Death appeared to be due to marked vascular congestion, and moderate interstitial and alveolar edema in the lungs suggestive of either an alteration in pulmonary arterial pressure, a change in capillary permeability, or a change in osmotic pressure in the blood leading to leakage of fluid from the circulation into the lungs. The acute intravenous toxicity of hirulog was examined in cynomolgus monkeys using a dose escalation design at doses of 5, 15, 50, and 100 mg/kg. No clinical signs of toxicity or mortality were observed.

Rats

Acute Intravenous Toxicity Study of Hirulog and Its Major Impurities in Rats (Biogen Study No. P8967-94-12).

Testing Laboratory: [redacted]

Study Started: May 11, 1994

Study Completed: January 17, 1995

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

Animals: Sprague Dawley rats were used in this study. On day of dosing, animals were 6 to 8 weeks old and body weight ranges were 242-282 g for males and 174-197 g for females.

Drug Batch: Hirulog, Lot No. 67A03Z; PB19-1, Notebook Ref. No. ASF01130-P38; PB19-2, Notebook Ref. No. ASF01130-P38; PB19-3, Notebook Ref. No. ASF01130-P38, and Saline, Lot No. 76-423-DK.

Methods: The acute intravenous toxicity of hirulog and its major potential impurities, [redacted] at a single dose of 50 mg/kg were examined in rats. Control rats received 0.9% NaCl. Each group consisted of 5 rats/sex/group. The dose volume was 2 mL/kg. Animals were observed daily for clinical signs of toxicity and mortality for 14 days after dosing. Body weights were measured prior to the start of treatment and on days 7 and 14. Blood for determination of hematological and clinical chemistry parameters were collected from fasted rats on days 8 and 15. Following the 14 day observation period, animals were sacrificed on day 15 and subjected to a gross examination.

Results: The acute intravenous toxicity of hirulog and its major potential impurities, [redacted] at a single dose of 50 mg/kg were examined in male and female rats. There was no mortality or clinical signs of toxicity. There were no gross pathological findings after a 14 day observation period. Body weights for male rats treated with hirulog, [redacted] were impaired by >10% over the 14 day observation period. In contrast, body weight gains for female rats treated with hirulog, [redacted] were unaffected.

Dogs

Acute Toxicity and Pharmacokinetic Profile of HirulogF and HirulogR After a Single Four-Hour Intravenous Infusion in Female Dogs (Biogen Study No. P8967-92-06).

Testing Laboratory: [redacted]

Study Started: September 30, 1992

Study Completed: April 11, 1994

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

Animals: Three female beagle dogs were used in this study. Animals were 6-7 months old and weighed between 6.9 and 8.1 kg.

Drug Batch: HirulogF (lot# 67X15W)
HirulogR (lot# 67Y01Z)

Methods: The potential toxicity and toxicokinetics of two different hirulog formulations were compared in female dogs. Hirulog was prepared by homogenous phase pilot scale. Three female dogs were dosed sequentially with a 4 hr infusion of saline, HirulogF (frozen solution of hirulog stored at -20°C), and HirulogR (liquid solution of hirulog stored at 2-8°C) on study days 1, 3, and 7, respectively. The two hirulog formulations were administered at 1.7 mg/kg/hr (1.0 mL/kg/hr) and saline was administered at 1.0 mL/kg/hr. Animals were observed daily for mortality. Animals were weighed prior to each treatment and at 1 week following the last dose. Animals were observed for clinical signs of toxicity at 1, 2, 3, 4, 6, and 8 hr after the completion of the infusion on days of treatment and daily between treatment days. Blood was collected for measurement of hematological and clinical chemistry parameters and venous blood gas analyses prior to treatment and at 4 and 6 hr after the start of infusion on treatment days 1, 3, and 7. Blood was collected for measurement of plasma drug levels and coagulation parameters prior to dosing and at 0.25, 0.5, 1, 2, 4, 4.25, 4.5, 4.75, 5, 6, 8, and 24 hr after the start of the infusion on days 3 and 7.

Results: Three female dogs were dosed sequentially with a 4 hr infusion of saline, HirulogF (frozen solution of hirulog stored at -20°C), and HirulogR (liquid solution of hirulog stored at 2-8°C) on study days 1, 3, and 7, respectively. Plasma drug and AUC values for the two formulations were similar. Hematological, biochemistry, and blood gas changes found with the two formulations were generally small and isolated suggesting little biological significance. Any differences in blood coagulation (i.e., APTT or PT) were not reported.

Monkeys

Acute Toxicity and Pharmacokinetic Profile of HirulogR and HirulogF After a Single Four Hour Intravenous Infusion in Female Cynomolgus Monkeys (Biogen Study No. P8967-92-07).

Testing Laboratory: [redacted]

Study Started: October 21, 1992

Study Completed: August 21, 1997

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

Animals: Two female cynomolgus monkeys (Macaca fascicularis) were used in this study.

Drug Batch: HirulogF (lot# 67X10W)
HirulogR (lot# 67Y01Z)

Methods: The potential toxicity and toxicokinetics of two hirulog formulations, HirulogR (refrigeration formulation) and HirulogF (frozen formulation), administered by intravenous infusion, were compared using two female cynomolgus monkeys (Macaca fascicularis). Hirulog was prepared by homogenous phase pilot scale. HirulogR at a total dose of 6.8 mg/kg was administered over a 4 hr period at an infusion rate of 1.7 mg/kg/hr (1 mL/kg/hr). HirulogF at total doses of 6.8 or 41.6 mg/kg were administered over 4 hr periods at infusion rates of 1.7 and 10.4 mg/kg/hr (1 mL/kg/hr), respectively. Saline was used as the vehicle control and infused at a rate of 1 mL/kg/hr. Each agent was infused over a 4 hr period on day 1 and observation continued until day 8. There was a wash-out period of at least 2 weeks between each agent. For each agent, animals were observed for clinical signs of toxicity at 1, 4, and 6 hr on day 1 and daily from days 2 through 8. Body weights were measured on day -1 prior to each infusion and on day 8 following each infusion. Blood for determination of hematological and clinical chemistry parameters was collected at 1 hr prior to the start of the infusion and at 3.85 and 8 hr after the start of the infusion. Blood for determination of the APTT was collected 1 hr prior to the start of the infusion, 1 hr after the start of the infusion, 10 min prior to the end of the infusion, and at 1 and 4 hr after completion of the infusion. Blood for determination of plasma drug levels was collected at 1 hr prior to the start of the infusion, at 0.25, 0.5, 1, 2, and 4 hr after initiation of the infusion, just prior to the stop of the infusion, and at 4.25, 4.5, 4.75, 5, 6, 8, and 24 hr after initiation of the infusion.

Results: There were no apparent differences in toxicity between two hirulog formulations, HirulogR (refrigeration formulation) and HirulogF (frozen formulation). Treatment with the two hirulog formulations prolonged the APTT in a similar manner. For HirulogR at 6.8 mg/kg, the APTT was elevated to 246.6, 300, 190, and 186.5% of the baseline at 1, 3.85, 5, and 8 hr after the start of the infusion, respectively. For HirulogF at 6.8 mg/kg, the APTT was elevated to 296, 289.5, 185, and 100.4% of the baseline at 1, 3.85, 5, and 8 hr after the start of the infusion, respectively. For HirulogF at 41.6 mg/kg, the APTT was elevated to 372 and 138% of the baseline at 5 and 8 hr after the start of the infusion, respectively. Plasma AUC values from 0-4.4 hr for 6.8 mg/kg HirulogR, 6.8 mg/kg HirulogF, and 41.6 mg/kg HirulogF were 13565,

8092, and 92100 ng·hr/mL, respectively. Differences between HirulogR and HirulogF at 6.8 mg/kg were attributed to difficulties in blood sampling. AUC values for HirulogF at 6.8 and 41.6 mg/kg increased with dose; although, the AUC at 41.6 mg/kg was 11.4 times that observed for 6.8 mg/kg. Clearance values for 6.8 mg/kg HirulogR, 6.8 mg/kg HirulogF, and 41.6 mg/kg HirulogF were 505.5, 818.3, and 459.4 mL/hr/kg, respectively. Total clearance values were greater than glomerular filtration (76 mL plasma/hr/kg), but less than renal plasma flow (1010 mL plasma/hr/kg). The volume of distribution at steady state for 6.8 mg/kg HirulogR, 6.8 mg/kg HirulogF, and 41.6 mg/kg HirulogF were 157.2, 1053.2, and 474 mL/kg, respectively. Volumes of distribution were greater than the blood volume suggesting distribution into tissues.

Subacute Toxicology

Rats

Fourteen Day Toxicity Study of Hirulog Administered Via Continuous Infusion to Sprague Dawley Rats (Biogen Study No. P8967-93-02).

Testing Laboratory: [redacted]

Study Started: September 21, 1993

Study Completed: December 2, 1994

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

Animals: Sprague Dawley rats (Tac:N(SD)fBR) were used in this study. For rats treated for 14 days, pretreatment mean body weight ranges were 308-315.4 g for males and 216.0-229.4 g for females.

Drug Batch: Hirulog, Lot No. 67W04T (homogenous phase pilot scale)

Methods: Rats received hirulog by continuous intravenous infusion at doses of 0, 80 (3.3 mg/kg/hr), and 1000 mg/kg/day (41.6 mg/kg/hr) for 14 days. Dose selection was based a range finding study in which male rats received hirulog by continuous intravenous infusion at a dose of 1200 mg/kg/day for 7 days (Biogen Study No. P90-051). Major histopathological findings included thymic hemorrhage and heart epicarditis. For the present study, the infusion rate was 2.0 mL/kg/hr. There were 5 rats/sex/group. The control group

received the vehicle, 0.9% NaCl. The sponsor also included a group composed of 5 rats/sex that received hirulog by continuous intravenous infusion at 80 mg/kg/day for 5 days; although, there was not a corresponding control group. Two additional groups received hirulog by continuous intravenous infusion at 500 (20.8 mg/kg/hr) and 1000 mg/kg/day for 28 days; although, there was not a corresponding control group. The group receiving 500 mg/kg/day was composed of 2 males and one female and the group receiving 1000 mg/kg/day was composed of one male and two females. Animals were observed twice daily for mortality/moribundity and once daily for clinical signs of toxicity. For animals treated for 14 days, body weights were measured prior to the start of treatment, on days 8 and 15, and prior to sacrifice. Ophthalmic examinations were performed before and after the 14 day treatment period. Blood for measurement of the APTT was collected at 6 hr after the start of the infusion on day 1, prior to termination of the infusion on day 15, and immediately prior to sacrifice on day 16. Blood for measurement of plasma hirulog concentration was collected at 6 hr after the start of the infusion on day 1 and prior to termination of the infusion on day 15. Blood for measurement of hematological and clinical chemistry parameters was collected immediately prior to sacrifice on day 16. Determination of fecal occult blood was performed on days 8 and 16. After the 14 day treatment, animals were fasted overnight, sacrificed on day 16, and subjected to a gross examination. Absolute and relative weights were determined for the adrenal glands, brain, kidneys, ovaries, and testes. Tissues collected and preserved for histopathological analysis included the heart, aorta, large intestine (cecum, colon, and rectum), liver, pancreas, salivary gland, small intestine (duodenum, jejunum, and ileum), stomach (forestomach and glandular), esophagus, trachea, lungs, adrenal glands, parathyroid glands, pituitary gland, thyroid glands, epididymides, seminal vesicles, testes, prostate, ovaries, uterus, vagina, skin, mammary gland, bone marrow (femur) lymph nodes (mandibular and mesenteric) spleen, thymus, brain (medulla/pons, cerebellum, and cerebral cortex), spinal cord (cervical, mid-thoracic, and lumbar), peripheral nerve, femur, skeletal muscle, kidneys, urinary bladder, administration site, tissue masses, gross lesions, and eyes. Histopatholgical evaluation was performed for only the control and 1000 mg/kg/day groups. There were significant deviations in dosing for several female rats in the 80 and 1000 mg/kg/day groups over the 14 day treatment period. Deviations for the 5 female rats in the 80 mg/kg/day group ranged from -33.29 to -90.16%. Deviations for 3 female rats in the 1000 mg/kg/day group ranged from -45.81 to +21.01%.

Results:

1. **Observed Effects**: Ruffled coats were observed for male and female rats treated with 1000 mg/kg/day for 14 days. Colored feces were observed for 1 male and 1 female treated with 1000 mg/kg/day. Eye or nose discharge was observed for both control and treatment groups, and may have been related to retro-orbital bleeding procedures.

2. **Mortality**: Mortality occurred for 1 male treated with 80 mg/kg/day and for 2 males and 2 females treated with 1000 mg/kg/day. One male (No. 8) from the 80 mg/kg/day group died on day 11 due to bacteremia. Lymphoid deposition was observed in several organs. Necrosis was found in the ileum. Arterial inflammation was adjacent to the mesenteric lymph node associated with bacteria. Male No. 13 from the 1000 mg/kg/day group was considered moribund on day 14 and sacrificed. Male No. 14 from the 1000 mg/kg/day group was found dead on day 15. Female No. 34 from the 1000 mg/kg/day group was found dead on day 3. Blood was found in its cage and death was attributed to extensive bleeding from the retro-orbital sinus on day 1. Female No. 31 from the 1000 mg/kg/day group was observed with swelling in the inguinal region from day 5 to 7 (regional response to catheter), and a laceration on the right thigh with bleeding on day 8. The animal was considered moribund on day 8 and sacrificed.

3. **Body Weights**: For the 14 day treatment period, body weight gain was impaired for male rats that received 80 mg/kg/day and for male and female rats that received 1000 mg/kg/day. Body weights for the control male rats prior to treatment and on day 15 were 314.6 and 375.4 g, respectively. Body weight gain for male rats treated with 80 mg/kg/day was 68.4% of the control. Male rats that received 1000 mg/kg/day lost 1% of their pretreatment body weight. Body weights for the control female rats prior to treatment and on day 15 were 219.5 and 241.5 g, respectively. Body weight gains for the female 80 and 1000 mg/kg/day groups were 101.8 and 73.4% of the control, respectively.

4. **Hematology**:

Hematology (Day 15): For the male 1000 mg/kg/day group, red blood cell counts, hemoglobin levels, hematocrit, and mean corpuscular hemoglobin content were reduced to 74.4, 70.3, 76.4, and 91.6% of respective control values (7.18 x 10^6 cells, 13.8 g/dL, 42.8%, and 32.3 g/dL). The reticulocyte count was elevated to 365.2% of the control (0.23 x 10^6 cells); although, this change was not significant. The percent neutrophils was reduced to 11% for male 1000 mg/kg/day group as compared to 23% for the control group

and the neutrophil count was reduced to 59.3% of the control (3.17 x 10^3); although these changes were not significant. The mean corpuscular hemoglobin content for the female 1000 mg/kg/day group was reduced to 94.5% of the control (32.6 g/dL). The reticulocyte count for the female 1000 mg/kg/day group was increased to 275% of the control (0.24 x 10^6); although, this change was not significant.

APTT: APTT values for the male 1000 mg/kg/day group from days 1 to 15 of treatment were not available. The APTT on days 1 and 15 for the 80 mg/kg/day group were increased to 194.3 and 161.3% of the control values (15.7 and 16.0 sec), respectively; although, these changes were not significant. By day 16, the APTT value for the 80 mg/kg/day group was 78% of the control value (16.8 sec). On days 1 and 15, the APTT for the female 80 mg/kg/day group was elevated to 163 and 150.6% of the control (14.1 and 16.6 sec), respectively; although, these changes were not significant. The APTT value for the female 1000 mg/kg/day group on day 15 was elevated to >106 sec. By day 16, the APTT values for the female 80 and 1000 mg/kg/day groups had returned to the control level.

Fecal Occult Blood: On day 8, 1 male of the 1000 mg/kg/day group was found to positive for fecal occult blood. On day 16, 2 females of the 1000 mg/kg/day group were found to be positive for fecal occult blood.

5. **Blood Chemistry (Day 15)**: Blood urea nitrogen levels for the male and female 1000 mg/kg/day groups were increased to 160 and 146.7% of the control (15 mg/dL), respectively. γ-glutamyl transferase activity were the male 80 and 1000 mg/kg/day groups were increased to 1 and 4 U/L, respectively, as compared to a control value of 0 U/L. Alanine and aspartate transferase activities for the male 1000 mg/kg/day groups were increased to 211.8 and 207.3% of respective control values (34 and 110 U/L); however, these changes were not significant.

6. **Ophthalmic Examination**: No treatment-related ophthalmic changes were found.

7. **Organ Weights**: Absolute and relative adrenal gland weights for the male 1000 mg/kg/day group were decreased to 56.7 and 48.1% of the control (0.0663 g and 0.0125%), respectively. Absolute and relative adrenal gland weights for the female 1000 mg/kg/day group were increased to 121.5 and 125.3% of the control (0.0688 g and 0.0320%), respectively. Absolute and relative kidney weight for the female 1000 mg/kg/day group were increased to 127.7 and 132.1% of the control (1.8276 g and 0.8463%), respectively.

8. **Gross Pathology**: Major gross pathological findings included pale or white foci in kidneys, splenomegaly, a red meningeal focus on the brain, and pale livers.

Gross pathological changes for rats that received hirulog by continuous intravenous infusion at doses of 0, 80, or 1000 mg/kg/day for 14 days.

Organ/Tissue	0 mg/kg/day		80 mg/kg/day		1000 mg/kg/day	
	M	F	M	F	M	F
Kidney						
-discoloration	0	0	0	0	1	0
-enlarged	0	0	1	0	2	1
-foci	0	0	1	0	0	0
Spleen						
-discoloration	0	0	0	0	1	0
-enlarged	0	0	1	0	2	1
-foci	0	0	1	0	0	0
Brain						
-foci	0	0	1	0	1	0
Heart						
-foci	0	0	0	1	1	0
Epididymis						
-foci	0	0	1	0	1	0
Thymus						
-foci	0	0	0	0	1	0
Liver						
-discoloration	0	2	0	1	4	0
-nodules	0	0	1	1	0	0

9. **Histopathology**: The target organs of toxicity appeared to be the kidney and liver. Histopathological changes in the kidney consisted of mild nephropathy, segmental zones of necrosis, acute and chronic inflammation, interstitial fibroplasia, and tubular epithelial regeneration. The sponsor attributed kidney damage to embolization rather than direct action of the drug. An increased incidence of Kupffer cell hypertrophy was found in livers from hirulog-treated animals and may have been related to phagocytosis. Hemorrhage was observed in the lungs (alveolar), brain (meningeal), thymus, rectum, prostate, seminal vesicles, and mesenteric lymph nodes. Extramedullary hematopoiesis (composed of erythroid precursor cells) was observed in the spleen and liver. Mild erythroid hyperplasia was found in bone marrow. Similar histopathological findings were observed for rats that received hirulog by continuous intravenous infusion at doses of 500 and 1000 mg/kg/day for 28 days (i.e., changes in the liver, spleen, thymus, lymph nodes and hemorrhage in several tissues).

Histopathological changes for rats that received hirulog by continuous intravenous infusion at doses of 0, 80, or 1000 mg/kg/day for 14 days.

Organ/Tissue	0 mg/kg/day		1000 mg/kg/day	
	Male	Female	Male	Female
Liver				
-Kupffer cell hypertrophy	1	0	2	3
-extramedullary hematopoiesis	0	0	2	1
-erythrophagocytosis	0	0	1	0
-inflammation, chronic	2	0	2	1
-increased mitoses	0	0	1	0
Kidneys				
-nephropathy	0	0	2	0
-inflammation, chronic	0	0	2	0
-inflammation, pelvis, acute	0	0	1	0
Brain				
-hemorrhage	0	0	1	0
Spleen				
-increased extramedullary hematopoiesis	1	0	2	2
Rectum				
-hemorrhage, serosal	0	0	1	0
Mesenteric lymph node				
-lymphoid deposition	0	0	1	0
-hemorrhage	0	0	1	1
-abscess	0	0	0	1
Peritoneal cavity				
-hemorrhage	0	0	0	1
-fibrosis	0	0	0	1
-inflammation, pyogranuloma	0	0	0	1
Bone marrow				
-erythroid hyperplasia	0	0	1	0
Lungs				
-interstitial edema	0	0	0	1
-inflammation, acute	0	0	0	1
-hemorrhage	0	1	0	1
-inflammation, chronic	1	0	0	0
-congestion	1	0	0	2
Seminal vesicles				
-hemorrhage	0	0	1	0
Prostate				
-inflammation, acute	0	0	2	0
-hemorrhage	0	0	1	0

10. **Plasma Drug Levels:** Plasma hirulog levels were proportional to dose at 6 and 336 hr after the start of the infusion. Plasma drug levels were similar at 6 and 336 hr after the start of the infusion. Plasma drug levels were similar between male and female rats.

Plasma hirulog levels (ng/mL) in rats at 6 and 336 hr after the start of intravenous infusion of hirulog at doses of 80 and 1000 mg/kg/day.

Sampling Time	80 mg/kg/day		1000 mg/kg/day	
	Male	Female	Male	Female
Day 1 (6 hr)	2550	2443	33025	32700
Day 15 (336 hr)	2948	2107	35845	39943

Rats received hirulog by continuous intravenous infusion at doses of 80 and 1000 mg/kg/day for 14 days. Only the control and 1000 mg/kg/day groups were evaluated for histopathological changes. The target organs of toxicity appeared to be the kidney and liver. Changes for the kidney consisted of mild nephropathy, segmental zones of necrosis, acute and chronic inflammation, interstitial fibroplasia, and tubular epithelial regeneration. An increased incidence of Kupffer cell hypertrophy was found in the 1000 mg/kg/day group. The sponsor attributed changes in the kidney to embolization rather than direct action of the drug. Hemorrhage due to the pharmacological action of the compound was observed in the lungs, brain, thymus, rectum, prostate, seminal vesicles, and mesenteric lympn nodes. Extramedullary hematopoiesis was found in the spleen and liver. Mild erythroid hyperplasia was found in bone marrow.

14 Day Intravenous Toxicity Study of Hirulog and Partially Degraded Hirulog in Rats (Biogen Study No. P8967-94-13).

Testing Laboratory: [redacted]

Study Started: May 17, 1994

Study Completed: January 22, 1995

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

Animals: Sprague Dawley rats were used in this study. At the start of treatment, animals were 6-9 weeks old, and body weight ranges were 277-340 g for male rats and 183-220 g for female rats.

Drug Batch: Hirulog, Lot No. 67Z18S (modified homogenous phase commercial scale; PB19-4, Lot No. PB19-4.

Methods: The toxicity of hirulog and partially-degraded hirulog (PB19-4) were evaluated in rats following intravenous bolus administration at 36 mg/kg/day for 14 days. Control animals received 0.9% NaCl. There were 10 rats/sex/group. The dose volume was 2 mL/kg. Animals were observed daily for clinical signs of toxicity and mortality. Body weights were measured prior to the start of treatment, on days 1, 8, and 14, and on day 15 after fasting overnight. Blood samples for determination of hematological and biochemical parameters were collected on day 15 from animals fasted overnight. Blood for determination of coagulation parameters, prothrombin time, partial thromboplastin time, and fibrinogen, were collected prior to and 5 min after dosing on day 14 and on day 15 prior to necropsy. Fibrinogen was measured on day 15 only. Urine for analysis was collected overnight (~16 hr) from day 14 to 15. Blood for determination of plasma hirulog levels was collected prior to and 15 min after dosing on day 14 and on day 15 prior to necropsy. All animals were sacrificed on day 15 and subjected to a gross examination. Organs and tissues were processed and analyzed by light microscopy as follows: all gross lesions, adrenal glands, bone and marrow (femur), brain, esophagus, eyes with optic nerve, heart with aorta, harderian glands, kidneys, large intestine (cecum, colon, rectum), liver, lungs (with bronchi), lymph nodes (mesenteric, mandibular), mammary gland with skin, ovaries, pancreas, pituitary gland, prostate gland, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle (thigh), skin (abdominal), small intestines (duodenum, jejunum, and ileum), spinal cord (thoracolumbar), stomach, testes with epididymes, thymus, thyroid with parathyroid glands, trachea, urinary bladder, uterus, vagina, and injection site (tail).

Results:

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

2. **Mortality**: None.

3. **Body Weights**: Body weight gains for female rats treated with hirulog or PB19-4 were impaired by >10%; although, male treatment groups were unaffected. Body weights for male controls on days 1 and 14 were 311 and 352 g, respectively. Body weight gains for male rats treated with hirulog or PB19-4 were 91.4 and 105.9% of the control, respectively. Body weights for female controls on days 1 and 14 were 203 and 239 g, respectively. Body weight gains for female rats treated with hirulog or PB19-4 were 87 and 82.9% of the control, respectively. Food consumption was not reported.

4. **Hematology and Blood Coagulation**:

Hematology: The segmented neutrophil count for male rats treated with hirulog or PB19-4 were decreased to 80.9 and 75.3% of the control (1.506 x 10^3 cells/µL), respectively. Monocyte counts for male rats treated with hirulog were decreased to 48.6% of the control (0.436 x 10^3/µL). Eosinophil counts for male rats treated with PB19-4 were increased to 237.3% of the control (0.067 x 10^3 cells/µL). White blood cell counts for female rats treated with hirulog and PB19-4 were increased to 112.4 and 123.6% of the control (8.9 x 10^3 cells/µL), respectively. The reticulocyte percentage for female rats treated with hirulog or PB19-4 were increased to 146.2 and 138.5% of the control (2.6%), respectively. The segmented neutrophil percentage and count for female rats treated with PB19-4 were reduced to 54.5 and 73.1% of the control (11% and 0.976 x 10^3 cells/µL), respectively. The lymphocyte counts for female rats treated with hirulog or PB19-4 were increased to 113.7 and 130.3% of the control (7.644 x 10^3 cells/µL), respectively. Monocyte count for female rats treated with PB19-4 were decreased to 58% of the control (0.150 x 10^3 cells/µL). Eosinophil counts for female rats treated with PB19-4 were increased to 219% of the control (0.100 x 10^3 cells/µL).

Blood Coagulation: On day 14, prothrombin times for male rats, 5 min after treatment with hirulog or PB19-4, were prolonged to 109.6 and 120 sec, respectively, as compared to a control value of 39 sec. On day 14, partial thromboplastin times for male rats, 5 min after treatment with hirulog or PB19-4, were prolonged to 274.1 and 300 sec, respectively, as compared to a control value of 104.5 sec. On day 14, prothrombin times for female rats, 5 min after treatment with hirulog or PB19-4, were both prolonged to 120 sec as compared to a control value of 13.3 sec. On day 14, partial thromboplastin times for female rats, 5 min after treatment with hirulog or PB19-4, were both prolonged to 300 sec as compared to a control value of 49.7 sec. Prothrombin and partial thromboplastin times for male and female treatment groups on day 14 prior to dosing or on day 15 had returned to control levels. Fibrinogen levels on day 15 for male and female treatment groups were not significantly different from the control.

5. **Blood Biochemistry and Urinalysis**:

Blood Biochemistry: For rats that received hirulog or PB19-4, small changes in the levels of glucose, phosphate, total bilirubin, triglyceride, and potassium were observed; although, there biological significance was minimal.

Urinalysis: The incidence of blood in the urine for male and female rats treated with hirulog or PB19-4 was slightly increased; although, its biological significance was probably minimal.

6. **Physical Effects**: Not reported.

7. **Organ Weights**: Not reported.

8. **Gross Pathology**: There were no significant treatment-related gross pathological observations.

9. **Histopathology**: The liver was the target organ of toxicity for rats treated with either hirulog or PB19-4. The sponsor considered liver changes following treatment with hirulog or PB19-4 to be a typical response to a foreign protein. The incidence of biliary hyperplasia was increased following treatment with either PB19-4 or hirulog as compared to the control. The incidence of biliary hyperplasia in PB19-4-treated female rats was higher as compared to hirulog.

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Histopathological changes for rats treated with either hirulog or partially-degraded hirulog (PB19-4) by intravenous bolus administration at a dose of 36 mg/kg/day for 14 days. There were 10 rats/sex/group.

Organ/Tissue	Control		Hirulog		PB19-4	
	Male	Female	Male	Female	Male	Female
Liver						
-biliary hyperplasia	0	0	1 (1)	3 (1)	2 (1)	9 (1)
-portal inflammation	5 (1	2 (1)	9(1.4)	5 (1)	10(1.5	9(1.4)
-extramedullary hematopoiesis	8 (1	2 (1)	6(1.2)	2 (1)	8(1)	6 (1)
-hepatocellular fatty change	1 (1	8(1.5)	0	6 (1)	2 (1)	8 (1.5)
-hepatocellular necrosis	0	0	0	0	1 (1)	0
-hemorrhage	0	0	0	0	1 (3)	0
Injection Site (tail)						
-perivascular hemorrhage	1 (2	0	1 (1)	2 (1)	1 (1)	2 (1)
-hemorrhage	0	1 (1)	0	2(1.5)	0	3 (1)
Lungs						
-inflammation	1 (2	2 (1)	2 (2)	2 (1)	4(1.5)	3(1.3)
-hemorrhage	1 (1	0	0	0	1 (1)	
Pancreas						
-chronic inflammation	1 (1	0	0	1 (1)	1 (2)	3 (1.3)

1 = minimal, (2) = mild, and (3) = moderate.

10. **Plasma Drug Levels**: Blood was collected for determination of plasma hirulog levels; however, no data was reported.

The toxicity of hirulog and partially-degraded hirulog (PB19-4) were evaluated in rats following intravenous bolus administration at 36 mg/kg/day for 14 days. There was no mortality or treatment-related clinical signs of toxicity. The liver was the target organ of toxicity for rats treated with hirulog or PB19-4. The incidence of biliary hyperplasia was increased following treatment with either PB19-4 or hirulog as compared to the control. The incidence of biliary hyperplasia in PB19-4-treated female rats was higher as compared to hirulog.

14 Day Intravenous Toxicity Study of PB19-6 (D-PHE12-Hirulog) in Rats (Biogen Study No. P8967-94-14).

Testing Laboratory: [redacted]

Study Started: August 18, 1994

Study Completed: August 28, 1997

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

Animals: Sprague Dawley rats were used in this study. At the start of treatment, animals were 7 weeks old, and body weight ranges were 203-236 g for male rats and 177-202 g for female rats.

Drug Batch: [redacted]

Methods: [redacted]

Animals were observed daily for clinical signs of toxicity and mortality. Body weights were measured on days 1, 7, 14, and 15. Blood for determination of coagulation parameters (i.e., prothrombin time, partial thromboplastin time, and fibrinogen levels) was collected on day 14 prior to dosing and 5 min after dosing, and on day 15. Prior to scheduled sacrifice on day 15, animals were fasted overnight. Blood for determination of hematological and clinical chemistry parameters was collected on day 15 prior to sacrifice. Urine for analysis was collected overnight on days 14 to 15. Animals were sacrificed on day 15 and subjected to a gross examination. Organ and tissues were collected for histopathological analysis as follows: adrenal glands, bone and bone marrow (femur), brain, esophagus, heart and aorta, kidneys, large intestine (cecum, colon, and rectum), liver, lungs with bronchi, lymph nodes (mandibular and mesenteric), mammary gland with skin, ovaries, pancreas, pituitary gland, eyes with optic nerve, injection site (tail), prostate gland, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle (thigh), skin (adjacent to mammary

tissue), small intestine (duodenum, jejunum, ileum), spinal cord (thoracolumbar), spleen, stomach, testes (with epididymides), thymus, thyroid gland with parathyroid glands, trachea, urinary bladder, and uterus with vagina. Any tissues with gross lesions were also collected for histopatholgical analysis.

Results:

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

2. **Mortality:** None.

3. **Body Weight:** There were no treatment-related effects on body weight gain. Body weights for male controls on days 1 and 14 were 215 and 304 g, respectively. Body weight gain for the male [redacted] group was 102.9% of the control. Body weights for female controls on days 1 and 14 were 191 and 221 g, respectively. Body weight gain for the female [redacted] group was 110.6% of the control.

4. **Hematology and Blood Coagulation:**

Hematology: Monocyte counts for the male [redacted] group were increased to 0.120 x 10^3 cells/µL as compared to a control value of 0. Eosinophil counts for the male [redacted] group were increased to 467% of the control (0.018 x 10^3 cells/µL). Monocyte counts for the female [redacted] group were decreased to 40% of the control (0.015 x 10^3 cells/µL). Eosinophil counts for the female [redacted] group were increased to 322% of the control (0.009 x 10^3 cells/µL).

Blood Coagulation: Five min after dosing on day 14, prothrombin time and partial thromboplastin time for the male [redacted] group were elevated to 16.3 and 50.1 sec as compared to respective control values of 11.1 and 25.6 sec. Five min after dosing on day 14, prothrombin time and partial thromboplastin time for the female [redacted] group were elevated to 16.2 and 52.4 sec as compared to respective control values of 10.1 and 27.0 sec. Prior to dosing on day 14 or on day 15, 24 hr after dosing on day 14, prothrombin and partial thromboplastin times for male and female treatment groups had returned to control levels. Fibrinogen levels were unaffected on days 14 or 15 by treatment with [redacted]

5. **Blood Chemistry and Urinalysis**:

Blood Chemistry: Glucose levels for male ——— group were decreased to 80.6% of the control (129 mg/dL). Slight decreases (generally < 10%) of albumin, total protein, calcium, cholesterol, and phosphate levels were observed for the male ——— group; however, these changes appeared to have little biological significance.

Urinalysis: Urinary bilirubin levels were slightly increased for the male ——— group (7-small, 3-moderate) as compared to the control (5-small). Urinary protein levels were slightly increased for the male ——— group (2 at 30 mg/dL, 6 at 100 mg/dL, and 2 at 2000 mg/dL) as compared to the control (1 at trace, 1 at 30 mg/dL, and 3 at 100 mg/dL). Urinary bilirubin levels were slightly increased for the female ——— group (9-small, 1-moderate) as compared to the control (4-negative, 1-small). Urinary protein levels were slightly increased for the female ———group (6 at 30 mg/dL and 4 at 100 mg/dL) as compared to the control (1 at trace, 4 at 30 mg/dL).

6. **Physical Effects**: Not performed.

7. **Organ Weights**: Not performed.

8. **Gross Pathology**: There were no observations of treatment-related gross pathological effects.

9. **Histopathology**: Target organs of toxicity with ——— appeared to be the liver, kidney, heart, and lungs. Treatment-related liver changes consisted of biliary hyperplasia, portal inflammation, and hepatocellular necrosis. The sponsor attributed these changes to intravenous administration of a protein. Further, they claimed that these changes were reversible; although, a recovery period was not included in the study. There was an increased incidence of chronic inflammation of the heart for rats treated with ——— which consisted of foci of macrophages and lymphocytes in the myocardium. The sponsor claimed that chronic inflammation of the heart is typical for rats >4 months old and test article either accelerated or enhanced the development of these changes in rats sacrificed at 9 weeks of age; however, no historical data was included to support this claim. Changes of the kidney are well known to occur as Sprague Dawley rats age. Changes in the kidneys and lungs were observed for rats treated with ——— although, the incidence of changes was low.

Histopathological changes produced by treatment of rats with [redacted] by the intravenous bolus administration into a caudal vein at a dose of 36 mg/kg/day for 14 days. There were 5 animals/sex in the control group and 10 animals/sex in the [redacted] group.

Organ/Tissue	Control		PB19-6	
	Male n = 5	Female n = 5	Male n = 10	Female n = 10
Liver				
-biliary hyperplasia	0	2	2 (1)	6 (1)
-portal inflammation	0	0	1 (1)	9 (1)
-extramedullary hematopoiesis	1	3	4	5
-hepatocellular necrosis	0	0	0	3
-hepatocellular fatty change	0	4	0	9
Kidney				
-tubular basophilia	0	0	1 (1)	0
-interstitial inflammation	0	0	2 (1)	0
-tubule dilation	0	1 (1)	2 (1)	3 (1.3)
-lumen hyaline droplets	0	0	1 (1)	1 (2)
-tubular eosinophilia	0	0	0	1 (2)
-focal mineralization	0	0	0	3 (1)
Heart				
-chronic inflammation	0	3	7 (1.3)	6
-minimal myocardial degeneration	2	1	5	2
Lung				
-acute inflammation	0	0	2 (2)	0
-edema	0	0	1 (2)	0
-lymphoid aggregates	0	0	0	4 (1.5)
-chronic inflammation	0	0	0	5 (1.2)

(1)=minimal, (2)=mild, (3)=moderate, and (4)=marked

[redacted]hirulog, was evaluated in rats by intravenous bolus administration into a caudal vein at a dose of 36 mg/kg/day for 14 days. There was no mortality or clinical signs of toxicity. Target organs of toxicity with [redacted] appeared to be the liver, kidney, heart, and lungs. Treatment-related liver changes consisted of biliary hyperplasia, portal inflammation, and hepatocellular necrosis. The sponsor attributed these changes to intravenous administration of a protein. Further, they claimed that these changes were reversible; although, a recovery period was not included in the study. There was an increased incidence of chronic inflammation of the heart for rats treated with [redacted] The sponsor claimed that chronic inflammation of the heart is typical for rats >4 months old and the test article either accelerated or enhanced the development of these changes in rats