

Table 34. Overall Dose Proportionality Factor of Ro 50-3821 in Rats

Dose (µg/kg/dose)	Theoretical Increases in Exposure (fold)	Observed Increases in Exposure (fold)	
		AUC _{0.083-144hr}	C _{max}
Day 1			
1	--	--	--
3	3.0	3.4	3.8
10	3.3	5.9	5.3
30	3.0	3.4	2.7
Day 22			
1	--	--	--
3	3.0	3.2	3.7
10	3.3	5.5	6.0
30	3.0	4.5	3.9
Day 85			
1	--	--	--
3	3.0	2.9	3.1
10	3.3	5.3	6.3

Antibody Analysis: Anti-EPO Ab was detected after 4 weeks of treatment (the earliest time point) (Table 35). There was no clear dose relationship in incidence of Ab positive in general. However, time-dependent trend was noted in 1 mcg/kg/dose group (6/16 after 4 weeks vs. 14/20 after 13 weeks). Some animals remained Ab positive after 8 week recovery period.

Table 35. Summary of Incidence of Anti-EPO Ab*

Dose (mcg/kg)	4 wk Sacrifice				13 wk Sacrifice			21 wk Sacrifice	
	Male		Female		Male	Female		Male	Female
	+	±	+	±	+	+	±	+	+
0	0/8	0/8	0/8	0/8	0/10	1/10	0/10	0/8	0/7
1	3/8	0/8	3/8	1/8	8/10	6/10	0/10	2/8	3/7
3	5/8	1/8	4/8	0/8	6/10	5/10	1/10	3/8	4/8
10	4/8	0/8	6/8	0/8	7/9 ^b	1/5 ^c	0/5	4/6	1/6
30	2/6 ^a	0/6	4/6 ^a	0/6	N/A	N/A	N/A	N/A	N/A

* Prepared by the reviewer based on the submission, + Positive, ±Ambiguous, N/A: no rats available, Data presented as No. of positive or ambiguous results/No. of samples assayed

^a Tube without serum for two rats

^b not done for one rat because of no enough serum

^c not done for four rats because of no enough serum

Other: N/A

Conclusion

According to the study report, most of the changes seen were the result of exaggerated pharmacological effects of Ro 50-3821/000, or the subsequent development of neutralizing antibody.

TK profiles administered by IV were different from that by SC. When administered by intravenous injection at the same dose levels using the same dosing schedule, much greater exposure (C_{max} and AUC) to Ro 50-3821 was achieved as compared to that in the subcutaneous injection study. In addition, unlike in the subcutaneous injection study, no accumulation was observed in this IV study.

Two males and five females receiving 10 mcg/kg/dose were found dead or were prematurely sacrificed due to morbidities. While information on two toxicokinetic rats was not available, data from two males and three females from the toxicity group revealed that these animals suffered from severe polycythemia. Chronic nephropathy, necrosis and/or inflammation of the kidney, stomach or intestine, and hemorrhage in the spinal cord and brain were the pathological findings in these animals.

In addition, increased trabecular bone formation in femur and lymphocytic depletion in the thymus were observed in the 30 mcg/kg/dose group in this intravenous injection study but not in subcutaneous injection study at the 4-week interim evaluation. After 13 weeks of treatment, kidney necrosis in 3 and 10 mcg/kg/dose groups, valvular inflammation and thrombosis in the heart, and vascular inflammation of the pancreas in 10 mcg/kg/dose were also noted.

Following 8 weeks of recovery, only stomach lesions remained but with less frequency and severity.

According to the study report, the pathological conditions seen in polycythemic animals that continuously received Ro 50-3821/000 without adjustment of dosing regimen are not likely to be a risk in clinical use of Ro 50-3821/000. During clinical use, the dosing schedule can be easily adjusted and hematocrit and hemoglobin concentrations will be maintained within the normal physiological range.

Reviewer's comments

The reviewer agreed with the conclusion above. The findings in this study illustrated the importance of clinical monitoring and dose adjustment, and consequence of polycythemia.

The NOAEL could not be established for this study.

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Study title: Ro 50-3821/000: A Twenty-Six Week Subcutaneous (Injection) Toxicity and Toxicokinetic Study in Rats, Followed by A Twelve-Week Recovery Period.

Key study findings:Mortality:

- Three deaths (Days 142-154) in the 3 mcg/kg/dose group: all three rats had severe polycythemia (hematocrit ranging from 67.2% to 73.5%), increased ALT, AST, total bilirubin, and urea nitrogen, two moderate myocardial degeneration, and one tubular necrosis and peritonitis.

Unscheduled sacrifice:

- Two rats in the 0.3 mcg/kg/dose group: one due to malignant lymphoma (Day 69) and another due to benign astrocytoma in the brain (Day 114)
- Two rats in the 3 mcg/kg/dose group (Days 93 and 118): both rats had low hematocrit (4.0 or 4.9%), positive serum anti-EPO Ab, myocardial degeneration (slight or marked), marked centrilobular hepatocellular vacuolation, and moderate erosion in the glandular stomach, accompanied by marked hypocellular bone marrow in one rat.
- Increases in ALT, AST, total bilirubin, and urea nitrogen in all rats except for one with benign astrocytoma.

Body weights:

- A 12% decrease in body weight gain in the 3 mcg/kg/dose male group (7% decrease in absolute body weight) when compared to controls

Hematology

- Dose-related increase in erythrocyte parameters (hematocrit: 43% in controls vs. about 60% in the 3 mcg/kg/dose group on Day 27), reversible
- Dose- and time-related increases in the incidence and severity of anisocytosis and polychromasia, completely reversible
- Hypochromia in anti-EPO Ab positive rats, reversible
- Dose-related decreases in platelet counts, reversible

Organ weights:

- Increases in relative brain, spleen, heart, kidney, and adrenal gland weights in male rats and absolute and relative heart and kidney weights in females in the 3 mcg/kg/dose group, at least partially reversible.

Histopathology:

- Drug-related congestion in most of the tissues and organs

- Dose-related erosions in the stomach (1/24 in the 1 mcg/kg group and 8/28 in the 3 mcg/kg group), accompanied by hemorrhage, edema, and inflammation in the adjacent mucosa.
- Decreased erythropoiesis in the bone marrow in almost all rats in the 1 (24/24) and 3 mcg/kg/dose (26/28) groups but not in 0.3 mcg/kg/dose group. Partially reversible
- Hypocellular bone marrow in 3 mcg/kg/dose group, reversible
- Dose-related increase in incidence and severity of tubular basophilia, tubular pigmentation, and tubular casts; Tubular necrosis and degeneration in 3 mcg/kg/dose group only, at least partially reversible.

Toxicokinetics:

- Serum concentrations peaked at 16 to 36 hours post subcutaneous administration, indicating a relatively slow absorption process
- Greater than (on Days 0 and 175) or approximately dose-proportional (on Day 84) increases in AUC; Less than (on Day 0) or approximately dose-proportional (on Days 84 and 175) increases in Cmax
- Accumulation in AUC for all dose groups and in Cmax for the 3 mcg/kg/dose group only after repeat dosing
- No consistent gender differences.

Antibody Analysis:

- Anti-EPO Ab detected after 13 weeks of treatment (the earliest time point)
- Time-dependent trend for males in 0.3 mcg/kg/dose group (11% after 13 weeks vs. 44% after 26 weeks)
- Incidence of Ab positive: females > males (11% for males vs. 55% for females after 13 weeks), 50% females remained Ab positive after 12 week recovery period.

NOAEL was established at 0.3 mcg/kg/dose for this study.

Study no.: 07437, Report No. 1003071

Volume #, and page #: Module 4 Volume 1.8, page 1-1297

Conducting laboratory and location: Department of Non-Clinical Drug Safety, Hoffmann-La Roche Inc., Nutley, NJ, Anti-EPO Ab analysis by _____

Date of study initiation: N/A, study report date: 2 October, 2002

GLP compliance: Yes, Non-GLP for anti-EPO Ab analysis

QA report: yes (X) no ()

Drug, lot #, and % purity: Ro 50-3821/000, Bulk Drug: Lot No. PZ0006P018 (G006.01E), Group B & C: 1 mcg/mL, Lot No. L200800, Group D: 3 mcg/mL, Lot No. L200810, purity, _____

Methods

Doses: 0 (vehicle: 10 mM sodium/potassium phosphate buffer, pH 7.0, with 7.73 mg/mL NaCl, Group A), 0.3 (Group B), 1 (Group C), or 3 (Group D) mcg/kg/dose, once weekly for 26 wks (26 doses) with a 12-week recovery period.

The initial release results for the 1 and 3 mcg/mL concentrations were 1.03 and 3.05 mcg/mL (103% and 102% of claim, respectively). The results for the 1 and 3 mcg/mL concentrations re-tested at the end of the study and were _____ mcg/mL _____ of claim, respectively), confirming the stability (at least 4 months) of these solutions during the usage period. All test results for the concentrations tested were within the acceptable range (0.7 - 1.3 mcg/mL for the 1 mcg/mL strength and 2.1 - 3.9 mcg/mL for the 3 mcg/mL strength, both within 70%-130% of claim) specified for these strengths in the Toxicology Specifications (dated 22 September 2000).

Species/strain: Rat/HsdBrlHan:WIST (Wistar Hannover)

Number/sex/group or time point: 12/sex/group

Route, formulation, volume, and infusion rate: subcutaneous in the area of the nape of the neck, dose volume: 0.3 mL/kg/dose for 0.3 mcg/kg/dose group and 1 mL/kg/dose for 1 or 3 mcg/kg/dose group.

Satellite groups used for toxicokinetics or recovery: 3 rats/sex/group/time point for TK, 5-8 rats/sex/group for recovery.

Age: approximately 6 weeks old

Weight: average of 208 grams for males and 147 grams for females

Sampling times: See other related sections.

Unique study design or methodology (if any): including neurologic examination

Observations and times:

Mortality: At least once daily during the treatment and recovery periods; at least twice, before and after dosing and on the day of dosing.

Clinical signs: At least once daily to record morbidity during the treatment and recovery periods; at least twice, before and after dosing and on the day of scheduled dosing; once weekly to record clinical signs during the treatment and recovery periods.

Neurologic examination: Once prior to initiation of treatment and after 25 weeks.

Body weights: Pre-dose (Day -5), once weekly during the treatment and recovery periods beginning on Day 0, and just prior to sacrifice (final body weight).

Food consumption: Once weekly for each cage of 2 rats during the treatment and recovery periods beginning on Day 0.

Ophthalmoscopy: Once prior to initiation of treatment and after 25 weeks.

ECG: Not performed.

Hematology: Every 4 or 5 weeks during the treatment and recovery periods. Blood samples were collected from the retro-orbital sinus of all rats (unfasted) in the toxicity subset under isoflurane/O₂ anesthesia.

Clinical chemistry: Serum from blood samples collected from 12 rats/sex/group after 26 weeks, and all remaining rats in all groups after 12 weeks of recovery.

Urinalysis: After 13 and 24 weeks of treatment, and after 12 weeks of recovery.

Gross pathology: 12 rats/sex/group after 26 weeks, and all remaining rats in all groups after 12 weeks of recovery. Animals 22M and 40M in 0.3 mcg/kg/dose group; 65M, 70M, 71M, 150F, and 153F in 3 mcg/kg/dose group either died early or were humane-sacrificed prior to the terminal necropsy. In order to maintain 12 animals in these groups at the terminal necropsy, animals 33M in 0.3 mcg/kg/dose recovery group, 73M, 74M, 75M, and 154F in 3 mcg/kg/dose recovery group were sacrificed as substitute animals at the terminal necropsy.

Organ weights: 12 rats/sex/group after 26 weeks, and all remaining rats in all groups after 12 weeks of recovery. Also see histopath table (Table 14).

Histopathology: 12 rats/sex/group after 26 weeks, and all remaining rats in all groups after 12 weeks of recovery. A histopathologic evaluation was conducted on all protocol-designated organs and tissues from all animals that died early, or were humane- or moribund-sacrificed, and all terminal and recovery-sacrificed animals in the control and 3 mcg/kg/dose groups; gross lesions were examined for rats in all groups. Target organs and tissues, as outlined in the histopath table (Table 14), were examined from all terminal- and recovery-sacrificed animals including 0.3 and 1 mcg/kg/dose groups.

Adequate Battery: yes (X), no ()

Peer review: yes (), no (X)

Toxicokinetic: Blood samples were collected from the retro-orbital sinus of rats under isoflurane/O₂ anesthesia prior to dosing (0 hour), 30 minutes, 3, 8, 16, 24, 36, 48, 72, 96, 120, and 144 hours after dosing on Days 0, 84, and 175 of the treatment period by a validated ELISA assay procedure. The lower limit of quantitation was _____ for Ro 50-3821. The precision (% CV) and accuracy (% Rel. Error) of the assay as determined from the analysis of quality control samples were within the acceptable range of _____, (CV ≤14.16%).

Watson™ (Version 6.1.1.03), a Hoffmann-La Roche, Inc. (HLR) validated laboratory information system, was used to evaluate the serum concentration data as well as to perform toxicokinetic analysis. The toxicokinetic parameters calculated were AUC_{0-144hr}, dose normalized AUC (AUC_{0-144hr}/Dose), C_{max}, and T_{max}. The C_{max} and T_{max} values were taken directly from the concentration-time profiles without any extrapolation. The AUCs were calculated using the linear trapezoidal rule. Dose proportionality evaluations were based on AUC_{0-144h} and C_{max} values. Accumulation of Ro 50-3821 following repeated doses was determined by the ratio of AUC_(0-144h) or C_{max} on Day 84 or 175 to the equivalent parameter on Day 0.

Antibodies: Development of anti-EPO Ab was determined after 13 and 26 weeks of treatment, and 12 weeks of recovery using a sandwich type ELISA with an additional competitive displacement step.

Results

Mortality: Two males (70M and 71M) and one female (150F) in the 3 mcg/kg/dose group were found dead on Days 154, 152, and 142, respectively. Various clinical signs included alopecia and unkempt were observed prior to the death. Animals 70M and 71M

showed signs of a red coloration in the urine or bedding, respectively. Animals 70M, 71M, and 150F were severely polycythemic with hematocrit ranging from 67.2% to 73.5%.

Grossly, animal 70M had moderate dark hemorrhage in alveoli, reddish brown mottling in the antero-ventral region of the lung. Microscopically, 70M had moderate myocardial degeneration and myocardial mineralization with an infiltration of mononuclear cells. Congestion of a variety of tissues, including the liver (moderate), lungs (moderate), and kidneys (marked), was also noted.

Animal 71M had moderate myocardial degeneration, a pale foci on the right kidney that correlated with microscopic finding of tubular necrosis in the cortex, yellowish brown fluid in the abdominal cavity as well as yellowish brown material on the visceral surface of the liver, spleen, and small and large intestine. There was ulceration and hemorrhage of the mucosal surface of the jejunum with necrotic debris and thrombosis in the mesentery, and acute inflammation on the serosal surface of the ileum (consistent with peritonitis).

Animal 150F had an enlarged spleen with marked congestion and mildly increased erythropoiesis. Massive congestion and marked hemorrhage in the lamina propria and lumen of the ileum were noted. Congestion in the lungs, liver, and jejunum were also noted.

Serum anti-EPO Ab was negative for 70M and 150F on Day 90 (first time point, hemolytic samples), but not tested for 71M (not enough material).

Unscheduled sacrifice:

Four rats were unscheduled sacrificed due to morbidities and described below.

In the 0.3 mcg/kg/dose group, two males (22M and 40M) were sacrificed on Days 114 or 69, respectively. Animal 22M was sacrificed based on decreasing body weight and food consumption, and clinical signs (e.g. decreased activity, weak, and skinny). Grossly, animal 22M had a gray-white area in the cerebrum. Microscopically, this animal was found to have a benign astrocytoma in the brain which may have contributed to this animal's condition.

Animal 40M was sacrificed based on a high white blood cell count (389 K/mcL) attributed to a markedly increased blast cell count, decreasing body weight and food consumption, and clinical signs (e.g. pale extremities and decreased activity). Grossly, animal 40M had an enlarged spleen, thymus, and mesenteric lymph nodes, and a reddish discoloration of the frontal lobe and brain stem. Microscopically, this animal was found to have a malignant lymphoma. Neoplastic lymphocytes were present in the liver, lungs, kidneys, spleen, brain, heart, thymus, epididymis, testis, and bone marrow.

In addition, mesothelioma was seen in the epididymis of a control male (2M) and this rat survived to the end of the study. None of these tumors were considered to be drug-related because of the low incidence, according to the study report.

In the 3 mcg/kg/dose group, one male (65M) was sacrificed on Day 93 based on decreasing body weight and food consumption, and clinical signs (e.g., pale eyes, ears, and extremities, cool to touch). Grossly, animal 65M had moderate enlargement of both ventricles of the heart, marked pale lungs, and a markedly yellowish liver. Microscopically, this animal had slight (grade 2) myocardial degeneration with moderate (grade 3) mononuclear cell infiltration, marked (grade 4) centrilobular hepatocellular vacuolation, a moderate erosion in the glandular stomach, and marked hypocellular bone marrow.

One female (153F) in the 3 mcg/kg/dose group was sacrificed on Day 118. Reduction in body weight and food consumption, and pale eyes and limbs were noted before the sacrifice. On the day of sacrifice, 153F was noted to be cold to the touch, weak, lethargic, inactive, and lying in a prostate position in the cage. Grossly, 153F had reddish discoloration of the left atrium and yellowish discoloration of the liver. Microscopically, 153F had a marked myocardial degeneration with moderate mononuclear cell infiltration, marked hepatocellular vacuolation, and multifocal areas of moderate coagulative necrosis with neutrophil accumulation and moderate erosions in the mucosa of glandular stomach.

Among four rats unscheduled sacrificed, low hematocrit values were noted in three rats [40M (12.2%), 65M (4.9%), and 153F (4.0%) on Day 90]. Serum anti-EPO Ab was positive for 65M and 153F on Day 90 (first time point), but not available for 40M (due to early sacrifice). In addition, marked hypocellular bone marrow was observed for 65M. The hematocrit values for 22M increased over time, and was 52.4% on the day of sacrifice (Day 114), which was higher than the mean value (44.6%) for control males measured on Day 118 (scheduled evaluation). Serum anti-EPO Ab was negative for 22M.

In addition, there were increases in alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, and urea nitrogen in all of the rats except for 22M. Microscopic findings that correlated with these changes observed in some of these animals included malignant lymphoma in the liver (40M), hepatic vacuolation (65M), and hepatic vacuolation and coagulative necrosis (153F).

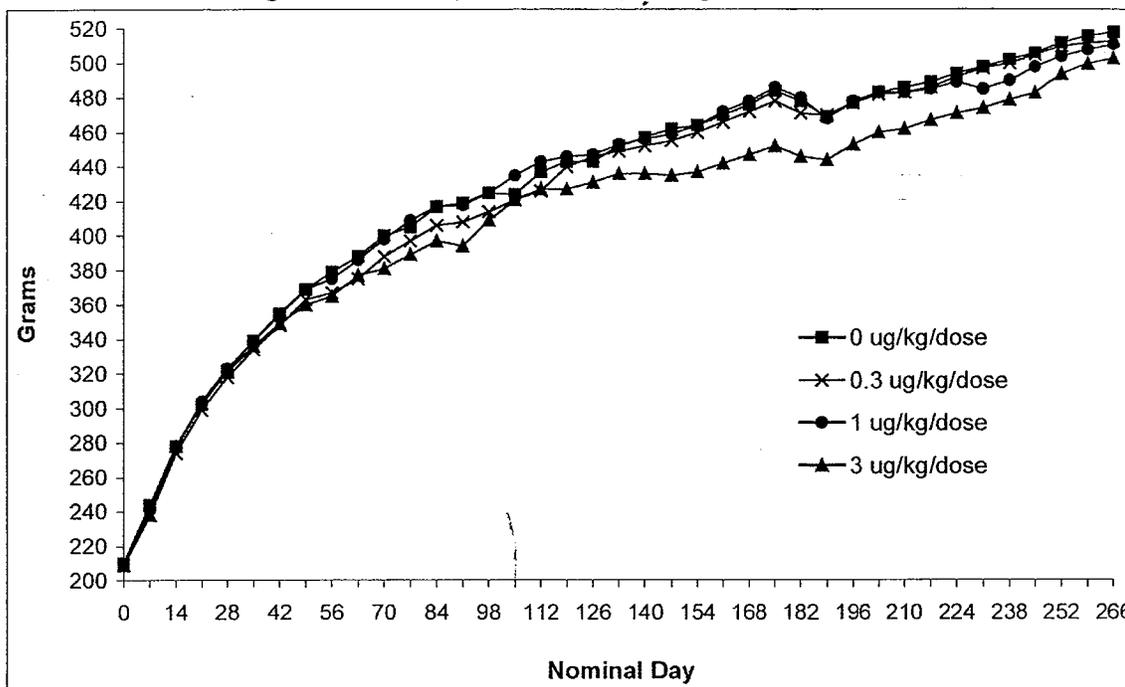
There were no mortalities or unscheduled sacrifices during the recovery period.

Clinical signs: According to the study report, there were no remarkable, drug-related clinical signs except for clinical signs described for animals that died or were unscheduled sacrificed in the mortality section above.

Body weights: A 12% decrease in body weight gain that resulted in a 7% decrease in absolute body weight after 26 weeks of treatment in the 3 mcg/kg/dose male group compared to controls (Figure 7). During the recovery period, mean body weight gain for males in all three treatment groups was generally similar to that for control males. A

statistically significant increase in mean body weight was noted for 3 mcg/kg/dose females on Day 161; however, this change was a transient effect noted on this day only, and was not considered to be drug-related, according to the study report.

Figure 7. Weekly Mean Body Weights for Males



Food consumption: No consistent increases or decreases in food consumption.

Neurological Examinations: No drug-related neurological findings, according to the study report.

Ophthalmoscopy: No remarkable drug related findings. Either a corneal opacity or a shrunken eye (shrunken globe) were observed in rats in all groups (2 rats in each dose groups) after 25 weeks of treatment that were attributed to repeated retro-orbital blood collection for monthly hematology evaluation.

ECG: Not performed.

Hematology:

Erythrocyte parameters

As expected from known pharmacological activity, RO50-3821 increased erythrocyte parameters [erythrocyte counts, hemoglobin, hematocrit, mean cell hemoglobin concentration (MCHC), red cell distribution width (RDW) and reticulocytes] in dose-related manner in both male (Table 36) and female (Table 37) rats. Statistically significant increase was observed in 1 and 3 mcg/kg/dose groups. After 12 weeks of

recovery, there was complete recovery in the 1 mcg/kg/dose group but only partial recovery in the 3 mcg/kg/dose group.

Although the reticulocyte counts increased in a dose-related manner in the early phase of the study, the effects tended to diminish over the course of the 26 week treatment period as some animals in each treatment group developed neutralizing anti-EPO Ab. Decrease in reticulocytes was noted in some rats as early as on Day 27 (the earliest time point tested) in 1 and 3 mcg/kg/dose groups. Majority of these rats were anti-EPO Ab positive on Day 90 (the earliest time point for Ab testing). In general, low erythrocyte parameters correlated well with positive anti-EPO Ab. For example, on Day 181 (terminal sacrifice), almost all Ab positive female rats (12/13 including one with ambiguous Ab result) had HCT<45% (range 14.4 -44.3%) except one rat (HCT 74.65%) while all Ab negative rats (4/4) had HCT>70% (range 73.8-76.7%).

Table 36. Summary of Hematology Parameters (Males)

Groups			Vehicle	0.3 mcg/kg	1 mcg/kg	3 mcg/kg
ERYTHROCYTES						
	MI/uL					
DAY 27	MEAN		8.08 D	8.43	8.92**	10.53**
DAY 55	MEAN		8.63 K	9.02	9.33**	12.82**
DAY 90	MEAN		8.85 K	9.21	9.42	11.98**
DAY 118	MEAN		8.71 K	8.81	8.98	12.29**
DAY 146	MEAN		8.85 K	8.84	9.15	12.55**
DAY 181	MEAN		8.74 K	8.74	9.06	11.67*
DAY 209	MEAN		9.10 D	8.18	7.83*	9.40
DAY 237	MEAN		9.31 K	8.76	8.08**	7.87**
DAY 265	MEAN		9.14 K	8.97	8.32	9.14
HEMOGLOBIN						
	G/DL					
DAY 27	MEAN		14.9 K	15.4	16.4**	20.1**
DAY 55	MEAN		15.1 K	15.6	16.3**	22.5**
DAY 90	MEAN		15.7 K	16.1	16.9	21.6**
DAY 118	MEAN		15.1 K	15.1	15.7	21.8**
DAY 146	MEAN		15.2 K	14.9	15.7	21.6**
DAY 181	MEAN		15.2 K	14.9	15.7	20.5*
DAY 209	MEAN		15.7 K	14.3	13.6*	16.1
DAY 237	MEAN		15.7 K	15.0	14.1*	14.8
DAY 265	MEAN		15.6 K	15.3	14.4	16.3
HEMATOCRIT						
	%					
DAY 27	MEAN		42.9 K	44.9	47.7**	58.4**
DAY 55	MEAN		43.9 K	45.5	47.6**	64.9**
DAY 90	MEAN		43.9 K	45.0	46.9	58.7**
DAY 118	MEAN		44.6 K	44.5	46.0	62.6**
DAY 146	MEAN		45.1 K	44.6	46.5	62.8**
DAY 181	MEAN		45.1 K	44.3	46.4	59.9*
DAY 209	MEAN		47.4 K	43.2	40.8*	47.8
DAY 237	MEAN		47.8 K	45.9	42.5*	45.1
DAY 265	MEAN		47.3 K	46.7	43.0	49.8
RETICULOCYTES						
	%					
DAY 27	MEAN		2.6 K	2.7	3.6	6.3**
DAY 55	MEAN		2.4 K	2.3	2.9*	4.3**
DAY 90	MEAN		1.8 K	1.9	2.3	4.4**
DAY 118	MEAN		2.1 K	2.2	2.4	4.0
DAY 146	MEAN		1.8 K	2.0	2.5	3.3**
DAY 181	MEAN		1.9 K	2.1	2.3	3.5**
DAY 209	MEAN		1.8 D	2.4	2.1	1.1
DAY 237	MEAN		1.8 D	1.9	2.2	3.9**
DAY 265	MEAN		2.0 D	2.1	1.8	1.4*

* p<0.05, ** p<0.01

Table 37. Summary of Hematology Parameters (Females)

Groups			Vehicle	0.3 mcg/kg	1 mcg/kg	3 mcg/kg
ERYTHROCYTES		NI/uL				
DAY 27	MEAN		7.75 D	8.12**	8.83**	10.94**
DAY 55	MEAN		8.03 D	7.76	9.25*	11.90**
DAY 90	MEAN		7.89 K	7.52	8.54	9.05
DAY 118	MEAN		7.75 K	7.15	8.38	8.37
DAY 146	MEAN		7.96 K	7.42	8.91	8.44
DAY 181	MEAN		7.64 K	7.16	8.93	7.82
DAY 209	MEAN		7.87 D	6.81	5.81	7.04
DAY 237	MEAN		7.93 D	7.26	7.56	6.46*
DAY 265	MEAN		8.02 D	7.52	7.55	7.10
HEMOGLOBIN		G/DL				
DAY 27	MEAN		14.5 D	15.1*	17.1**	21.4**
DAY 55	MEAN		14.7 D	14.1	17.3*	21.9**
DAY 90	MEAN		14.9 K	14.1	16.4	17.4
DAY 118	MEAN		14.4 K	13.4	15.9	16.0
DAY 146	MEAN		14.7 K	13.6	16.6	15.9
DAY 181	MEAN		14.2 K	13.3	16.8	15.1
DAY 209	MEAN		14.8 K	12.9	12.8	13.2
DAY 237	MEAN		14.6 D	13.3	14.2	12.3
DAY 265	MEAN		14.9 K	13.7	14.1	13.3
HEMATOCRIT		%				
DAY 27	MEAN		42.7 D	44.4*	49.7**	62.4**
DAY 55	MEAN		42.7 D	41.2	50.4**	62.9**
DAY 90	MEAN		41.8 K	39.5	45.7	47.6
DAY 118	MEAN		42.1 K	39.0	46.4	45.8
DAY 146	MEAN		43.6 K	40.2	49.1	46.0
DAY 181	MEAN		42.1 K	39.4	49.8	43.9
DAY 209	MEAN		44.3 K	38.2	38.3	39.0
DAY 237	MEAN		43.6 K	39.7	42.9	36.9
DAY 265	MEAN		44.6 K	41.4	42.3	39.6
RETICULOCYTES		%				
DAY 27	MEAN		2.5 D	2.5	4.1**	6.7**
DAY 55	MEAN		2.2 K	1.9	3.0	3.0
DAY 90	MEAN		2.2 K	2.0	2.8	2.9
DAY 118	MEAN		2.0 K	2.2	2.6	2.9
DAY 146	MEAN		2.2 K	2.2	2.9	3.1
DAY 181	MEAN		2.0 D	2.1	2.9	2.2
DAY 209	MEAN		2.0 D	1.9	2.5	1.4
DAY 237	MEAN		2.4 K	1.9	2.0	2.8
DAY 265	MEAN		1.8 D	2.3	1.9	1.7

* p<0.05, ** p<0.01

Four, eight, or 12 weeks after cessation of treatment, there were decreases in erythrocyte and reticulocytes counts, and increases in mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) in some male and/or female rats given 1 and/or 3 mcg/kg/dose as compared to controls. These findings, generally seen in polycythemic animals that did not develop neutralizing antibodies, were considered the result of the recovery process and continued until their red blood cell mass approached normal levels. Although there was a slight but statistically significant increase in MCV, and a slight but statistically significant decrease in reticulocytes in the 3 mcg/kg/dose males, these parameters were generally similar to controls at the end of the recovery period.

Anisocytosis and polychromasia

Dose- and treatment-time related increases in incidence and severity of RBC anisocytosis and polychromasia were noted (Table 38). RBC anisocytosis and polychromasia were observed in majority of rats as early as Day 27 (the earliest time point tested) in 3 mcg/kg/dose group but no longer detected on Day 237 and afterwards, indicating complete recovery.

Table 38. Summary of RBC Anisocytosis and Polychromasia*

Dose (mcg/kg)	0	0.3	1	3
Anisocytosis				
Day 27	0/40	0/40	0/36	33/40 (2+)
Day 55	0/39	1/39 (2+)	1/39 (2+)	30/37 (2+=20, 3+=10)
Day 90	0/38	0/37	2/36 (2+)	26/40 (2+=9, 3+=17)
Day 118	0/40	0/34	9/39 (2+)	19/34 (2+=4, 3+=15)
Day 146	0/40	0/38	6/38 (2+)	20/36 (3+=14, 4+=6)
Day 181	0/39	1/37 (2+)	10/39 (2+)	18/35 (3+=14, 4+=4)
Day 209	0/15	0/13	1/15 (2+)	3/11 (2+)
Day 237	0/16	0/14	0/14	0/11
Polychromasia				
Day 27	0/40	0/40	0/36	36/40 (2+)
Day 55	0/39	0/39	0/39	17/37 (2+=16, 3+=1)
Day 90	0/38	1/37 (2+)	0/36	22/40 (2+=21, 3+=1)
Day 118	0/40	0/34	3/39 (2+)	10/34 (2+=9, 3+=1)
Day 146	0/40	0/38	2/38 (2+)	16/36 (2+)
Day 181	0/39	0/37	1/39 (2+)	12/35 (2+)
Day 209	0/15	0/13	0/15	0/11
Day 237	0/16	0/14	0/14	0/11

* Prepared by the reviewer based on data submitted, the incidence presented as # of rats present anisocytosis or polychromasia/# of rats tested, and severity as (1+=rare, 2+=few, 3+=moderate, 4+=numerous).

Macrocytes, hypochromia, and anti-EPO Ab

Macrocytes, hypochromia, and target cells were observed mainly after 13 weeks of treatment and were seen in some rats in all drug groups (Table 39). All rats with hypochromia also had positive results for anti-EPO Ab on Day 90 and/or Day 181. Two rats (134F and 159F) continued to exhibit these changes after 4 weeks of recovery, thus indicating a prolonged antibody response. Hypochromia was no longer observed after 8 week recovery and Ab analysis showed Ab negative on Day 265 for these rats.

Platelet counts

Dose-related decrease in platelet counts was noted (Table 40). The decrease was statistically significant in the 1 and 3 mcg/kg/dose groups at several time points. The decreases in platelet counts were considered, according to the study report, to be artifactual changes associated with high erythrocyte counts, decreased mean cell volume, increased variability in erythrocyte size, and limitations in the analyzer's ability to accurately count platelets under these conditions. Furthermore, during the treatment period, due to the polycythemic condition of the animals, mean platelet volume (MPV) could not be calculated for most animals in the 3 mcg/kg/dose group, a few individual animals in the 1 mcg/kg/dose group at earlier weekly time points, and for most rats at later time points. At the end of the 12-week recovery period, the platelet counts were comparable to the controls.

There was a slight increase in the prothrombin time (PT) in rats treated with 3 mcg/kg/dose following 26 weeks of treatment (males: 16.9 ± 2.0 sec in 3 mcg/kg/dose vs. 14.7 ± 0.5 in controls, $p < 0.01$; females: 15.9 ± 2.7 sec in 3 mcg/kg/dose vs. 14.7 ± 0.3 in controls). At the end of 12 weeks of recovery, PT was comparable to controls (males: 16.6 ± 0.8 sec in 3 mcg/kg/dose vs. 16.4 ± 0.6 for controls). This effect was likely an artifactual change and considered unrelated to the effects of Ro 50-3821/001 by the study report. The increase in the PT time was likely due to relatively higher plasma concentrations of the anticoagulant (sodium citrate) used for blood sample collection — there was increased red blood cell mass and therefore decreased plasma volume. Prothrombin time in the 3 mcg/kg/dose males reversed to normal by the end of the 12-week recovery period, as red cell mass return to more normal levels.

Table 39. Summary of Macrocytes, Hypochromia, and Target Cells*

Dose (mcg/kg)	0	0.3	1	3
Macrocytes				
Day 27	0/40	0/40	0/36	0/40
Day 55	0/39	0/39	0/39	0/37
Day 90	0/38	0/37	0/36 (2+)	3/40 (2+=2, 3+=1)
Day 118	0/40	0/34	3/39 (2+)	1/34 (2+)
Day 146	0/40	0/38	0/38	0/36
Day 181	0/39	0/37	1/39 (2+)	18/35 (3+=14, 4+=4)
Day 209	0/15	0/13	0/15	0/11
Day 237	0/16	0/14	0/14	0/11
Hypochromia				
Day 27	0/40	0/40	0/36	0/40
Day 55	0/39	1/39 (3+)	0/39	0/37
Day 90	0/38	2/37 (2+=1, 3+=1)	1/36 (3+)	4/40 (2+=3, 3+=1)
Day 118	0/40	3/34 (2+)	2/39 (2+)	3/34 (2+=2, 3+=1)
Day 146	0/40	0/38	0/38	3/36 (2+)
Day 181	0/39	0/37	1/39 (2+)	4/35 (2+)
Day 209	0/15	0/13	1/15 (2+)	1/11 (2+)
Day 237	0/16	0/14	0/14	0/11
Target Cells				
Day 27	0/40	0/40	0/36	0/40
Day 55	0/39	0/39	0/39	0/37
Day 90	0/38	2/37 (1+=1, 2+=1)	2/36 (2+)	4/40 (2+=3, 3+=1)
Day 118	0/40	1/34 (1+)	2/39 (1+=1, 2+=1)	1/34 (2+)
Day 146	0/40	0/38	0/38	1/36 (2+)
Day 181	0/39	0/37	1/39 (2+)	3/35 (2+)
Day 209	0/15	0/13	0/15	1/11 (1+)
Day 237	0/16	0/14	0/14	0/11

* Prepared by the reviewer based on data submitted, the incidence presented as # of rats with the positive finding/# of rats tested, and severity as (1+=rare, 2+=few, 3+=moderate, 4+=numerous).

Table 40. Summary of Platelet Counts

Groups			Vehicle	0.3 mcg/kg	1 mcg/kg	3 mcg/kg
Male rats						
PLATELETS	K/uL					
DAY 27	MEAN		893 D	925	851	773**
DAY 55	MEAN		814 D	922	859	709
DAY 90	MEAN		847 D	749	805	702**
DAY 118	MEAN		828 D	973	856	720
DAY 146	MEAN		858 K	947	855	619**
DAY 181	MEAN		876 K	951	815	557**
DAY 209	MEAN		827 D	904	831	774
DAY 237	MEAN		856 D	980	904	770
DAY 265	MEAN		894 D	950	846	758
Female rats						
PLATELETS	K/uL					
DAY 27	MEAN		890 D	906	809*	713**
DAY 55	MEAN		800 D	866	721	606**
DAY 90	MEAN		752 D	766	695	588**
DAY 118	MEAN		764 K	839	732	674
DAY 146	MEAN		782 K	814	722	642
DAY 181	MEAN		722 K	759	646	674
DAY 209	MEAN		729 D	918*	775	604
DAY 237	MEAN		810 D	869	738	695
DAY 265	MEAN		752 D	878	776	746

Clinical chemistry:

The following changes were noted.

- Increases in total bilirubin in 3 mcg/kg/dose groups but only statistically significant in males (0.22 mg/dL vs. 0.1 in controls in males, $p < 0.01$). Additionally, a few individual animals in the 0.3 and 1 mcg/kg/dose groups exhibited mild to moderate increases in total bilirubin. Total bilirubin in both sexes was comparable to controls at the end of the recovery period.
- Decreases in Unsaturated Iron Binding Capacity [UIBC, 352 mcg/dL in male controls vs. 285 in 0.3 mcg/kg ($p < 0.05$), 302 in 1 mcg/kg, 259 in 3 mcg/kg ($p < 0.05$)], reversible (341 mcg/dL in controls vs. 362 in 0.3 mcg/kg, 326 in 1 mcg/kg, 270 in 3 mcg/kg). Based on the facts that there was no clear dose relationship and there was wide intra-animal variability, these changes were not attributed to treatment by the study report.
- Decreases in glucose in the males of 3 mcg/kg/dose group ($p < 0.01$, 108 mg/dL vs. 159 in controls), reversible.
- Increases in serum potassium in the males treated with 3 mcg/kg/dose group ($p < 0.05$, 5.0 mmol/L vs. 4.6 in controls), reversible.
- Increases in the urea nitrogen values of a few individual rats were observed in all treated groups. While these increases did not result in statistically significant changes, the results for these animals were slightly higher than the results observed for most of the other animals within their respective groups.

Urinalysis: Decreases in urine calcium ($p < 0.05$ for males only, 26.4 mg/dL in controls vs. 15.1 in 3 mcg/kg/dose group on Day 92) and the corresponding urine calcium/creatinine ratio ($p < 0.01$ for males, 0.26 in controls vs. 0.14 in 3 mcg/kg/dose group on Day 92) in 3 mcg/kg/dose group, reversible.

Gross pathology:*Unscheduled Sacrifice*

See the section on mortality above.

Terminal Sacrifice

The most consistent drug-related finding was a slight to moderate reddening of the glandular mucosa of the stomach. This change was evident in 1/12, 2/12, and 6/15 males in the 0.3, 1, or 3 mcg/kg/dose group, respectively. This finding correlated with vascular congestion in most animals examined, and hemorrhage or erosions in a few of the affected animals. Reddening of the glandular stomach mucosa was not observed in any of drug groups at the end of 12 weeks of recovery.

Recovery Sacrifice

No significant macroscopic findings were seen at necropsy after 12 weeks of recovery.

Organ weights: There were statistically significant increases in relative brain, spleen, heart, kidney, and adrenal gland weights in male rats (Table 41), and in absolute and relative heart and kidney weights in females in the 3 mcg/kg/dose group after 26 weeks. Congestion in these organs due to polycythemia was considered to be the primary cause of the observed weight changes according to the study report.

At the end of recovery, statistically significant increase in relative spleen weight was noted for males given 3 mcg/kg/dose only. Only minimal splenic congestion was evident in two males. In addition, statistically significant increases in absolute but not relative heart, spleen (0.53250 g vs. 0.65833, $p \leq 0.05$), ovary, and kidney weights were noted in females given 3 mcg/kg/dose, probably due to significantly increased final body weights.

Table 41. Summary of Relative Organ Weight (Mean±SD)^a

Dose (mcg/kg)	After 26 week treatment				After 12 week recovery			
	0 n=12 (M)	3 n=12 (M)	0 n=12 (F)	3 n=12 (F)	0 n=8 (M)	3 n=5 (M)	0 n=8 (F)	3 n=6 (F)
Brain	0.45874± 0.036	0.49953± 0.045*	0.79409± 0.060	0.76773± 0.055	0.44569± 0.048	0.47453± 0.030	0.81204± 0.045	0.71457± 0.076*
Spleen	0.15994± 0.017	0.23236± 0.078**	0.23149± 0.029	0.27365± 0.148	0.13952± 0.013	0.16826± 0.019*	0.21599± 0.030	0.23347± 0.056
Heart	0.25858± 0.016	0.29956± 0.026**	0.33023± 0.031	0.40742± 0.051**	0.25169± 0.021	0.26157± 0.018	0.33414± 0.038	0.35961± 0.039
Kidneys	0.53526± 0.037	0.61806± 0.058**	0.66911± 0.042	0.73431± 0.068*	0.51496± 0.042	0.55425± 0.095	0.69296± 0.055	0.70917± 0.064
Adrenal Glands	0.01298± 0.002	0.01568± 0.003*	0.03478± 0.004	0.03563± 0.007	0.01257± 0.002	0.01479± 0.002	0.03102± 0.003	0.03139± 0.005

^a Prepared by the reviewer according to the submitted data, * $p \leq 0.05$, ** $p \leq 0.01$

Histopathology:

The most consistent drug-related change observed was congestion (hyperemia) of the vasculature in majority of the tissues and organs examined including brain, heart, kidneys, liver, spleen, stomach, and thymus. The congestion in the brain, heart, and kidneys was the probable cause of increases in the absolute and relative weights of these organs. An additional finding was erosions in the stomach, noted in 1/24 (one male) and 8/28 (5/15 males including one moribund-sacrificed animal and 3/13 females) in the 1 and 3 mcg/kg/dose groups, respectively. These erosions were accompanied by hemorrhage, edema, and inflammation in the adjacent mucosa.

Decreased erythropoiesis was noted in the sternum and femoral bone marrow in almost all rats in the 1 (24/24 rats) and 3 mcg/kg/dose (13/15 males, 13/13 females) groups but not in 0.3 mcg/kg/dose group. Average grades were 1.6 (range 1-2) for females and 1.8 (range 1-3) for males in 1 mcg/kg/dose group and 1.9 for females (range 1-4) and 2.0 (range 1-4) for males in 3 mcg/kg/dose group (grade 1: minimal, Grade 2: slight, Grade 3: moderate, Grade 4: marked). Decreased erythropoiesis was observed only in 3 mcg/kg/dose group with reduced incidence and severity after 12 week recovery period (2/5 males with average grade 1.0 and 0/7 females). Hypocellular bone marrow was noted in 3 mcg/kg/dose group at 26 week sacrifice [1/15 males (65M) with grade 4.0 (marked) and 3/14 females (143F, 146F, and 153F) with grade 3.0 (moderate)] but not noted after 12 week recovery period.

Erythropoiesis was observed in the spleen of 7/28 rats [3/15 males (69M, 70M, and 73 M) and 4/13 females (144F, 145F, 150F, and 151F)] in the 3 mcg/kg/dose group, but also in 3/24 rats (2/12 males, 3M and 7M, and 1/12 females, 87F) in control group. Erythropoiesis was also noted in the liver of 2 females (81F and 85F) in control group.

There were numerous macrophages containing large amounts of brownish pigment (considered to be hemosiderin) in the spleen. This pigment was seen in both control and drug groups but it was more intense in the latter.

Drug-related kidney lesions were observed as evidenced by dose-related increase in incidence and severity of tubular basophilia, tubular pigmentation, and tubular casts (Table 42). Furthermore, tubular necrosis and degeneration were observed in high dose group only. These lesions were, at least partially, reversible as evidenced by reduced incidence and severity after 12 week recovery period. These changes were considered to be most likely due to an exacerbation of the early stages of chronic progressive glomerulonephropathy, a spontaneous disease of rats that occurs more frequently in males, according to the study report.

Table 42. Summary of Histopathology Findings in Kidneys^a

Group	Ctrl		0.3 mcg/kg		1 mcg/kg		3 mcg/kg	
	M	F	M	F	M	F	M	F
Basophilic tubule								
Terminal sacrifice	2/12 (1.0*)	2/12 (1.0*)	1/13 (1.0*)	1/12 (1.0*)	4/12 (1.3, 1*-2**)	0/12	13/15 (2.0, 1.0*-4**)	5/13 (1.0*-**)
Recovery sacrifice	1/8 (1.0*)	0/8	2/7 (1.5*, 1-2)	0/8	0/8	0/8	2/5 (1.0, 1.0*-1.0**)	0/7
Pigmentation tubular^b	0/12	5/12 (1.0**)	0/13	0/12	1/12 (1.0**)	0/12	7/15 (2.0**, 1**-4**)	3/13 (1.7**, 1**-2**)
Tubular casts								
Terminal sacrifice	0/12	0/12	0/13	0/12	1/12 (1.0**)	1/12 (1.0**)	3/15 (1.7, 1*-2**)	2/13 (1.0, 1.0*-1.0**)
Recovery sacrifice	0/8	1/8 (1.0*)	0/7	1/8 (1.0*)	0/8	0/8	1/5 (1.0**)	1/7 (1.0*)
Congestion								
Terminal sacrifice	0/12	0/12	0/13	0/12	9/12 (1.4, 1**-2**)	8/12 (1.6, 1**-2**)	11/15 (2.6, 1**-4**)	11/13 (1.9, 1-3)
Recovery sacrifice	0/8	0/8	0/7	0/8	0/8	0/8	3/5 (1.0**)	3/7 (1.0**)
Necrosis tubular^b	0/12	0/12	0/13	0/12	0/12	0/12	2/15 (2.5, 2*-3*)	0/13
Degeneration tubular^b	0/12	0/12	0/13	0/12	0/12	0/12	1/15 (2**)	0/13

^aPrepared by the reviewer based on data submitted, presented as incidence (mean severity, range)

^bonly noted in terminal sacrifice rats

*unilateral ** bilateral

Toxicokinetics:

Parameters and Concentrations vs. Time Profiles

The inter-subject variability of serum Ro 50-3821 concentration at the 0.3 and 1 mcg/kg/dose levels was rather high due to very low (near to the detection limit: 1.0 ng/mL) serum concentrations in some animals and/or a limited number of animals (only 1 or 2 animals/sex/group/time point) with detectable drug concentrations. Thus, the data for these two groups were not reliable.

In general, serum concentrations peaked at 16 to 36 hours post-dose across all treatment groups (Figure 8, Table 43), indicating a relatively slow absorption process from the injection site following subcutaneous administration. Greater than (on Days 0 and 175) or approximately dose-proportional (on Day 84) increases in AUC were observed (Table 43). Less than (on Day 0) or approximately dose-proportional (on Days 84 and 175) increases in C_{max} were observed.

Figure 8. Concentration-Time Profile of Ro 50-3821 in Rats (Day 175)

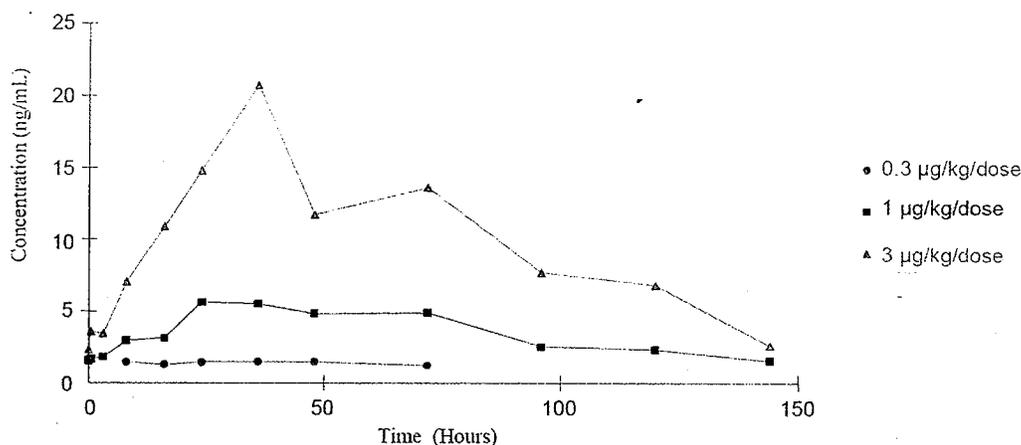


Table 43. Toxicokinetic Parameters for Ro 50-3821

Day 0

Dose (µg/kg/dose)	Parameters	Units	Male	Female	Overall
0.3	AUC	ng*Hours/mL	15.7	40.3	39.9
	AUC/Dose	ng*Hours/mL/µg/kg	52.3	134	133
	Cmax	ng/mL	1.46	1.89	1.89
	Tmax	Hours	16	36	36
1	AUC	ng*Hours/mL	168	184	227
	AUC/Dose	ng*Hours/mL/µg/kg	168	184	227
	Cmax	ng/mL	3.35	6.18	4.22
	Tmax	Hours	36	16	16
3	AUC	ng*Hours/mL	667	770	735
	AUC/Dose	ng*Hours/mL/µg/kg	222	257	245
	Cmax	ng/mL	9.41	11.7	10.6
	Tmax	Hours	36	24	36

Day 84

Dose (µg/kg/dose)	Parameters	Units	Male	Female	Overall
0.3	AUC	ng*Hours/mL	122	69.5	148
	AUC/Dose	ng*Hours/mL/µg/kg	407	232	493
	Cmax	ng/mL	1.17	1.67	1.67
	Tmax	Hours	36	24	24
1	AUC	ng*Hours/mL	242	377	308
	AUC/Dose	ng*Hours/mL/µg/kg	242	377	308
	Cmax	ng/mL	3.39	4.91	4.15
	Tmax	Hours	24	24	24
3	AUC	ng*Hours/mL	1030	1450	1240
	AUC/Dose	ng*Hours/mL/µg/kg	343	483	413
	Cmax	ng/mL	12.1	22.9	17.5
	Tmax	Hours	24	24	24

Day 175

Dose (µg/kg/dose)	Parameters	Units	Male	Female	Overall
0.3	AUC	ng*Hours/mL	52.5	91.1	93.5
	AUC/Dose	ng*Hours/mL/µg/kg	175	304	312
	Cmax	ng/mL	1.60	1.46	1.49
	Tmax	Hours	36	8	36
1	AUC	ng*Hours/mL	541	494	516
	AUC/Dose	ng*Hours/mL/µg/kg	541	494	516
	Cmax	ng/mL	6.80	5.88	5.63
	Tmax	Hours	24	36	24
3	AUC	ng*Hours/mL	1320	1610	1460
	AUC/Dose	ng*Hours/mL/µg/kg	440	537	487
	Cmax	ng/mL	20.3	21.0	20.7
	Tmax	Hours	36	36	36

Table 44. Overall Dose Proportionality Factor of Ro 50-3821 in Rats

Dose (µg/kg/dose)	Theoretical Increases in Exposure (fold)	Observed Increases in Exposure (fold)		
		Day 0	Day 84	D175
		AUC _{0-144hr}		
1	3.3	5.69	2.08	5.52
3	10	18.4	8.38	15.6
		C _{max}		
1	3.3	2.23	2.49	3.78
3	10	5.61	10.5	13.9

Compared to 13-week study in rats given Ro 50-3821/000 subcutaneously once per week, the overall AUC and Cmax values at the 1 and 3 mcg/kg/dose levels on Days 0 and 84 in both studies were comparable (see Table 45).

Table 45. TK Parameter Comparison of 13-week with 26-week Studies in Rats

Dose (µg/kg/dose)	AUC (ng•h/ml)		Cmax (ng/ml)	
	Day 0			
	13-week Study	Present Study	13-week Study	Present Study
1	199	227	3.97	4.22
3	754	735	13.5	10.6
	Day 84			
1	369	308	5.26	4.15
3	1460	1240	20.3	17.5

Changes During Repeated Administration

Generally, there was no accumulation in Cmax for the 0.3 and 1 mcg/kg/dose groups on Days 84 and 175, but there was slight accumulation for the 3 mcg/kg/dose group on Days

84 and 175 (see Table 46). A small degree of accumulation in AUC was observed across all drug groups with accumulation factors ranging from 1.36 to 3.71.

Table 46. Repeated Dose Factor of Ro 50-3821 in Rats

Dose ($\mu\text{g}/\text{kg}/\text{dose}$)	$\text{AUC}_{\text{day84}}/\text{AUC}_{\text{day0}}$	$\text{AUC}_{\text{day175}}/\text{AUC}_{\text{day0}}$
0.3	3.71	2.34
1	1.36	2.27
3	1.69	1.99
	$\text{Cmax}_{\text{day84}}/\text{Cmax}_{\text{day0}}$	$\text{Cmax}_{\text{day175}}/\text{Cmax}_{\text{day0}}$
0.3	0.884	0.788
1	0.983	1.33
3	1.65	1.95

Gender Differences

Although the exposure in females was higher than that in males at some time points (Table 47), there was no consistent trend for gender differences.

Table 47. Gender Differences of Ro 50-3821 in Rats

Dose ($\mu\text{g}/\text{kg}/\text{day}$)	Day 0	Day 84	Day 175
	$\text{AUC}_{\text{female}}/\text{AUC}_{\text{male}}$		
0.3	2.57	0.570	1.74
1	1.10	1.56	0.913
3	1.15	1.41	1.22
	$\text{Cmax}_{\text{female}}/\text{Cmax}_{\text{male}}$		
0.3	1.29	1.43	0.913
1	1.84	1.45	0.865
3	1.24	1.89	1.03

Antibody Analysis:

Anti-EPO Ab was detected after 13 weeks of treatment (the earliest time points) (Table 48). There was no clear dose relationship in incidence of Ab positive, in general. However, time-dependent trend was noted for males in 0.3 mcg/kg/dose group (11% after 13 weeks vs. 44% after 26 weeks), and more females than males were Ab positive after 13 weeks (11% for males vs. 55% for females). Three of six females remained Ab positive after 12 week recovery period.

Table 48. Incidence of Positive Anti-EPO Abs^a

Dose (µg/kg/dose)	13 doses		26 doses		Recovery	
	Males	Females	Males	Females	Males	Females
0 (vehicle control)	0/20 (0%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	1/8 (13%)	0/8 (0%)
0.3	2/19 ^b (11%)	11/20 (55%)	8/18 ^d (44%)	11/20 (55%)	0/6 ^d (0%)	2/8 (25%)
1.0	12/20 (60%)	11/20 (55%)	11/20 (55%)	7/19 ^f (37%)	0/8 (0%)	0/8 (0%)
3.0	6/19 ^c (32%)	13/20 (65%)	7/13 ^e (54%)	12/18 ^g (67%)	1/5 ^h (20%)	3/6 ^d (50%)

^a No. of positive results/No. of samples assayed.

^b One animal was sacrificed prior to scheduled sampling.

^c An insufficient quantity of serum was available for analysis for one animal, and the results were ambiguous for three animals.

^d Two animals died or were sacrificed prior to scheduled sampling.

^e Three animals died or were sacrificed prior to scheduled sampling, and an insufficient quantity of serum was available for analysis for four animals.

^f No serum was available for analysis for one animal, and the results were ambiguous for three animals.

^g Two animals died or were sacrificed prior to scheduled sampling, and the results were ambiguous for one animal.

^h Three animals were sacrificed prior to scheduled sampling, and the results were ambiguous for one animal.

There was, however, a good correlation between the presence of anti-EPO Ab and reductions in hematocrit at the individual animal levels. When the reductions in hematocrite levels were followed, there was an apparent dose-related trend with the number of affected animals increasing from a few in the 0.3 mcg/kg/dose group to most of the animals being affected in the 3 mcg/kg/dose group.

The presence of anti-EPO Ab in animals 65M and 153F (3 mcg/kg/dose group) was associated with reductions in hematocrit levels (65M - 4.9%; 153F - 4.0%) subsequent to Ro 50-3821/000 induced polycythemia. One animal, 40M in the 0.3 mcg/kg/dose group, had a low hematocrit (12.2%), but tested negative for anti-EPO Abs.

Anti-EPO Ab was detected in some animals at the end of the recovery period. One control male and one male in 3 mcg/kg/dose group tested positive at the end of the recovery period, but negative on Days 90 and 181. The significance of the positive results observed in these animals at the end of the recovery period is not clear, according to the study report. Two females in 0.3 mcg/kg/dose group and three females in 3 mcg/kg/dose groups remained positive at the end of the recovery period. However, their hematocrit values were similar to hematocrit values seen in the control groups.

Other: N/A

Conclusion:

According to the study report, the drug-related findings were secondary to exaggerated pharmacological effects. No evidence of drug-related neoplasia or unexpected hyperplasia was observed in this chronic study. The NOAEL in rats after 26 weeks of treatment with Ro 50-3821 was 0.3 mcg/kg/dose when administered once weekly by subcutaneous injection.

Reviewer comments:

Exaggerated pharmacology effects such as polycythemia or anti-EPO neutralizing antibodies-induced hypochromia were observed.

Heart, liver, stomach, and kidney lesions were observed in dead or unscheduled sacrificed rats.

Dose-related erosions in the stomach accompanied by hemorrhage, edema, and inflammation in the adjacent mucosa were noted.

Dose-related increase in incidence and severity of tubular lesions (basophilia, pigmentation, casts, necrosis, and degeneration) was noted. However, such lesions were not observed in 13 week SC rat study even in higher dose (10 mg/kg), suggesting treatment time-dependency.

Deficiencies:

The following deficiencies were identified.

Ab analysis:

- Only qualitative analysis was conducted.
- Assay validation was conducted using dog rather than rat serum.
- The earliest time point was on Day 90.
- High incidence of no sufficient sample (up to 4 rats in one group).
- Ab positive in two males in recovery group while previous Ab negative (1 in control and 1 in 3 mcg/kg/dose).
- Non GLP.

As discussed previously, these deficiencies had negative impact on quality and interpretation of data. For example, two rats including one in control group were Ab positive in recovery sample only, which alerted the concern regarding the quality of assay and/or potential contamination. Quantitative analysis with earlier time points would facilitate the data analysis and correlation of erythrocyte parameters and Ab production.

TK

The ELISA was not sufficiently sensitive to accurately measure the serum Ro 50-3821 concentrations at 0.3 and 1 mcg/kg/dose dose levels. The serum Ro 50-3821 concentrations were mainly either below or near the detection limit: 1.0 ng/mL, resulting in high inter-subject variability, thus TK data were not reliable.

Summary of repeat dose toxicity studies

Five repeat dose toxicity studies (See Table 5 for the study design) were conducted and reviewed above. The key findings were summarized below.

Mortality:

Drug-related deaths and moribund sacrifices due to:

- Severe polycythemia (exaggerated erythropoiesis-expected pharmacology effect, dose-related) and secondary lesions
- Severe anemia (ant-EPO Ab effect, no clear dose relationship in general)

Common abnormalities (mainly based on 15 rat data, only one moribund sacrifice in dog studies):

- Aberrations in skin and eye color (red or blue if polycythemia or pale if anemia)
- Aberrations in erythrocytes parameters (increase if polycythemia or decrease if anemia)
- Increases in total bilirubin, ALT, AST, and urea nitrogen
- Myocardial degeneration
- Necrosis in kidneys, liver, or ileum/cecum
- Erosion in the glandular stomach

Other unscheduled sacrifice:

- Two in the 0.3 mcg/kg/dose group: one with benign astrocytoma in the brain (Day 114) and another with malignant lymphoma (Day 69).

Body weights and food consumptions:

- Dose-related reduction in body weights and food consumptions.

Ophthalmoscopy:

- Drug- and time- related hyperemia and dilated retinal blood vessels in dogs at Week 13 but not at Week 4, reversible.

Hematology:

- Dose- and time-related aberrations in erythrocyte parameters, at least partially reversible

Histopathology:

- Drug-related congestion in the tissues and organs, at least partially reversible
- Increased hematopoiesis in bone marrow, dose-related trend in some studies, at least partially reversible, erythroid hyperplasia relating to polycythemia.
- Decreased hematopoiesis in bone marrow (0, 24/24, and 26/28 in 0.3, 1, and 3 mcg/kg/dose groups, respectively, in 26 wk SC rat study), no clear dose relationship, partially reversible, erythroid hypoplasia relating to no circulating reticulocytes.
- Increased hematopoiesis in spleen and liver, at least partially reversible
- Dose- and time-related erosions in the stomach, rats only (1/24 and 8/28 in 1 and 3 mcg/kg groups, respectively, at 26 week, rat SC study)
- In 26 wk SC rat study, dose-related increase in incidence and severity of renal tubular basophilia, tubular pigmentation, and tubular casts, accompanied by tubular necrosis and degeneration in 3 mcg/kg/dose group only, at least partially reversible; tubular basophilia in some dogs of 10 mcg/kg/dose group (SC) at Week 13 sacrifice.

Toxicokinetics:

- Higher systemic exposure by IV than by SC.
- Tmax values from 16 to 87 hr post dosing, indicating a relatively slow absorption process following subcutaneous injection.
- Greater than dose-proportional increases in AUC and Cmax, in general (between 1 and 10 mcg/kg/dose, no longer from 10 to 30 mcg/kg/dose).
- Accumulation during the first 4 weeks of dosing by SC but not by IV, in general.
- No consistent gender differences.
- Lower exposure levels on Day 85 than those on Day 22 and/or Day 1 in some studies, probably due to the interference resulted from anti-EPO Ab.

Antibody Analysis:

- Anti-EPO Ab detected after 4 weeks of treatment (the earliest time point tested)
- Time-dependent trend in low dose groups (e.g. 11% after 13 weeks vs. 44% after 26 weeks in 0.3 mcg/kg/dose group, rat SC study).
- No clear dose relationship except for the dog IV study (dose-related trend: 2/20, 9/20, 14/20, and 8/10 Ab positive in the 1, 3, 10, and 30 mcg/kg/dose groups, respectively, at Week 4).
- In general, positive animals at interim sacrifice remained positive at terminal sacrifice and some remained positive after recovery period.

NOAELs were established at 1 mcg/kg/dose based on 4 week rat SC study and at 0.3 mcg/kg/dose based on 26 week rat SC study. NOAELs could not be established based on other studies.

2.6.6.4 Genetic toxicology N/A**2.6.6.5 Carcinogenicity**

Study title: Ligand-receptor binding study of RO0503821 (CERA) with normal human tissues

Key study findings: Two staining patterns were revealed: 1). Membrane of hematopoietic progenitors in bone marrow: low intensity and frequency, usually high-staining affinity, physiologically or toxicologically important, probably reflective of the binding of RO0503821 to EPO-R on the cell surface; 2) Cytoplasmic granules/globules in multiple cells and tissues (including neural elements): variable intensity, frequency, and staining affinity; probably reflective of binding to EPO-R and cross-reactive with EPO-R or cytoplasmic compartments interpreted to represent possible proteasomes.

Study no.: Study No. IM946, HLR Study No. 08392, Report No. 1015621

Volume #, and page #: 1-64

Conducting laboratory and location: _____

Date of study initiation: April 7, 2004

GLP compliance: yes, except that purity and concentration analyses of the test ligand were not conducted under GLP conditions by Hoffman-La Roche, Inc. (certificate of analysis included).

QA report: yes (X) no ()

Drug, lot #, and % purity: CERA-ALEXA 488 (CERA conjugated to the fluorescent dye _____, Lot, No.: AW 08, Molar coupling ratio EPO: _____ 1:5, Concentration: 3.78 mg/mL (OD 280), Purity (SEC): _____

EPO-/- (Erythropoietin conjugated to the fluorescent dye _____, Lot. No.: AW 06, Molar coupling ratio EPO: _____ 1:5, Concentration: 4.05 mg/mL (OD 280), Purity (SEC) _____

EPO-/- Erythropoietin conjugated to the fluorescent dye _____, Lot. No.: AW 07, Molar coupling ratio EPO: _____ 1:5, Concentration: 4.6 mg/mL (OD 280), Purity (SEC): _____

Methods

The purpose of this study was to evaluate binding of RO0503821 on cryosections of normal human tissues. Three sources per each tissue (see Table 49 for tissues examined) were tested. The positive control material was _____ (_____. The negative control material was _____

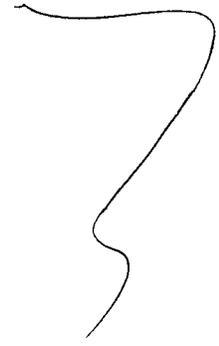
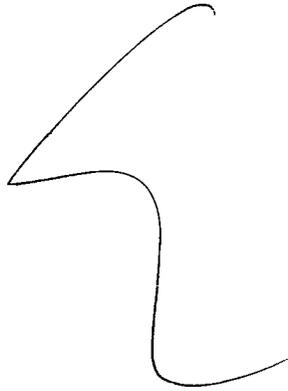
Table 49. Normal Human Tissues Used in Cross-Reactivity Testing^a

Normal Human Tissues*	Cross-Reactivity Testing
1. Adrenal	Y
2. Bladder	Y
3. Blood cells (Leukocytes)	Y
4. Bone Marrow	Y
5. Breast	Y
6. Cerebellum	Y
7. Cerebral cortex	Y
8. Colon	Y
9. Endothelium	Y
10. Eye	Y
11. Fallopian tube	Y
12. Gastrointestinal tract	Y
13. Heart	Y
14. Kidney (glomerulus, tubule)	Y
15. Liver	Y
16. Lung	Y
17. Lymph node	Y
18. Ovary	Y
19. Pancreas	Y
20. Parathyroid	Y
21. Pituitary	Y
22. Placenta	Y
23. Prostate	Y
24. Skin	Y
25. Spinal cord	Y
26. Spleen	Y
27. Striated muscle	Y
28. Testis	Y
29. Thymus	Y
30. Thyroid	Y
31. Ureter	Y
32. Uterus (cervix, endometrium**)	Y

^a Prepared by the reviewer based on data submitted.

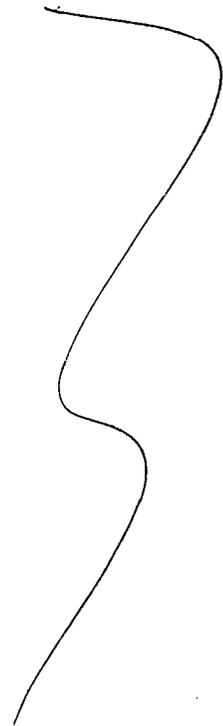
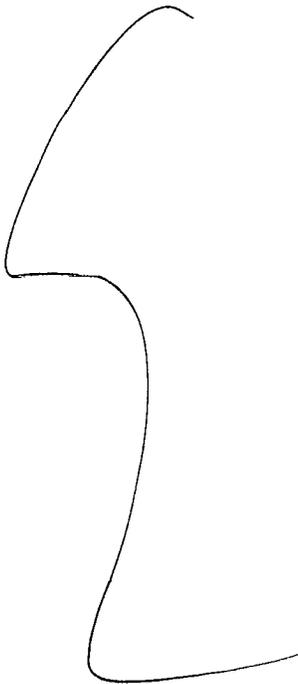
* In addition, peripheral nerve, salivary gland, and tonsil were tested.

** Uterus (endometrium): only two sources, mucosal epithelium not present in three examined cryosections. Recorded as M (Missing), no further donors with adequate mucosa available.



Results:

Study validation:



Study findings:

Two staining patterns for CERA.  were revealed and summarized below.

Membrane of hematopoietic progenitors in bone marrow:

CERA  stained the membrane of occasional hematopoietic progenitor cells in the bone marrow of two donors (Table 50) and binding was judged equivocal to weak by

the study report. Weak staining was observed with EPO- (the positive control ligand) in the bone marrow of 2/3 donors as well. The weak level of staining might indicate low receptor density display by these cells or occupation by endogenous EPO in vivo thus unavailability for staining with exogenous EPO or related ligands in vitro. The study report judged the staining as pharmacologically or toxicologically significant based on high staining affinity (usually). The staining was probably reflective of the binding of RO0503821 to EPO-R on cell surfaces.

Table 50

Table 1. Ligand-Receptor Binding Study of CERA with Normal Human Tissues

Tissue	Source	Test Article Ligand CERA		Negative Control Ligand CERA- EPO-		Positive Control Ligand EPO-	Assay Control
		20 µg/mL	2 µg/mL	20 µg/mL	2 µg/mL	20 µg/mL	
Positive Control Slide		3+	2-3+	Neg-1+	Neg-1+	3-4+	Neg
Negative Control Slide		Neg	Neg	Neg	Neg	Neg	Neg
Bone Marrow							
Hematopoietic progenitors (membrane)		±-1+ (occas.)	±-1+ (occas.)	Neg	Neg	1+ (occas.)	Neg
Other elements		Neg	Neg	Neg	Neg	Neg	Neg
Bone Marrow							
Hematopoietic progenitors (membrane)		±-1+ (occas.)	Neg	Neg	Neg	1-2+ (occas.)	Neg
Other elements		Neg	Neg	Neg	Neg	Neg	Neg
Brain - cerebrum (cortex)							
Glial cells (cytoplasmic granules/ globules [possible proteasomes])		Neg	±-1+ (occas.)	Neg	Neg	1-2+ (rare)	Neg
Other elements		Neg	Neg	Neg	Neg	Neg	Neg
Brain - cerebrum (cortex)							
Glial cells (cytoplasmic granules/ globules [possible proteasomes])		1-2+ (occas.)	Neg	Neg	Neg	1-2+ (occas.)	Neg
Other elements		Neg	Neg	Neg	Neg	Neg	Neg
Brain - cerebrum (cortex)							
Glial cells (cytoplasmic granules)		Neg	Neg	Neg	Neg	1-2+ (occas.)	Neg
Other elements		Neg	Neg	Neg	Neg	Neg	Neg

Lung	Neg	Neg	Neg	Neg	Neg	Neg
Lung						
Macrophages (alveolar, interstitial, cytoplasmic granules/ globules [possible proteasomes])	1-2+ (rare)	Neg	Neg	Neg	2-3+ (rare to occas.)	Neg
Other elements	Neg	Neg	Neg	Neg	Neg	Neg
Lung	Neg	Neg	Neg	Neg	Neg	Neg
Prostate						
Acinar epithelium (cytoplasmic granules/ globules [possible proteasomes])	Neg	Neg	Neg	Neg	±-1+ (rare)	Neg
Other elements	Neg	Neg	Neg	Neg	Neg	Neg
Prostate						
Acinar epithelium (cytoplasmic granules/ globules [possible proteasomes])	±-1+ (rare)	±-1+ (rare)	Neg	Neg	1-2+ (occas. to frequent)	Neg
Other elements	Neg	Neg	Neg	Neg	Neg	Neg
Prostate						
Acinar epithelium (cytoplasmic granules/ globules [possible proteasomes])	1-2+ (occas.)	1-2+ (occas.)	Neg	Neg	1-2+ (occas. to frequent)	Neg
Other elements	Neg	Neg	Neg	Neg	Neg	Neg
Salivary Gland						
Acinar epithelium and duct epithelium (cytoplasmic granules/ globules [possible proteasomes])	±-1+ (frequent)	±-1+ (frequent)	±-1+ (occas.)	Neg	1-2+ (frequent)	Neg
Other elements	Neg	Neg	Neg	Neg	Neg	Neg
Salivary Gland						
Acinar epithelium and duct epithelium (cytoplasmic granules/ globules [possible proteasomes])	2+ (frequent)	1-2+ (frequent)	±-1+ (occas. to frequent)	±-1+ (occas. to frequent)	2-3+ (frequent)	Neg
Other elements	Neg	Neg	Neg	Neg	Neg	Neg
Salivary Gland						
Acinar epithelium and duct epithelium (cytoplasmic granules/ globules [possible proteasomes])	1-2+ (frequent)	±-1+ (occas. to frequent)	±-1+ (occas. to frequent)	±-1+ (occas. to frequent)	2-3+ (frequent)	Neg
Other elements	Neg	Neg	Neg	Neg	Neg	Neg
Thyroid	Neg	Neg	Neg	Neg	Neg	Neg
Thyroid						
Epithelium (cytoplasmic granules/ globules [possible proteasomes])	2+ (frequent)	Neg	Neg	Neg	2-3+ (frequent)	Neg
Other elements	Neg	Neg	Neg	Neg	Neg	Neg
Thyroid	Neg	Neg	Neg	Neg	Neg	Neg

± = equivocal, 1+ = weak, 2+ = moderate, 3+ = strong, 4+ = intense, Neg = Negative, M = Missing [or inadequate for evaluation), occas = occasional.

Cytoplasmic granules/globules in various cells and tissues:

CERA stained cytoplasmic granules/globules and/or cytoplasm in glial cells in brain (cerebrum [2/3 donors] and cerebellum [1/3 donors]). Cytoplasmic granules/globules were also stained in epithelial cells in prostate (2/3 donors), salivary gland (3/3 donors), thyroid (1/3 donors), and adenohypophysis of the pituitary gland (1/3 donors). In the lung, there was staining of cytoplasmic granules/globules in alveolar and interstitial macrophages (1/3 donors). In these tissues, the CERA-stained granules or globules were of similar or slightly larger size than lipofuscin or hemosiderin granules. Location within the cell was consistent with proteasome granules according to the study report. Staining was generally judged low to intermediate affinity, due to absence or reduced staining at 2 mcg/mL CERA compared to that at 20 mcg/mL. However, high-affinity staining was observed in prostate from two donors and salivary gland from one donor. According to the study report, the staining probably reflected binding to EPO-R in cytoplasmic compartments, proteasomes. The interpretation as proteasomes was consistent with the physiological breakdown of EPO-R in cytoplasmic proteasomes (specialized lysosomal granules) reported in published literatures (Verdier et al, 1998, 2000; Yen et al., 2000). Therefore, binding evidence of CERA to inactive cytoplasmic EPO-R was not considered pharmacologically or toxicologically relevant by the study report.

Table 51**Incidence Table of Positive Cytoplasmic Granules/Globules (Possible Proteasomes) Staining**

Normal Tissue	CERA- (20 µg/mL)	EPO- (20 µg/mL)
Brain – cortex – glial cells	weak to moderate (1/3)	weak to moderate (3/3)
Brain – cerebellum		
glial cells	weak (1/3)	weak to moderate (3/3)
Purkinje cells	negative	weak to moderate (1/3)
granule cells	negative	weak to moderate (2/3)
Gastrointestinal tract – small intestine – epithelium	negative	moderate to strong (1/3)
Heart – cardiomyocytes	negative	weak to moderate (1/3)
Lung – alveolar interstitial macrophages	weak to moderate (1/3)	moderate to strong (1/3)
Pancreas – acinar and duct epithelium	negative	weak to strong (2/3)
Pituitary		
Neurhypophysis – glial cells	negative	weak to moderate (3/3)
Adenohypophysis - epithelium	weak (1/3)	weak to moderate (3/3)
Prostate – acinar epithelium	equivocal to moderate (2/3)	equivocal to moderate (3/3)
Salivary gland – acinar and duct epithelium	equivocal to moderate (3/3)	weak to strong (3/3)
Spinal cord – glial cells	negative	equivocal to weak (1/3)
Testis – seminiferous tubule cells or epididymis epithelium	negative	weak (2/3)
Thyroid – epithelium	moderate (1/3)	moderate to strong (1/3)
Ureter – epithelium	negative	weak (2/3)

The tissue binding profile of CERA was generally comparable to that of EPO (see Table 51). Positive membrane staining of CERA seen in the bone marrow of two donors was confirmed by positive staining with EPO. Bone marrow of one donor was negative for both ligands. In most tissues, a positive cytoplasmic staining with CERA- was confirmed by positive staining with EPO-; however, EPO- resulted in positive cytoplasmic staining in some additional tissues and cell types (such as cytoplasmic granules/globules in cardiac myocytes in heart acinar and/or duct epithelium in pancreas, epithelium in small intestine and epithelium in ureter) for which no positive staining was revealed with CERA.

Conclusion

The study report concluded that the experimental condition and detection method utilized for this study were sensitive and specific for EPO-R. Cell membrane binding pattern, which is considered a pharmacologically and toxicologically relevant binding pattern, was only seen in the hematological progenitor cells in the bone marrow, which were the intended target cells for CERA. Overall, the tissue binding profile of CERA was comparable to that of EPO.

Reviewer's comments:

The study was adequately conducted and specific binding of membrane of hematopoietic progenitors in bone marrow and cytoplasmic granules/globules in various cells and tissues probably reflected binding to EPO-R and cross-reactive with EPO-R or cytoplasmic compartments interpreted to represent possible proteasomes.

Study title: The Influence of RO0503821 on Proliferation in Established Erythropoietin Receptor Positive and Negative Cell Lines

Key study findings: Neither RO0503821 nor Epoetin beta stimulated the proliferation of the EPO-R positive cell lines HepG2 and K562 or the proliferation of the EPO-R negative cell line RT112. Both RO0503821 and Epoetin beta stimulated the growth of the UT-7 cell line whose proliferation is dependent on the presence of growth factors such as GM-CSF or EPO.

Study no.: CERA_PZ_Prolif_001, Report No. 1019698

Volume #, and page #: 1-15

Conducting laboratory and location: _____

Date of study initiation: N/A

GLP compliance: No

QA report: yes () no (X)

Drug, lot #, and % purity: Epoetin beta, 2.66 mg/mL (1 mg = 200,000 U), Id.: 1817965; RO0503821, 5.1 mg/mL, Id.: 10041273

Methods

Cell lines:

EPO-R positive: HepG2 (a hepatocellular carcinoma cell line) and K562 (a human chronic myeloid leukemia cell line)

EPO-R negative: RT112 (human urinary bladder transitional cell carcinoma cell line)

Positive control: UT-7 (a human acute myeloid leukemia cell line with EPO-R), growth factor dependent, the proliferation stimulated by Epoetin beta.

In vitro Assay: BrdU Assay (microtiter plates)

Results

Neither Epoetin beta nor RO0503821 stimulated proliferation of HepG2, K 562, or RT-112 cells (see Tables 52-54).

**Table 52. Proliferation of HepG2 Cells
in the Absence or Presence of Epoetin beta or RO0503821**

Cell line	MTP plate	Average (E)				Average (E)			
		plus Epoetin beta	STDEV	without Epoetin beta	STDEV	plus RO0503821	STDEV	without RO0503821	STDEV
HepG2	1	1.338	0.075	1.201	0.110	1.167	0.134	1.335	0.081
	2	1.515	0.046	1.551	0.137	1.291	0.107	1.528	0.041
	3	1.065	0.108	1.138	0.121	1.040	0.145	1.158	0.097
	4	1.345	0.111	1.183	0.060	1.170	0.083	1.339	0.086

5×10^3 HepG2 cells were seeded into wells of 96 well plates in the absence (without) or presence (plus) of 270 nM (= 1000 IU/mL) Epoetin beta or 270 nM RO0503821. Extinction E average was calculated based on OD from 12 wells.

**Table 53. Proliferation of K 562 Cells
in the Absence or Presence of Epoetin beta or RO0503821**

Cell line	MTP plate	Average (E)				Average (E)			
		plus Epoetin beta	STDEV	without Epoetin beta	STDEV	plus RO0503821	STDEV	without RO0503821	STDEV
K 562	1	1.172	0.230	1.324	0.101	1.012	0.173	1.135	0.238
	2	1.158	0.228	1.305	0.099	0.999	0.173	1.114	0.230

5×10^3 K 562 cells were seeded into wells of 96 well plates in the absence (without) or presence (plus) of 270 nM (= 1000 IU/mL) Epoetin beta or 270 nM RO0503821. Extinction E average was calculated based on OD from 12 wells.

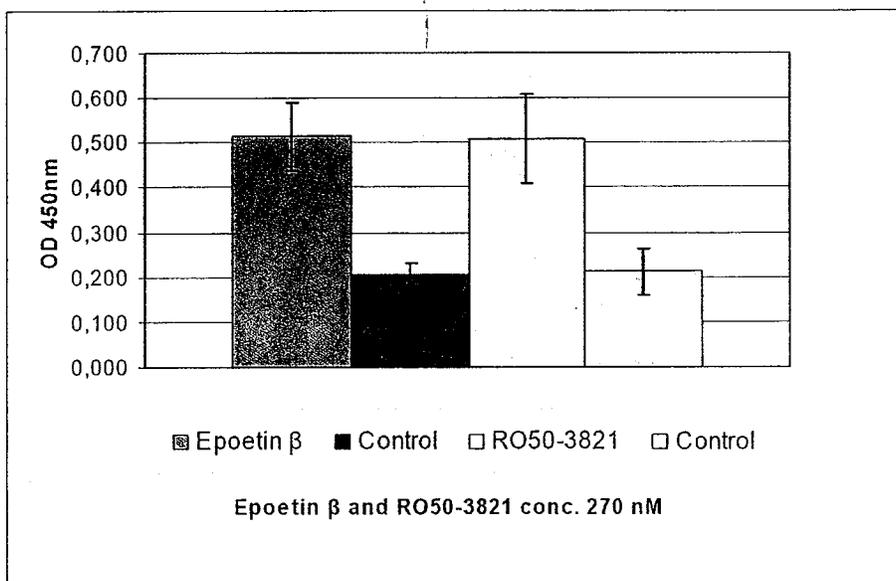
Table 54. Proliferation of RT-112 Cells in the Absence or Presence of Epoetin beta or RO0503821

Cell line	MTP plate	Average (E)				Average (E)			
		plus Epoetin beta	STDEV	without Epoetin beta	STDEV	plus RO0503821	STDEV	without RO0503821	STDEV
RT-112	1	0.831	0.125	0.859	0.093	0.943	0.081	0.921	0.089
	2	0.953	0.104	0.708	0.097	1.030	0.124	0.941	0.071

5x10³ RT-112 cells were seeded into wells of 96 well plates in the absence (without) or presence (plus) of 270 nM (= 1000 IU/mL) Epoetin beta or 270 nM RO0503821. Extinction E average was calculated based on OD from 12 wells.

Positive control UT-7 cells proliferated in the presence of either Epoetin beta or RO0503821 (Fig. 9).

Figure 9. Proliferation of UT-7 Cells in the Presence or Absence of Epoetin beta or RO0503821



2x10⁴ RT-112 cells were seeded into wells of 96 well plates in the absence (control) or presence of 270 nM (= 1000 IU/mL) Epoetin beta or 270 nM RO0503821. Extinction E average was calculated based on OD from 16 wells.

Conclusions

According to the study report, RO0503821 and Epoetin beta did not stimulate any of the chosen tumor cell lines that are not growing in a growth factor dependent manner regardless whether the EPO-R is expressed on the cell surface or not.

Reviewer's comments

The reviewer agreed with the conclusion above.

2.6.6.6 Reproductive and developmental toxicology**Fertility and early embryonic development**

Study title: Ro 50-3821/000: Fertility and General Reproduction Toxicity Study of Ro 50-3821/000 Administered Subcutaneously to Rats

Key study findings:

- Dose-related but not statistically significant increase in nonviable embryos.
- Dose-related reduction in the absolute and relative weights of the seminal vesicles and the prostate.

Based on no remarkable drug-related adverse effect in reproductive parameters in this study, the reproductive NOAEL for Ro 50-3821/000 in the male and female rats could be established at 50 mcg/kg/dose.

Study no.: Roche Study No. 08140, Study No. 208-046, Report No. 1010684

Volume #, and page #: 1- 244

Conducting laboratory and location: The prepared formulations analyzed at Hoffmann-La Roche Inc., Nutley, New Jersey.

Date of study initiation: 20 May 2003

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: Ro 50-3821/000, Bulk drug: Lot No. G003.03E, Group 2: 5 mcg/mL, Lot No. L2072253, Group 3: 20 mcg/mL, Lot No. L207263, Group 4: 50 mcg/mL, Lot No. L207273, purity:

Methods

Doses: 0 (vehicle), 5, 20, or 50 mcg/kg/dose. Males were dosed once weekly beginning 28 days before cohabitation, through cohabitation (maximum 21 days), and continuing through sacrifice. Females were dosed once weekly beginning 15 days before cohabitation, through cohabitation (maximum 21 days), and continuing through Day 7 of presumed gestation.

Species/strain: Rat —:CD (SD)IGS BR VAF/Plus

Number/sex/group: 25/sex/group

Route, formulation, volume, and infusion rate: subcutaneous injection, volume: 1 mL/kg

Satellite groups used for toxicokinetics: N/A

Study design:

Table 55. Summary of Study Design

Group	Dose ^a (mcg/kg/dose)	Concentration (mcg/ml)	Dose Volume (ml/kg)	Number of Rats per Sex	Assigned Numbers	
					Male Rats	Female Rats
I	0	0	1	25	10801 - 10825	10901 - 10925
II	5	5	1	25	10826 - 10850	10926 - 10950
III	20	20	1	25	10851 - 10875	10951 - 10975
IV	50	50	1	25	10876 - 10900	10976 - 11000

a. The test article was considered 100% active/pure for the purpose of dose calculations.

Parameters and endpoints evaluated:

- Hematological evaluation: Blood samples were collected on the first day of cohabitation from the female rats and on the second day of cohabitation from the male rats.
- Sperm evaluations: Sperm concentration (sperm per gram of tissue weight, using a homogenate from the left cauda epididymis) and motility (using a sample collected from the left vas deferens) were evaluated using computer-assisted sperm analysis (CASA).
- Estrous cycling: Evaluated by examination of vaginal cytology for 14 days before initiation of dose administration, for 14 days before cohabitation, and then until spermatozoa and/or a copulatory plug (DG 0) noted during the cohabitation period. Female rats that did not mate with a male rat within the first 14 days of cohabitation were assigned an alternate male rat that had mated (same dose group) and remained in cohabitation for a maximum of seven additional days.
- Males: after completion of the cohabitation period, all surviving male rats were sacrificed and a gross necropsy of the thoracic, abdominal, and pelvic viscera was performed.
- Mated females: Cesarean sectioned on presumed gestation day 13 and a gross necropsy of the thoracic, abdominal, and pelvic viscera was performed. The number and distribution of corpora lutea were recorded. The uterus of each rat was excised and examined for pregnancy, number and distribution of implantation sites, and viable and nonviable embryos.

Results

Mortality: No drug related deaths.

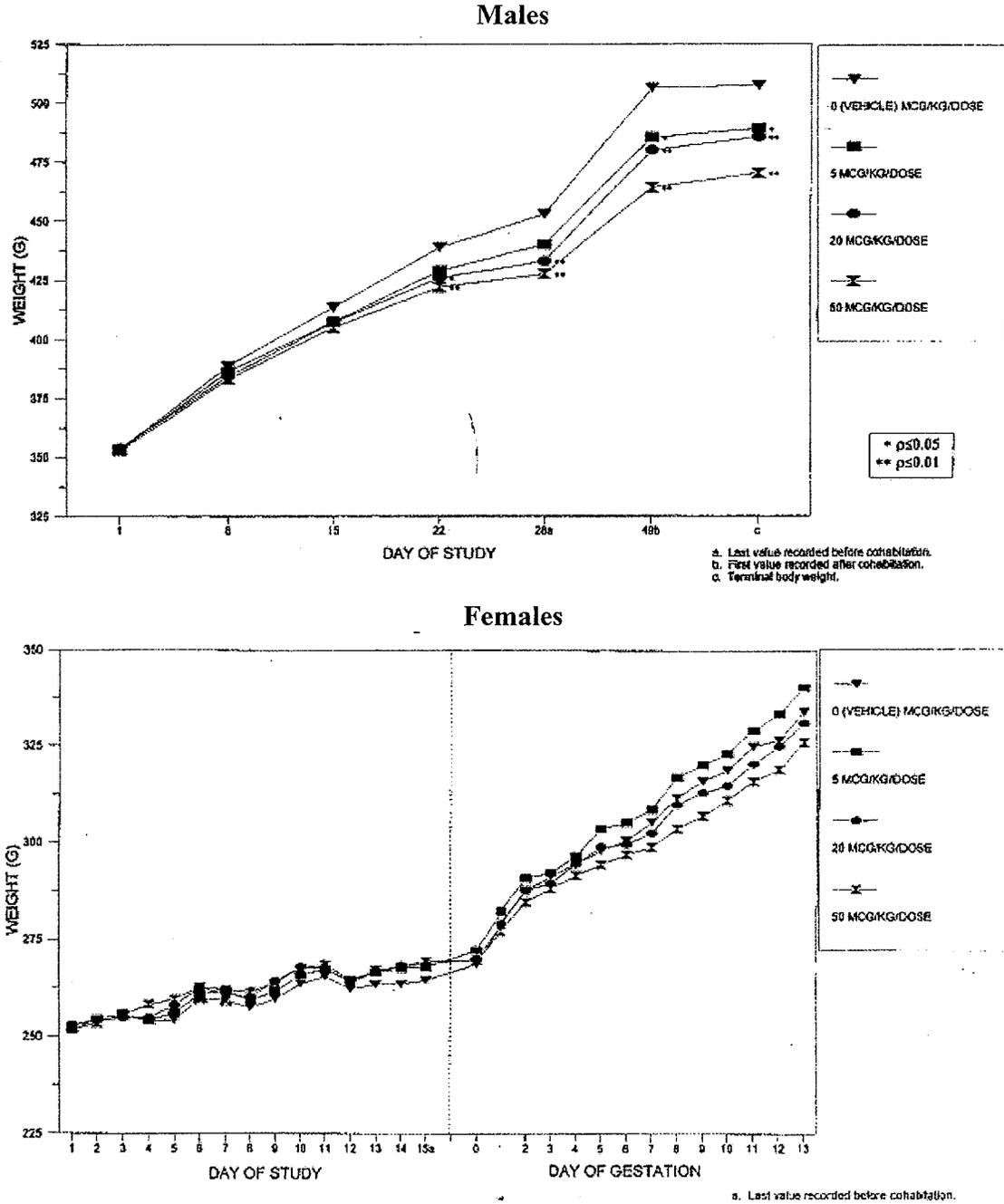
Two rats (#10858 and #10894 in 20 or 50 mcg/kg/dose group, respectively) were sacrificed due to moribund condition on Days 32 and 31, respectively. The deaths were attributed to trauma sustained from the blood collection procedures (from the retro-orbital sinus), according to the study report. In addition, Rat 10894 had a large spleen.

Clinical signs: No remarkable drug related signs.

Body weight:

Dose- and time- related reduction in body weight were observed in males (Figure 10). This reduction in body weight was observed in females in the 50 mcg/kg/dose group only and during the entire gestation period only.

Figure 10. Summary of Body Weight



Food consumption:

Reduction in absolute (g/day) and relative (g/kg/day) feed consumption values were noted in males in the 5, 20, and 50 mcg/kg/dose groups and females in the 20 and 50 mcg/kg/dose groups.

Hematology:

Dose-related increases in red blood cell count, hemoglobin, hematocrit, red cell distribution width, and reticulocyte counts were noted in the 5, 20, and 50 mcg/kg/dose dose groups.

Toxicokinetics: N/A

Estrous Cycling: The number of estrous stages per 14 days did not differ significantly among the groups and no rat had six or more consecutive days of diestrus or estrus.

Sperm Evaluation: No drug-related effects on sperm motility, count, and density.

Necropsy:Organ Weights:

Dose-related absolute and relative weights of the seminal vesicles with and without fluid and the prostate were significantly reduced ($p < 0.01$) in all drug groups. The absolute weight of the left epididymis was significantly reduced ($p < 0.01$) in the 50 mcg/kg/dose dose group.

Gross Findings:

Dose-related increase in the incidence of large spleen ($p < 0.01$ in the 50 mcg/kg/dose group).

The numbers of female rats with a red pancreas and pale areas on the spleen were increased in the 5, 20, and 50 mcg/kg/dose groups; the incidences were statistically significant at 50 mcg/kg/dose.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):*Males*

All mating and fertility parameters (number of days in cohabitation, rats that mated, fertility index, rats with confirmed mating dates during the first and second weeks of cohabitation, and rats pregnant per rat in cohabitation) were not affected by Ro 50-3821/000 at doses as high as 50 mcg/kg/dose.

Females

Pregnancy occurred in 24 (96.0%), 25 (100%), 25 (100%), and 25 (100%) rats in the 0, 5, 20, and 50 mcg/kg/dose groups, respectively. The litter averages for corpora lutea, implantations, litter sizes, and viable embryos were comparable among the four groups.

No dam had a litter consisting of only nonviable embryos, and all placentae appeared normal.

Dose-related increase in nonviable embryos was noted. The total numbers of nonviable embryos were 19 (0.8 ± 1.5 , mean \pm SD/litter), 26 (1.0 ± 1.2), 38 (1.5 ± 1.6), and 45 (1.8 ± 2.8) in the 0, 5, 20, and 50 mcg/kg/dose groups, respectively. Dose-related increase in post implantation loss as evidenced by increased % nonviable conceptuses/litter [5.2 ± 9.9 (mean \pm SD), 7.0 ± 8.4 , 9.9 ± 10.2 , and 10.7 ± 15.4 in the 0, 5, 20, and 50 mcg/kg/dose groups, respectively) was also noted.

Conclusion:

When male and female rats were administered a subcutaneous dose of Ro 50-3821/000 once weekly at 5, 20, and 50 mcg/kg/dose, there was no effect on estrous cycling, mating, fertility, and sperm parameters.

Based on finding of no remarkable drug-related adverse effect in reproductive parameters in this study, the reproductive NOAEL for Ro 50-3821/000 in the male and female rats could be established at 50 mcg/kg/dose.

Reviewer's comments:

The reviewer agreed with the conclusion above.

Embryofetal development

Teratology studies were conducted in rats and rabbits and reviewed separately below.

A.

Study title: Ro 50-3821/000: A Teratology and Toxicokinetic Study of Ro 50-3821/000 Administered Subcutaneously to Pregnant CD®(SD)IGS BR VAF/Plus® Rats

Key study findings:

- Reduction in body weights and body weight gains in both 20 and 50 mcg/kg groups (-6.25% and -10.96% in body weight gains, respectively, for DGs 6 to 18).
- Reduction in absolute and relative food consumptions in both 20 and 50 mcg/kg groups during the dosing periods.
- Dose-related reduction in fetal weights (5.32 ± 0.28 , 5.02 ± 0.28 , 4.17 ± 0.25 , and 4.57 ± 0.44 g/litter for 0, 5, 20, and 50 mcg/kg groups, respectively).

- Dose-related developmental delays for fetuses from all drug groups ($p \leq 0.05$ or $p \leq 0.01$, reduction of ossification sites for caudal vertebrae, fore and hind limb phalanges and metatarsals).
- Only three caudal vertebrae present resulting in a thread-like tail in one fetus in 50 mcg/kg/dose group.

NOAEL is less than 5 mcg/kg/dose for both maternal and fetal toxicities.

Study no.: 08085, Report No. 1010681, _____ Protocol Number: 208-043

Volume #, and page #: 1-297

Conducting laboratory and location: _____ The analyses of prepared formulations at Hoffmann-La Roche Inc., Nutley, New Jersey.

Hematological analysis at _____ Bioanalyses of Ro 50-3821 in serum samples at _____ and toxicokinetic evaluations at Hoffmann-La Roche Inc., Nutley, New Jersey. The formulation analysis, hematological analysis, bioanalytical and toxicokinetic reports were forwarded to _____ to be included in the final report.

Date of study initiation: 14 April, 2003 (GD 0)

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: Ro 50-3821/000 (Bulk Drug: Lot No. G003.03E, Group 2: 5 mcg/mL, Lot No. L207013, Group 3: 20 mcg/mL, Lot No. L207023, Group 4: 50 mcg/mL, Lot No. L207033; **purity:** _____

Methods

Doses: 0, 5, 20, or 50 mcg/kg/dose (Group I to IV) on DGs 6, 9, 12, and 15 (total 4 doses).

Species/strain: rat ~ CD®(SD)IGS BR VAF/Plus®

Number/sex/group: 25/female/group

Route, formulation, volume, and infusion rate: subcutaneous injection, dose volume: 1 mL/kg

Satellite groups used for toxicokinetics: 9/female/group/time point, blood sample collection prior to the first dose, on presumed DG 6 (at 1, 4, 12, 24, 48 and 72 hours (prior to second dose) post dosing) and DG 15 [at 0 (prior to last dose), 1, 4, 12, 24, 48, 72, 96, 120 and 144 hours post dosing].

Study design: Daily observations made for effects of the drug, abortions, premature deliveries. Blood samples were collected for TK and the final sample taken on presumed GD 21, prior to necropsy.

Parameters and endpoints evaluated: Caesarean-sectioning observations were based on 23, 24, 23, and 24 pregnant rats with one or more live fetuses in Groups I through IV, respectively. The litter averages for corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, percent resorbed conceptuses, and percent live male fetuses were evaluated. Fetal evaluations were based on 330, 354, 319, and 354 live, DG 21 Caesarean-delivered fetuses in 23, 24, 23, and 24 litters in the 0, 5, 20, and 50

mcg/kg/dose groups, respectively. Each of these fetuses was examined for gross external alterations. Of these respective fetuses, 160, 174, 155, and 172 fetuses were examined for soft tissue alterations, and 177, 180, 164, and 182 fetuses were examined for skeletal alterations and fetal ossification site averages.

Results

Mortality (dams): No mortalities or unscheduled sacrifices.

Clinical signs (dams): increased incidence of localized alopecia on the limbs in the 50 mcg/kg/dose group [4/25 dams, not in 5 and 20 mcg/kg/dose groups] compared to the control (1/25).

Body weight (dams): reduction in the 20 and 50 mcg/kg groups but not in 5 mcg/kg group. The animals in 20 and 50 mcg/kg/dose groups had reduced body weight gains (-6.25% and -10.96%, respectively) for DGs 6 to 18 (last dose on DG 15), (-7.55% and -6.54%, respectively) for DGs 0 to 21. The reduction of body weight gains in the 50 mcg/kg/dose group was statistically significant ($p \leq 0.01$) during DGs 12 to 15 when compared to that in the controls.

Food consumption (dams): Absolute and relative food consumptions were reduced for both 20 mcg/kg and 50 mcg/kg groups during the dosing periods. Absolute (g/day) feed consumption values were significantly decreased ($p \leq 0.05$) in the 20 mcg/kg/dose group over DGs 15 to 18 and significantly decreased ($p \leq 0.01$) in the 50 mcg/kg/dose group over DGs 12 to 15 and 15 to 18. Relative (g/kg/day) feed consumption values were significantly decreased ($p \leq 0.05$ or $p \leq 0.01$) in the 20 and 50 mcg/kg/dose groups over DGs 12 to 15 and 15 to 18. These dose groups had significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) relative feed consumption values for the entire dose period (calculated as DGs 6 to 18), the entire period after the initiation of treatment (DGs 6 to 21), and the entire gestation period (DGs 0 to 21). The animals in 20 and 50 mcg/kg/dose groups had reduced relative feed consumption values (-6.01% and -10.56%, respectively) for DGs 18 to 21.

Pharmacology effects:

- Expected drug-related increases in maternal hematological parameters (DG 21 sample).
- Dose-related increase in the incidence of enlarged spleens in dams at necropsy (0/25, 0/25, 1/25, and 16/25 in 0, 5, 20, and 50 mcg/kg groups, respectively), expected exaggerated pharmacological activity.

Toxicokinetics:

- Slow absorption following subcutaneous injection, reaching peak serum concentrations between 12 to 48 hours (T_{max}) post dosing
- Slow elimination resulting in a substantial level of Ro 50-3821 at 72-hours post dosing on DG 6 and 144 hours post dosing on DG 15

- Greater than dose proportional increases in AUC and C_{max} as well as drug accumulation after repeated dosing in 20 mcg/kg/dose group
- Approximate dose proportional increases in AUC and C_{max} on both study days and no drug accumulation after repeated dosing at dose of 50 mcg/kg/dose
- The systemic exposure on gestation day 6 is about 60-80% lower than that in normal female rats. However, given the facts that 1) the AUC calculated in the present study has a shorter time interval, 0-72 vs. 0-168 hour, and 2) high inter-subject variability observed in the present study, it was concluded that the systemic exposure of Ro 50-3821 between normal female rats and pregnant rats are in general comparable, according to the study report.

One possible explanation for this outcome is that the higher strength of formulation (50 mcg/mL) used for the high-dose group may have limited absorption from the subcutaneous injection site, compared to lower strength formulations (5 and 20 mcg/mL) used for low- and mid-dose group, according to the study report.

Terminal and necropsic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.): No other gross lesions were revealed in dams by necropsy except that enlarged spleen occurred in the 20 and 50 mcg/kg/dose groups. No abortions and premature deliveries occurred during the study. Litter parameters (the litter averages for corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, percent resorbed conceptuses, and percent live male fetuses) were comparable among the four dose groups. No dam had a litter consisting of only resorbed conceptuses, and there were no dead fetuses. All placentae appeared normal.

Offspring (malformations, variations, etc.):

- Dose-related reduction in fetal weights (5.32 ± 0.28 , 5.02 ± 0.28 , 4.17 ± 0.25 , and 4.57 ± 0.44 g/litter for 0, 5, 20, and 50 mcg/kg groups, respectively, $p \leq 0.01$, drug groups vs. control group). Fetus weights within the historical control value range of the Testing Facility in 5 mcg/kg/dose group and at the bottom of the range in 20 and 50 mcg/kg/dose groups.
- Dose-related developmental delays for fetuses from all drug groups ($p \leq 0.05$ or $p \leq 0.01$, reduction of ossification sites for caudal vertebrae, fore and hind limb phalanges and metatarsals).
- Three caudal vertebrae were present in one fetus (9376-5) in the 50 mcg/kg/dose group. This fetus had a thread-like tail. No additional increase in the incidences of malformations in the fetuses.

NOAEL is less than 5 mcg/kg/dose for both maternal and fetal toxicities.

Tabulated Summary of Toxicity and Toxicokinetic Findings: Teratology Study in Rats

Dose (µg/kg/dose)	Toxicokinetic Data			Toxicity/Teratology Findings
	Gestation Day of Sampling	Ro 50-3821		
		C _{max} (µg/ml)	AUC ₀₋₇₂ (µg•hr/ml)	
5	6 15	0.0204 0.0257	1.03 1.13	Fetal weights were significantly reduced. Increased the incidence of reversible developmental delays (reduced the average numbers of ossification sites per fetus). Increased red blood cell parameters (pharmacological effect of RO 50-3821/000).
20	6 15	0.0729 0.278	3.49 9.39	Body weight and body weight gains reduced. Decreased food consumption. Fetal weights were significantly reduced. Increased the incidence of reversible developmental delays (reduced the average numbers of ossification sites per fetus). Increased red blood cell parameters (pharmacological effect of RO 50-3821/000). Enlarged spleen (conforms to the anticipated pharmacological effect of Ro 50-3821/000, stimulation of extramedullary hematopoiesis).
50	6 15	0.209 0.318	11.3 13.1	Body weight and body weight gains reduced. Decreased food consumption. Fetal weights were significantly reduced. Increased the incidence of reversible developmental delays (reduced the average numbers of ossification sites per fetus). Increased red blood cell parameters (pharmacological effect of RO 50-3821/000). Enlarged spleen (conforms to the anticipated pharmacological effect of Ro 50-3821/000, stimulation of extramedullary hematopoiesis).

B.

Study title: Ro 50-3821/000: A Teratology and Toxicokinetic Study of Ro 50-3821/000 Administered Subcutaneously to Pregnant New Zealand White [Hra:(NZW)SPF] Rabbits

Key study findings:

- Two unscheduled sacrifice in 20 mcg/kg group on DG 28 or DG 29 due to miscarriage and premature delivery. Two does had severely reduced feed consumption, abnormal feces, thick, tan, and red mottled liver, and one late resorption.
- Dose-related reduction in body weights, body weight gains, and food consumptions in does of 5, 20, and 50 mcg/kg groups [body weight (kg) on DG 29: 4.28±0.30, 4.00±0.29 (p≤0.05 vs. controls), 3.88±0.39 (p≤0.01), and 3.88±0.42 (p≤0.01) in 0, 5, 20, and 50 mcg/kg groups, respectively].
- Dose-related increased % resorbed conceptuses /litter (4.6 ± 7.7, 9.7 ±21.8, 12.5 ±23.2, and 13.1 ±15.9 in 0, 5, 20, and 50 mcg/kg groups, respectively).
- Dose-related reduction in fetal weights [46.65±3.46, 41.43±6.90 (p≤0.05 vs. controls), 38.42 ±6.64 (p≤0.01), and 38.17±7.53 (p≤0.01) g/litter for 0, 5, 20, and 50 mcg/kg groups, respectively].

- Dose-related increased numbers of fetuses with alterations (20.9, 27.7, 25.3, and 38.8% in 0, 5, 20, and 50 mcg/kg groups, respectively).
- Dose-related increase in incidence of hyoid with angulated alae, flat ribs,
- Dose-related increase in incidence of incomplete or no ossification.
- Two rabbits with skeletal malformations in the 50 mcg/kg/dose group (one with absent 1st digit metacarpal and phalanx on each forelimb resulting in absent polex and another with fused 4th and 5th cervical vertebrae centra).

NOAEL is less than 5 mcg/kg/dose for both maternal and fetal toxicities.

Study no.: Study No. 208-044, HLR Study No. 08086, Report No. 1010682, Protocol Number: 208-044

Volume #, and page #: 1-429

Conducting laboratory and location:

Formulation analysis at Hoffmann-La Roche Inc., Nutley, New Jersey; Hematological evaluation at _____; Bioanalyses of Ro 50-3821 in serum samples at _____ and toxicokinetic evaluations at Hoffmann-La Roche Inc., Nutley, New Jersey. The formulation analysis, hematological analysis, bioanalytical and toxicokinetic reports were forwarded to _____ to be included in the final report.

Date of study initiation: 13-16 April, 2003 (GD 6)

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: Ro 50-3821/000 (Bulk Drug: Lot No. G003.03E, Control Article: Group I: 0 mcg/mL, Lot No. L207003; Test Article: Group II: 5 mcg/mL, Lot No. L207013, Group III: 20 mcg/mL, Lot No. L207023, Group IV: 50 mcg/mL, Lot No. L207033; purity: _____

Methods

Doses: 0, 5, 20, or 50 mcg/kg/dose (Group I to IV), once every three days from Day 6 to 18 of presumed gestation (DGs 6, 9, 12, 15, and 18).

Table 56. Summary of Study Design

Dose Group	Dose (mcg/kg/dose)	Concentration (mcg/ml)	Dose Volume (ml/kg)	Number of Rabbits	Assigned Rabbit Numbers	
					Main Study	Toxicokinetic Study
I	0	0	1	20	2201 - 2220	N/A
II	5	5	1	20 + 3 ^a	2221 - 2240	2281 - 2283
III	20	20	1	20 + 3 ^a	2241 - 2260	2284 - 2286
IV	50	50	1	20 + 3 ^a	2261 - 2280	2287 - 2289

The test article was considered 100% active for the purpose of dose calculations.

N/A - Not applicable.

a. Three rabbits per group were assigned to toxicokinetic sample.

All Ro 50-3821 active sterile solutions for injection were found to meet assay requirements of 80% to 120% of claim. The vehicle did not contain any Ro 50-3821.

Doses were selected by the Sponsor on the basis of a pilot teratology study (Study No. 07968) performed with Ro 50-3821/000 in the rabbit.

Species/strain: rabbit/New Zealand White [HRA:(NZW)SPF]

Number/sex/group: 20/female/group

Route, formulation, volume, and infusion rate: subcutaneous injection, dose volume: 1 mL/kg

Satellite groups used for toxicokinetics: 3/female/group (II-IV), blood samples collected prior to the first dose, on DG 6 [at 3, 6, 12, 24, 36, 48, and 72 hours postdosing (prior to second dose)], and on DG 18 [at 0 (prior to last dose), 3, 6, 12, 24, 36, 48, 72, 120, and 168 hours postdosing].

Study design: at least twice/day for viability and at least once/day for general appearance during the predosing period; Once before dosing, once between 30 to 60 minutes after dosing and once daily during the postdosing period for clinical signs, abortions, premature deliveries, and deaths; Body weights on DG 0, the day of arrival at the Testing Facility, daily during the dosing and postdosing periods, and on the day of sacrifice; Feed consumption values daily after arrival at the Testing Facility; whole blood samples from the main study rabbits for hematological evaluation on DG 21.

Parameters and endpoints evaluated: number and distribution of corpora lutea; number and distribution of implantation sites; live and dead fetuses and early and late resorptions; size, color, and shape of placentae; the litter averages for corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, percent resorbed conceptuses, and percent live male fetuses; gross external alterations; soft tissue alterations; skeletal alterations; and fetal ossification site averages.

Pregnancy occurred in 19, 20, 20, and 20 does in 0, 5, 20, and 50 mcg/kg dose groups, respectively. Caesarean-sectioning observations were based on 18 (excluding one unscheduled sacrifice), 20, 18 (excluding two unscheduled sacrifices), and 20 pregnant does with one or more live fetuses in 0, 5, 20, and 50 mcg/kg groups, respectively. Fetal evaluations were based on 153, 155, 146, and 160 live, DG 29 Caesarean-delivered fetuses in 18, 20, 18, and 20 litters in 0, 5, 20, and 50 mcg/kg groups, respectively.

Results

Mortality (dams): All does survived until scheduled sacrifice except for one in control (0 mcg/kg) group and two in 20 mcg/kg/dose group.

Control Group

One control doe (2202) was sacrificed on DG 14 due to moribund condition. This doe had abnormal feces (soft, liquid, or mucoid) and ungroomed coat since DG 7, red

substance in the cage pan on DGs 11 and 13, scant feces on DG 13, and no feces, apparent dehydration and emaciation on DG 14. The doe lost 662 g of body weight after DG 6 and feed consumption values were severely reduced during this same period. Necropsy revealed a trichobezoar (hairball) in the stomach but all other tissues appeared normal. The litter consisted of seven fetuses in uterus, which appeared normal for their developmental age, according to the study report.

Twenty mcg/kg dose group

In the 20 mcg/kg/dose group, two does were unscheduled sacrificed due to abortion and premature delivery and the events were briefly summarized below. The abortion and premature delivery were not considered related to the drug because the incidence was not dose-dependent, according to the study report.

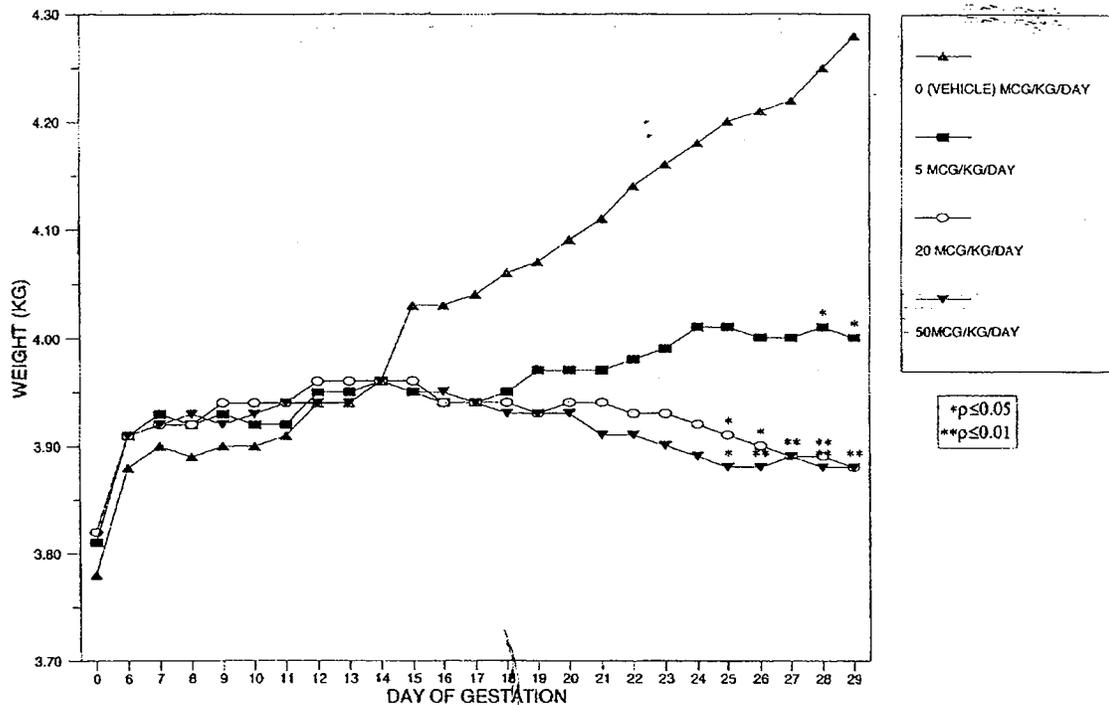
Doe 2245 was sacrificed on DG 28 after aborting. A red substance and two conceptuses were found in the cage pan. This doe had localized alopecia and no feces present in the cage pan on DG 28. Body weight gain was normal but feed consumption was severely reduced from DG 16. Gross necropsy revealed a thick, mottled tan and red liver but all other tissues appeared normal. The litter consisted of ten implantations. One live fetus and one late resorption were aborted and eight live fetuses were removed from the uterus. All live fetuses appeared normal at fetal gross, visceral, and skeletal examination.

Doe 2251 delivered a dead pup on DG 29 and was sacrificed. This doe had abnormal feces (soft or liquid, discolored, scant, dried, or no feces) and ungroomed coat since DG 15. The doe was emaciated on DG 29 and lost 981 g of body weight after DG 9 and feed consumption values were reduced or severely reduced after DG 9. Necropsy revealed a thick, tan and red mottled liver but all other tissues appeared normal. The litter consisted of one delivered dead pup, eight fetuses in uterus and one late resorption in uterus. The delivered pup and all fetuses appeared normal at fetal gross, visceral and skeletal examination.

Clinical signs (dams): The incidences of abnormal stool (soft/liquid, scant, mucoid, dried, light brown/green or no feces) were increased in all drug groups.

Body weight (dams): Dose-related reduction in body weight and body weight gains was noted (Figure 11). Body weights (kg) on DG 29 were 4.28 ± 0.30 , 4.00 ± 0.29 ($p \leq 0.05$ vs. controls), 3.88 ± 0.39 ($p \leq 0.01$), and 3.88 ± 0.42 ($p \leq 0.01$) in 0, 5, 20, and 50 mcg/kg/dose groups, respectively. Body weight change from DG6 to DG29 were $+ 0.38 \pm 0.17$, $+ 0.09 \pm 0.22$ ($p \leq 0.01$ vs. controls), $- 0.02 \pm 0.38$ ($p \leq 0.01$), and $- 0.03 \pm 0.32$ ($p \leq 0.01$) in 0, 5, 20, and 50 mcg/kg/dose groups, respectively.

Figure 11. Maternal Body Weight



Food consumption (dams): Dose-related absolute and relative feed consumption reduction was noted in 5, 20, and 50 mcg/kg groups (in general, over the same periods that the reduced body weight gains were observed).

Pharmacology effects: Anticipated dose-related increases in maternal hematological parameters (red blood cells, hemoglobin, hematocrit, mean corpuscular volume, red cell distribution width, relative reticulocyte count, and absolute reticulocyte count, DG 21 samples). In addition, mean corpuscular hemoglobin concentration values were significantly reduced ($p \leq 0.01$) in all drug groups.

Toxicokinetics:

- Slow absorption following subcutaneous injection, reaching peak serum concentrations between 18 to 52 hours (T_{max}) post dosing
- Slow elimination resulting in a substantial level of Ro 50-3821 at 72-hours post dosing on DG 6 (29.6 to 451 ng/mL) and 168-hours post dosing on DG 18 (7.52 to 132 ng/mL), indicating that the animals were continuously exposed to Ro 50-3821 from DG 6 through DG 25.
- Greater than dose proportional increases in AUC and C_{max} at doses of 20 and 50 mcg/kg/dose on both study days, in general (Table 57)
- Drug accumulation after repeated dosing in all dose groups with drug accumulation factors for AUCs ranged from 2.3 to 3.6.

Table 57. TK Parameters in Developmental Toxicity Study in Rabbits

Dosage ($\mu\text{g}/\text{kg}/\text{dose}$)	AUC _{0-72h} ($\mu\text{g}\cdot\text{h}/\text{ml}$)	Cmax ($\mu\text{g}/\text{ml}$)	Tmax (hr)	AUC/Dose ($\mu\text{g}\cdot\text{hr}/\text{ml}/\mu\text{g}/\text{kg}/\text{dose}$)
			Gestation day 6 (the 1 st dose)	
5	1.50 \pm 0.333	0.0839 \pm 0.0893	52 \pm 18	0.299 \pm 0.0655
20	9.30 \pm 4.18	0.194 \pm 0.0928	36 \pm 21	0.465 \pm 0.209
50	24.6 \pm 6.70	0.476 \pm 0.0913	48 \pm 0	0.492 \pm 0.134
			Gestation day 18 (the last dose)	
5	3.69 \pm 0.806	0.0592 \pm 0.0113	18 \pm 10	0.737 \pm 0.161
20	33.1 \pm 6.66	0.587 \pm 0.132	21 \pm 17	1.66 \pm 0.332
50	57.0 \pm 8.60	0.964 \pm 0.161	40 \pm 6.9	1.14 \pm 0.172

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

Terminal sacrificed does:

One doe (2270) in the 50 mcg/kg dose group had firm, black areas (about 1.0 cm in diameter) on the gallbladder and one doe (2229) in the 5 mcg/kg dose group had an absent gallbladder. A clear, fluid-filled mass (0.5 cm x 0.5 cm x 0.2 cm) was noted on the left kidney of one doe (2232) in 5 mcg/kg dose group. No gross lesions were considered drug-related because none were dose-dependent, according to the study report.

Litters

Dose-related increased resorptions were noted (Table 58). These increases in resorptions rates were not statistically different from the concurrent control values but above the average historically observed at the Testing Facility and therefore considered drug-related, according to the study report.

Table 58. Total, Early and Late Resorptions*

Dose (mcg/kg)		0	5	20	50
Does (pregnant + c-section on DG 29)	N	18	20	18	20
Total (resorptions/doe)	M \pm SD	0.4 \pm 0.7	0.8 \pm 1.6	1.2 \pm 2.3	1.2 \pm 1.4
Early	N	2	12	8	14
	M \pm SD	0.1 \pm 0.5	0.6 \pm 1.4	0.4 \pm 1.9	0.7 \pm 1.2
Late	N	6	3	14	10
	M \pm SD	0.3 \pm 0.6	0.2 \pm 0.4	0.8 \pm 1.6	0.5 \pm 0.9
Does w/ any resorptions	N (%)	6 (33.3)	6 (30.0)	6 (33.3)	12 (60.0)

* Prepared by the reviewer based on the submission; Historical data: mean (range), 0.4 (0-1.4)/litter for total resorption, 0.2 (0-1.1)/litter for early resorption, 0.2 (0-0.8)/litter for late resorption, 28.9% (0-80%) for does with any resorptions

No other caesarean-sectioning or litter parameters were affected by the drug at doses up to 50 mcg/kg/dose. The litter averages for corpora lutea, implantations, litter sizes, live fetuses, and percent live male fetuses were comparable among the four dose groups and

did not significantly differ. No dam had a litter consisting of only resorbed conceptuses, and there were no dead fetuses. All placentae appeared normal.

Offspring (malformations, variations, etc.):

Key changes in fetal parameters were summarized in Table 59 and listed below.

Table 59. Fetal Parameters in Developmental Toxicity Study in Rabbits

Dose (mcg/kg)		0	5	20	50
Litters evaluated	N	18	20	18	20
Fetuses evaluated	N	153	155	146	160
Live fetal body weights (g/litter)	M±SD	46.65±3.46	41.43±6.90*	38.42±6.64**	38.17±7.53**
% resorbed conceptuses/litter	M±SD	4.6±7.7	9.7±21.8	12.5±23.2	13.1±15.9
Litters w/ fetuses w/ any alterations	N (%)	15 (83.3)	17 (85.0)	13 (72.2)	17 (85.0)
Fetuses w/ any alterations	N (%)	32 (20.9)	43 (27.7)	37 (25.3)	62 (38.8)**
% fetuses w/ any alterations/litter	M±SD	20.6±15.6	28.1±23.4	27.1±25.8	38.4±29.4
Hyoid: ala, angulated					
Litter incidence	N (%)	3 (16.7)	5 (25.0)	7 (38.9)	9 (45.0)
Fetal incidence	N (%)	3 (2.0)	14 (9.0)**	12 (8.2)*	22 (13.8)**
Caudal vertebrae: misaligned					
Litter incidence	N (%)	1 (5.6)	5 (25.0)	1 (5.6)	2 (10.0)
Fetal incidence	N (%)	1 (0.6)	6 (3.9)	1 (0.7)	2 (1.2)
Ribs, flat					
Litter incidence	N (%)	0 (0)	0 (0)	1 (5.6)	2 (10.0)
Fetal incidence	N (%)	0 (0)	0 (0)	1 (0.7)	6 (3.8)**
Sternal centra, fused					
Litter incidence	N (%)	3 (16.7)	1 (5.0)	5 (27.8)	1 (5.0)
Fetal incidence	N (%)	5 (3.3)	1 (0.6)*	8 (5.5)	2 (1.2)
Sternal centra, incompletely ossified					
Litter incidence	N (%)	1 (5.6)	3 (15.0)	6 (33.3)	7 (35.0)
Fetal incidence	N (%)	1 (0.6)	7 (4.5)	6 (4.1)	18 (10.0)**
Sternal centra, not ossified					
Litter incidence	N (%)	0 (0)	0 (0)	0 (0)	1 (5.0)
Fetal incidence	N (%)	0 (0)	0 (0)	0 (0)	1 (0.6)
Sternal centra, asymmetric					
Litter incidence	N (%)	1 (5.0)	0 (0)	0 (0)	2 (10.0)
Fetal incidence	N (%)	1 (0.6)	0 (0)	0 (0)	2 (1.2)
Pelvis, pubis, not ossified					
Litter incidence	N (%)	0 (0)	1 (5.0)	0 (0)	1 (5.0)
Fetal incidence	N (%)	0 (0)	1 (0.6)	0 (0)	3 (1.9)

Prepared by the reviewer based on the submission. * $p \leq 0.05$, ** $p \leq 0.01$

Historic controls [mean (range)]:

Fetal weights (g/litter): 44.15 (39.85-51.13).

Dead or resorbed conceptuses (%/litter): 4.9 (0-13.5)

Hyoid: ala, angulated, litter, 0-5 (0-25%), fetal, 0-8 (0-4.8%).

Caudal vertebrae: misaligned, litter, 0-2 (0-11.1%), fetal, 0-2 (0-1.3%).

Ribs, flat, litter, 0-1 (0-5.0%), fetal, 0-7 (0-4.1%).

Sternal centra, fused, litter, 0-4 (0-25.0%), fetal, 0-7 (0-4.8%).

Sternal centra, incompletely or not ossified, litter, 0-3 (0-15.0%), fetal, 0-4 (0-2.2%).

Sternal centra, asymmetric, litter, 0-1 (0-5.6%), fetal, 0-1 (0-0.7%).

Pelvis, pubis, incompletely or not ossified, litter, 0-1 (0-5.0%), fetal, 0-1 (0-0.6%).

- Dose-related reduction in fetal weights
- Dose-related increased % resorbed conceptuses /litter
- Dose-related increased numbers of fetuses with alterations
- Dose-related increase in incidence of hyoid with angulated alae and flat rib
- Dose-related increase in incidence of incomplete or no ossification

Skeletal Malformations

Two fetuses in the 50 mcg/kg group had skeletal malformations. Fetus 2277-1 had absent 1st digit, metacarpal and phalanx on each forelimb, resulting in an absent pollex on each forelimb (historic control 0-1) at external examination. In addition, this fetus had an internasal bone, fused sternal centers and flat ribs at skeletal examination. Only digit absence was noted in historic controls (range/study: 0-1). Another fetus (2265-2) had fused 4th and 5th cervical vertebrae centra (not recorded in historic controls) in addition to a unilaterally ossified cervical vertebrae, misaligned caudal vertebrae, and incompletely ossified 1st sternal centra.

NOAEL is less than 5 mcg/kg/dose for both maternal and fetal toxicities.

**Table Tabulated Summary of Toxicity and Toxicokinetic Findings:
Teratology Study in Rabbits**

Dose (µg/kg/dose)	Toxicokinetic Data			Toxicity/Teratology Findings
	Gestation Day of Sampling	Ro 50-3821		
		C _{max} (µg/ml)	AUC _{0-72 hr} (µg·hr/ml)	
5	6	0.0839	1.50	Body weight and body weight gains reduced. Decreased food consumption. Incidences of abnormal stool increased. Increased red blood cell parameters (pharmacological effect of Ro 50-3821). Fetal weights were significantly reduced.
	18	0.0592	3.69	
20	6	0.194	9.30	Body weight and body weight gains reduced. Decreased food consumption. Incidences of abnormal stool increased. Increased red blood cell parameters (pharmacological effect of Ro 50-3821). Fetal weights were significantly reduced. Increased number of resorptions.
	18	0.587	33.1	
50	6	0.476	24.6	Body weight and body weight gains reduced. Decreased food consumption. Incidences of abnormal stool increased. Increased red blood cell parameters (pharmacological effect of Ro 50-3821). Fetal weights were significantly reduced. Increased number of resorptions. Significantly increased number of fetuses with alterations.
	18	0.964	57.0	

Conclusion

According to the study report, the maternal NOAEL of Ro 50-3821/000 was less than 5 mcg/kg/dose because all doses caused adverse clinical observations and reductions in body weight gain and feed consumption. The developmental NOAEL was less than 5

mcg/kg/dose because all doses caused decreases in fetal body weights. Fetal toxicities included increased resorptions, decreased fetal body weights, and increased number of fetuses with alterations. However, there was no evidence of increased incidences of malformation. Based on these data, Ro 50-3821/000 should not be identified as a selective developmental toxicant.

Reviewer's comments

Administration route should be the route provided a greater systemic exposure (higher AUC, IV rather than SC if one route used).

Although in low incidence (two fetuses), bone malformation in 50 mcg/kg group is a concern especially considering the fact that severe bone malformation (one rat) was also observed in rat study.

Prenatal and postnatal development

Study title: Ro 50-3821/000: A Developmental and Perinatal/Postnatal Reproduction Toxicity Study of Ro 50-3821/000 Administered Subcutaneously in Rats, Including a Postnatal Behavioral/Functional Evaluation

Key study findings:

F₁ physical development:

- Increased deaths in drug groups from Birth to Lactation Day 21, but no clear dose relationship; pale liver and lungs in one dead rat in the 50 mcg/kg/dose group.
- One moribund sacrifices in the 20 mcg/kg/dose group and three deaths in the 50 mcg/kg/dose groups during the first week postweaning, pale liver in the rat of the 20 mcg/kg/dose group.
- Dose-related increase in incidences of abdominal distension during the first 3 weeks of postweaning.
- Dose-related decrease in pup body weights, at least partially reversible.
- Dose-related increased incidence of pups with pale lungs and/or liver.

F₁ behavioral evaluation:

- Dose-related delay (approximately one to two days) in eye opening and the development of the air righting reflex.

F₁ reproduction:

- Dose-related delay (approximately two to four days) of preputial separation in males.
- Dose-related increased number of days in cohabitation.

NOAEL of F1 generation could not be determined (less than 5 mcg/kg/dose) based on significant reduction in growth rate, especially during lactation and early postweaning periods.

Study no.: HLR Study No. 08141, Study No. 208-047, Report No. 1016154

Volume #, and page #: 1-448

Conducting laboratory and location: _____ The prepared formulations analyzed at Hoffmann-La Roche Inc., Nutley, New Jersey.

Date of study initiation: 25 May 2003

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: Ro 50-3821/000, Bulk Drug: Lot No. G003.03E, Group 2: 5 mcg/mL, Lot No. L207253, Group 3: 20 mcg/mL, Lot No. L207263, Group 4: 50 mcg/mL, Lot No. L207273, purity: _____

Methods

Doses: 0 (Group 1), 5 (Group 2), 20 (Group 3), or 50 (Group 4) mcg/kg/dose, on presumed gestation days 6, 13, and 20 and lactation days 5, 12, and 19

Species/strain: rat _____ CD® (SD) IGS BR VAF/Plus®

Number/sex/group: 25 presumed pregnant female/group

Route, formulation, volume, and infusion rate: subcutaneous injection, dose volume: 1 mL/kg

Satellite groups used for toxicokinetics: N/A

Study design, and parameters and endpoints evaluated:

The followings were evaluated for the F1 generation offsprings.

- Viability
- Growth
- Reflex and physical development (Surface righting, pinna unfolding, hair growth, incisor eruption, eye opening, auditory startle, and air righting reflex were monitored daily beginning on Days 1, 2, 7, 9, 12, 13, and 14 postpartum, respectively; pupil constriction was evaluated only on DL 21)
- Learning and memory (Beginning at 24 ± 1 day postpartum, one male rat and one female rat from each litter were evaluated in a passive avoidance test for learning, short-term retention, and long-term retention; Beginning at approximately 70 days postpartum, one male rat and one female rat from each litter were evaluated in a water-filled M-maze for overt coordination, swimming ability, learning, and memory)
- Reproductive performance (Female rats were evaluated for the age of vaginal patency, beginning on Day 28 postpartum; Male rats were evaluated for the age of preputial separation, beginning on Day 39 postpartum; At approximately 90 days of age, the F1 generation rats within each dose group were assigned to cohabitation. All surviving female rats were sacrificed on DG 21, Caesarean-sectioned, and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Female rats were examined for number, distribution of corpora lutea, implantation sites, live and dead fetuses, and early and late resorptions. All

surviving male rats were sacrificed after completion of the 21-day cohabitation period. A gross necropsy was performed. Testes and epididymides of male rats were excised and paired organ weights were recorded). The F2 litters were weighed, examined externally, and saved.

Results

F₀ in-life:

Mortality: All females survived to scheduled sacrifice.

Clinical Signs: No drug-related adverse clinical signs.

Body Weight: Body weight gain was significantly reduced on DGs 18 to 20 in the 50 mcg/kg/dose group.

Food Consumption: Absolute (g/day) and relative (g/kg/day) food consumption values were unaffected during the gestation period, but significantly reduced during the lactation period in 20 and 50 mcg/kg/dose groups.

Natural Delivery and Litter Findings: Pregnancy occurred in 25, 25, 24, and 23 dams in the 0, 5, 20, and 50 mcg/kg/dose groups, respectively. All pregnant dams delivered litters. Natural delivery or litter observations were unaffected by Ro 50-3821/000.

F₀ necropsy:

Dose-dependent increase in incidences of rats with a red pancreas, red thymus, red areas of the fundic region of the stomach.

Dose-dependent increase in incidences of rats with enlarged spleen.

F₁ physical development:

Mortality:

Birth to Lactation Day 21: See Table 60.

Table 60. Summary of Deaths of F1 Generation Pups (Birth to Lactation Period)*

Dose (mcg/kg/dose)	0	5	20	50
Stillborn ^a	3/3 ^b	2/2	1	1
DEATH	1/1	4/4	5/2	4/4
-Pale liver & lung	0	0	0	1 (Day 20 ^c)
-No milk in stomach	1	0	0	2 (Day 2)
-Milk harden in stomach	0	0	1 (Day 18)	0
-All tissues appeared normal	0	3 (Days 1, 3, 4)	1 (Day 12)	1
-Autolysis		1 (Day 18)	3 (Days 3, 15, 17)	1 (Day 1)
Moribund sacrifice	1 ^d	0	0	0

*prepared by the reviewer based on submitted study report.

^a all tissues appeared normal for all stillborns

^b # of pups/# of litters

^c days postpartum

^d no necropsy conducted

Higher incidences of deaths were noted in drug groups than that in the control group. However, no clear dose relationship was noted. One dead rat (on DP 20) in the 50 mcg/kg/dose group had pale liver and lungs.

Postweaning:

Two male rats (# 14288 and 14293 on Days 3 and 4 postweaning, respectively) and one female rat (# 14391 on Day 7 postweaning) in the 50 mcg/kg/dose group were found dead and one female rat (# 14366 on Day 4 postweaning) in the 20 mcg /kg/dose group was moribund sacrificed during the first week postweaning. These deaths and moribund condition were attributed to failure to thrive postweaning, related to reduction of the body weight, according to the study report. Rat # 14366 had impaired righting reflex and bradypnea. Rat # 14366 was also cold to touch and had pale liver (all lobes). Rat # 14391 had dehydration. No clinical signs were recorded for rats 14288 and 14293. All tissues appeared normal for rats # 14391, 14288, and 14293 on necropsy.

One male rat (# 14294) in 50 mcg/kg/dose group was moribund sacrificed on Day 99 postweaning, the condition was attributed to an injury to the palate caused by misaligned incisors and unrelated to maternal administration of the drug, according to the study report.

Clinical Signs:

Birth to Lactation Day 21:

No drug-related adverse clinical signs.

Lactation Day 21 to Terminal Sacrifice:

Dose-related increase in incidences of abdominal distension (0/25, 0/25, 4/25, and 9/25 for males and 0/25, 0/25, 1/25, and 8/25 for females in 0, 5, 20, and 50 mcg/kg/dose groups, respectively) during the first 3 weeks of postweaning.

Body Weight:

Birth to Lactation Day 21:

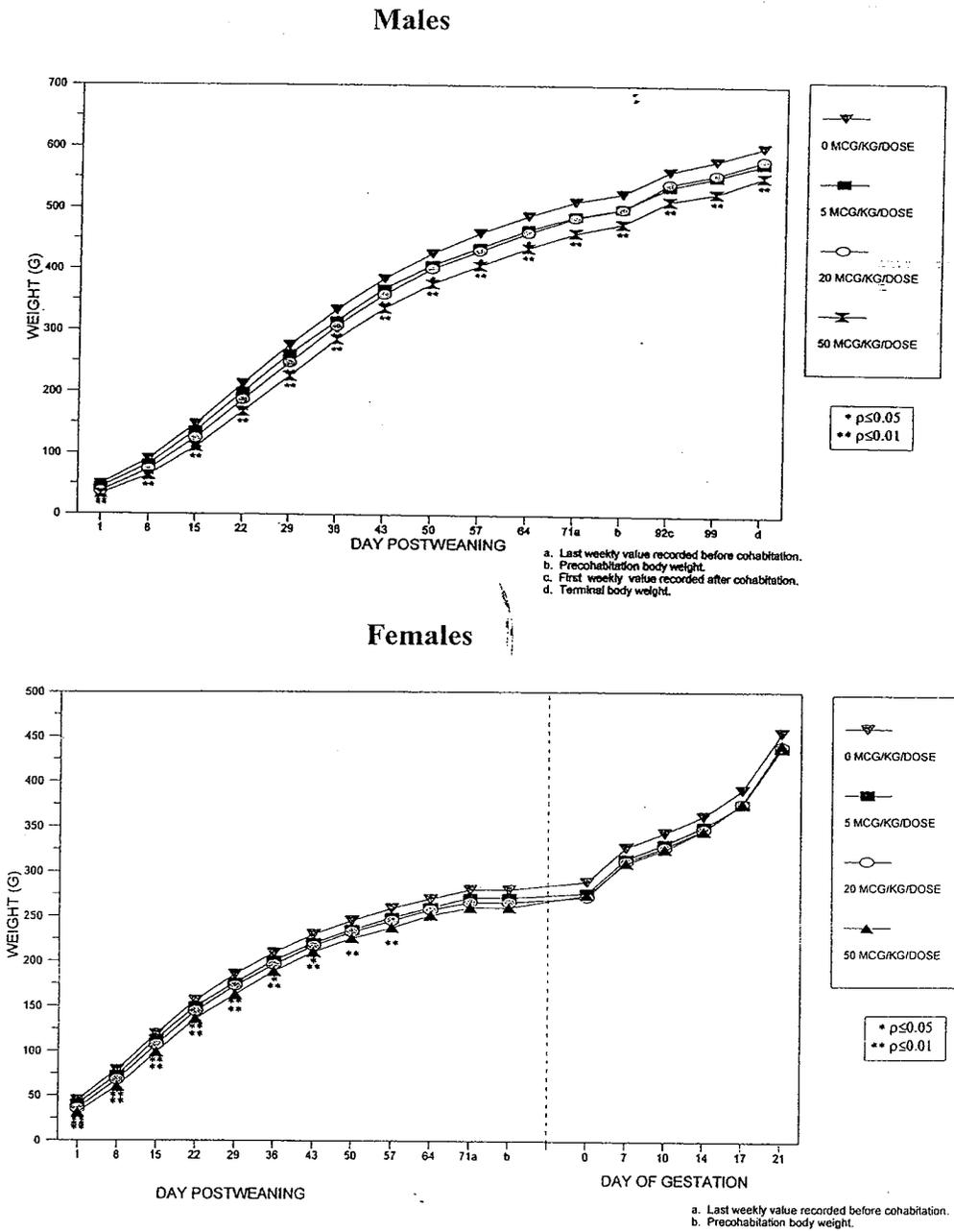
Dose- and time-related decrease in pup body weights during lactation period (Figure 12) with statistical significance in males only.

Postweaning:

Dose- and time-related decrease in pup body weights.

Gestation: Dose-related decrease in pup body weights with statistical significance in 50 mcg/kg/dose group only.

Figure 12. Body Weight of F1 Rats



Food Consumption:

Postweaning: Decrease in absolute (g/day) food consumption while increase in relative (g/kg/day) food consumption in drug groups.

Gestation: Absolute and relative food consumption values were comparable among all groups.

Necropsy Findings:

Lactation Day 21 Sacrifice: Table 61. Dose-related pale organs were noted.

Table 61. Summary of Key Histopathology Findings of F1 Generation Pups (Birth to Lactation Period)*

Dose (mcg/kg/dose)	0	5	20	50
Kidney, right, pelvis, slight dilation	0	2/1 ^a	0	1
Liver pale (all lobes)	0	0	3/1	4/2
Lung pale (all lobes)	0	0	0	8/3
Intestines, portion protruded through umbilicus	0	1	0	0
Spleen: numerous tan areas	0	0	0	1

*prepared by the reviewer based on submitted study report

^a # of pups/# of litters

Terminal Sacrifice: Reduced absolute organ weights for testes and epididymides in 20 and 50 mcg/kg/dose groups, but comparable relative weights, indicating the reductions in the organ weights reflected the reductions in terminal body weights and not a direct effect of drug on the reproductive organs.

F₁ behavioral evaluation:

Reflex and Physical Development:

Dose-related delay in eye opening (16/25, 12/25, 7/24, and 2/23 litters with 100% pups with eye opening on Day 15 postpartum, 25/25, 22/25, 20/24, and 18/23 litters with 100% pups with eye opening on Day 16 postpartum, in the 0, 5, 20, and 50 mcg/kg/dose groups, respectively)

Dose-related delay in the development of the air righting reflex (25/25, 19/25, 11/24, and 5/23 litters with 100% pups on Day 19 postpartum, 25/25, 25/25, 21/24, and 18/23 litters with 100% pups on Day 21 postpartum, in the 0, 5, 20, and 50 mcg/kg/dose groups, respectively).

According to the study report, these development delays were within one or two days and were not considered toxicologically meaningful.

Learning/Memory: No remarkable drug-related differences in the values for learning, short-term retention, long-term retention, or response inhibition in F1 generation, as evaluated by performance in a water maze or passive avoidance paradigm.

F₁ reproduction:

Sexual Maturation:

Dose-related delay (approximately two to four days) of preputial separation [a sexual maturation parameter in male F1 generation, 45.7 ± 1.7 (mean \pm SD), 47.0 ± 2.1 , 47.5 ± 2.6 ($p < 0.01$), 49.6 ± 2.5 ($p < 0.01$) days in 0, 5, 20, and 50 mcg/kg/dose groups, respectively], which was attributed to the reduced body weights, according to the study report.

Reproductive Performance:

Dose-related increased number of days in cohabitation (2.4 ± 1.7 , 3.3 ± 2.1 , 3.6 ± 3.7 , and 4.0 ± 3.8 days for males, 2.4 ± 1.7 , 3.3 ± 2.1 , 3.3 ± 3.6 , and 4.8 ± 5.2 days for females in 0, 5, 20, and 50 mcg/kg/dose groups, respectively). No other effects on mating and fertility parameters were observed.

Reproductive and Fetal Parameters:

A total of 22, 24, 22, and 23 F1 generation rats were pregnant and 22, 24, 21, and 22 Caesarean-sectioned on gestation day 21 in the 0, 5, 20, and 50 mcg/kg/dose groups, respectively. No Caesarean-sectioning or litter parameters were affected by maternal dosages of Ro 50-3821/000 as high as 50 mcg/kg/dose. No fetal gross external alterations were observed.

F₂ findings: No drug related effects on reproductive and fetal parameters including live fetal body weights.

Conclusion:

According to the study report, once weekly dose of Ro 50-3821/000 to pregnant female rats at 0, 5, 20, and 50 mcg/kg/dose during gestation and lactation period was generally well tolerated and did not adversely affect pregnancy parameters, natural delivery, or litter observations. Survival rate of offspring were reduced in 50 mcg/kg/dose maternal dose group. A significant increase in the appearance of abdominal distension was also observed in the F1 offspring of the 50 mcg/kg/dose group.

Although pup body weights were comparable across all maternal dose groups at birth, but pup body weights were lower in the drug groups than that in the control group during lactation and early postweaning period, resulting in dose-dependent reduction in mean body weight gains. Based on significant reduction in growth rate, the NOAEL of F1 generation was determined to be less than 5 mcg/kg/dose. However, no meaningful changes were observed for reflex and physical development, learning, or memory in F1 generation of any maternal dose groups. Other than an increase in number of cohabitation days prior to mating, no effects on other reproductive parameters were observed in F1 generation.

Reviewer's comment:

Of interest, dose-related increased incidences of pale organs (liver only in 20 mcg/kg/dose group, liver only or liver and lungs in 50 mcg/kg/dose group) were observed in F1 pups. In chronic toxicity studies, the pale tissues were correlated with severe anemia seen in some animals who developed anti-EPO Ab. Unfortunately, no hematology parameters and antibody data were monitored in this study.

Dose-related delay in eye opening and in the development of the air righting reflex were noted. These development delays were not considered toxicologically meaningful by the study report because of small scale (one or two days). Due to the dose-related nature, the reviewer considered the delays as toxicity findings, probably secondary to reduced body weight. However, the delays would not have permanent consequence.

2.6.6.7 Local tolerance

According to the study reports, all parenteral formulations of RO0503821 showed excellent injection site tolerability for both intravenous and subcutaneous injection, and no signs of hemolytic potential were observed.

2.6.6.8 Special toxicology studies**Formulation Bridging Studies**

Additional nonclinical studies including a 4-week toxicity and toxicokinetic study, and local tolerance study were conducted to bridge the changes in clinical formulations. The formulations used were listed below.

- Preliminary Formulation: 100 mcg/mL injectable solutions of RO0503821 (G006.02E) by preliminary process formulated in phosphate buffer, pH 7, containing sodium chloride and no preservatives
- Final Formulation: 100 mcg/mL injectable solutions of RO0503821 (G002.03E) by final process (1F) formulated in an aqueous solution containing sodium phosphate, sodium sulfate, mannitol, methionine and poloxamer 188, pH 6.2, and no preservatives

The key study findings were briefly summarized based on the submission below.

Local tolerance studies

Additional local tolerance studies were conducted via SC and IV injections in rabbits. According to the study report, all formulations tested, including the final clinical formulation that included these new excipients and adjusted to pH 6.2, showed excellent tolerability.

Four-week comparability toxicity and toxicokinetic study

A 4-week GLP comparability toxicity and toxicokinetic study was conducted in rats comparing preliminary and final formulations. The results of this study showed that the two formulations were comparable with respect to the toxicokinetic profiles (AUC_{0-168h} , C_{max} , dose proportionality, accumulation index, and gender effect), anti-RO0503821 antibody development, pharmacodynamic effects, and toxicity profiles after once weekly SC injections for 4 weeks at 0, 5, or 50 mcg/kg/dose.

2.6.6.9 Discussion and Conclusions

Erythropoietin (EPO), a hormone produced primarily in the kidneys, stimulates the production of erythrocytes in bone marrow and is essential for the maintenance of normal erythrocyte counts. Anemia is a hallmark of chronic kidney disease and is considered caused mainly by EPO deficiency. The recombinant EPO hormones, epoetin alfa marketed as Epogen, and darbepoetin alfa marketed as Aranesp, have been approved by FDA to treat anemia associated with chronic renal failure (CRF) with approved dosing schedules ranging from three injections per week to once every second week. Patients with chronic renal anemia generally require life-long therapy. A less frequent administration regimen will provide convenience for patients.

RO0503821 (pegserepoetin beta, Mircera) is a pegylated EPO receptor (EPO-R) activator and developed by Roche as a long-lasting EPO-R activator. RO0503821 is intended for the treatment of anemia associated with CRF including patients on dialysis and patients not on dialysis. Compared to EPO, RO0503821 shows a different activity profile at the receptor level characterized by a slower association to and faster dissociation from the receptor in vitro, the reduced receptor mediated elimination, and an increased in vivo erythropoiesis stimulation activity and half-life. These pharmacological properties are the foundation for an up to once monthly administration regimen, an advantage over other EPO products. Stimulation of erythropoiesis by RO0503821 was specific and dose-dependent.

Extensive toxicity studies were performed to evaluate the safety of RO0503821. Despite of some deficiencies in study designs and conducts, the potential risks of RO0503821 were considered to be adequately evaluated. Main drug-related safety issues relevant to clinical use and safety monitoring are polycythemia and anemia and further discussed below. In addition, drug-related developmental and renal toxicities are discussed.

Polycythemia-related toxicities

Polycythemia was noted in repeat dose toxicity studies in a dose-dependent manner due to exaggerated erythropoiesis, an expected pharmacological effect. Drug-related severe polycythemia resulted in deaths and unscheduled moribund sacrifices. The markedly increased erythrocyte mass and the corresponding increase in blood viscosity could predispose animals to vascular congestion, thrombosis, and hemorrhage, subsequently

necrosis, and/or inflammation in critical organs such as brain, heart, kidneys, liver, and stomach.

Correlatedly, RO0503821 and other erythropoiesis-stimulating agents (ESAs) increased the risk for death and for serious cardiovascular events in controlled clinical trials when administered to target a Hb of greater than 12 g/dL. There was an increased risk of serious arterial and venous thromboembolic events, including myocardial infarction, stroke, congestive heart failure, and hemodialysis graft occlusion.

Therefore, careful dose titrating and close Hb monitoring are critical to the safe use of RO0503821. The lowest effective dose should be used to minimize the potential risks.

Neutralizing antibody formation-induced anemia

As expected, when human protein is introduced to animals, some rats and dogs developed anti-EPO antibodies (anti-EPO Ab) following repeat administrations of RO0503821. There was no clear dose relationship regarding Ab development, in general. Nevertheless, severe anemia following repeat drug treatment occurred and resulted in unscheduled moribund sacrifices. Severe anemia was considered to result from RO0503821-induced neutralizing Ab which neutralized not only RO0503821 but also endogenous EPO. Of interest, although there was no direct evidence due to the deficiencies of study design, possible severe anemia as evidenced by pale organs occurred in F1 pups and resulted in deaths and unscheduled moribund sacrifices when F0 females were given RO0503821 during pregnancy and lactation. These data suggest that anti-EPO Ab can be transferred to fetuses during pregnancy and subsequently result in severe anemia in pups. Fortunately, in clinical studies with 1789 patient, no patients developed newly detectable Ab nor was there any evidence of pure red cell aplasia (PRCA) in patients receiving RO0503821. However, cases of PRCA and of severe anemia associated with neutralizing anti-EPO Ab have been reported in patients treated with ESAs. Therefore, close monitoring of drug efficacy and Ab production is critical to the safe use of RO0503821.

Reproductive and developmental toxicities

Although no remarkable drug-related adverse effect in reproductive parameters was observed in adult rats at doses up to 50 mcg/kg, drug-related adverse effects on embryo and fetal development including bone malformations (very low incidence) were noted in 50 mcg/kg/dose group. The delays in physical and reproductive development were also noted and likely secondary to the drug-related reduction in body weights in theory although there is no direct evidence to support the notion.

Kidney lesions

Dose- and time-related increase in incidence and severity of kidney lesions including necrosis, tubular basophilia, and glomerular thrombosis was observed. These lesions persisted after the recovery periods and were considered secondary to polycythemia related adverse effects. In a 13 week Sc dog study, dose-related, increased incidence of

segmental glomerular sclerosis and interstitial fibrosis in the kidneys were noted in recovery animals. These kidney lesions observed in the recovery groups were interpreted as scarring of the glomerular thrombi and tubular basophilia observed in the terminal sacrifice groups. Although no exacerbation of kidney disease was reported in clinical trials, precaution should be exercised especially considering the persistent nature of such lesions and existing CRF in intended patient population.

Conclusions

Extensive toxicity studies were performed to evaluate the safety of RO0503821. Despite of some deficiencies in study designs and conducts, the potential risks of RO0503821 were considered to be adequately evaluated according to the current standard. Main drug-related safety issues relevant to clinical use and safety monitoring are polycythemia and anemia. Polycythemia resulted from expected, exaggerated pharmacology (erythropoiesis) effect. Severe polycythemia resulted in deaths, unscheduled moribund sacrifices, and lesions in critical organs such as heart, kidneys, liver, and stomach. Anemia was considered resulting from RO0503821-induced neutralizing Ab, an expected reaction to a foreign protein, and resulted in unscheduled moribund sacrifices. In addition, drug related adverse effects on embryo and fetal development including bone malformations and delays in physical and reproductive developments were noted. Furthermore, the available data suggest that anti-EPO Ab can be transferred to fetuses during pregnancy, resulting in anemia in pups. Persistent kidney lesions were also noted. Although these risks may less likely occur in a well controlled clinical setting, the toxicity data illustrated the importance of careful dose titrating and close Hb monitoring in safe use of RO0503821. The lowest effective dose should be used to minimize the potential risks.

2.6.6.10 Tables and Figures N/A

2.6.7 TOXICOLOGY TABULATED SUMMARY N/A

OVERALL CONCLUSIONS AND RECOMMENDATIONS

RO0503821 (pegserepoetin beta, Mircera) is a pegalated EPO receptor (EPO-R) activator and developed by Roche as a long-lasting EPO-R activator. Compared to EPO, RO0503821 demonstrated a different activity profile at the receptor level characterized by a slower association to and faster dissociation from the receptor in vitro, the reduced receptor mediated elimination, and an increased in vivo erythropoiesis stimulation activity and half-life. These pharmacological properties are the foundation for an up to once monthly administration regimen. Stimulation of erythropoiesis by RO0503821 was specific and dose-dependent.

Extensive toxicity studies were performed to evaluate the safety of RO0503821. Despite of some deficiencies in study designs and conducts, the potential risks of RO0503821 were considered to be adequately evaluated according to the current standard. Main drug-related safety issues relevant to clinical use and safety monitoring are polycythemia

and anemia. Polycythemia resulted from exaggerated erythropoiesis, an expected pharmacological effect. Drug-related severe polycythemia resulted in deaths, unscheduled moribund sacrifices, and lesions in critical organs such as heart, kidneys, liver, and stomach. Anemia was considered to result from RO0503821-induced neutralizing Ab, an expected reaction to a foreign protein. Severe anemia resulted in unscheduled moribund sacrifices. The available data also suggest that anti-EPO Ab can be transferred to fetuses during pregnancy and subsequently result in severe anemia in pups. In addition, drug related adverse effects on embryo and fetal development including bone malformations (very low incidence) and delays in physical and reproductive development were noted.

Taken together, drug-induced polycythemia or anemia, and secondary lesions in heart, kidneys, liver, and stomach were key toxicity findings. These findings are expected effects due to either exaggerated pharmacological effect or drug-induced neutralizing Ab production. However, the risks may less likely occur in a well controlled clinical setting. Therefore, the available pharmacology and toxicology data support the approval of RO0503821 from pharmacology and toxicology perspective.

Recommendations: Approval

Suggested labeling:





Signatures (optional):

Reviewer Signature *[Signature]* 5/14/07
Supervisor Signature *[Signature]* 5/14/07 Concurrency Yes No

APPENDIX/ATTACHMENTS N/A