

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:
BLA 125164

PHARMACOLOGY REVIEW(S)



DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

BLA NUMBER: 125164

PRODUCT: Methoxypolyethylene glycol epoetin beta
(Mircera)

INTENDED CLINICAL POPULATION: Patients with anemia associated with chronic renal failure including patients on dialysis and patients not on dialysis

SPONSOR: Hoffmann-La Roche Inc.

REVIEW DIVISION: Division of Medical Imaging and Hematology Products (DMIHP)

PHARM/TOX REVIEWER: Yanli Ouyang, MD, PhD, DABT
[Signature] 11/5/07

PHARM/TOX SUPERVISOR: Adebayo Laniyonu, Ph.D.
[Signature] 11/5/07

Mircera was recommended for approval in the perspective of pharmacology/toxicology during the first circle. No additional pharmacology/toxicology studies have been recommended. Formal primary, secondary, and tertiary BLA reviews have been filed during the first circle. There is no additional pharmacology/toxicology issue to review during the second cycle.

Supervisory Pharmacologist Memo

BLA: 125164
Drug: Pegserepoetin beta (Mircera)
Sponsor: Hoffmann-La Roche Inc.

Mircera is a pegalated erythropoietin receptor (EPO-R) activator. It differs from EPO beta through integration of an amide bond between either the N-terminal amino group or the ϵ -amino group of lysine and methoxy polyethylene glycol (PEG) butanoic acid. Binding to EPO-R leads to erythropoiesis stimulation. Mircera is proposed for the treatment of anemia associated with chronic renal failure (CFR) including patients on dialysis and patients not on dialysis. The proposed starting dose for the CFR treatment is an intravenous or subcutaneous administration of 0.6 mcg/kg body weight once every two weeks.

Dr. Ouyang reviewed the preclinical Pharmacology and Toxicology section of BLA 125164. She did not identify any outstanding nonclinical issues and concluded that the studies conducted supported safety and efficacy from preclinical Pharm/Tox perspectives. She recommended approval of the BLA. This secondary review is based on Dr. Ouyang's review. Please see Dr. Ouyang's review for details.

Cardiovascular safety evaluation was considered adequate. There were no notable effects on any of the lead II ECG variables including QTc and hemodynamics and respiratory parameters. Neurological evaluation conducted as part of a 26 week rat tox study was unremarkable.

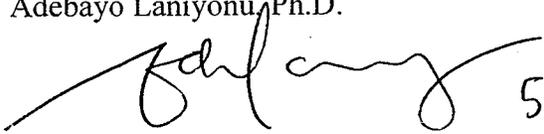
Mircera systemic clearance was slower than that of epoetin beta with the apparent elimination $t_{1/2}$ prolonged by approximately 2- and 7-11- folds in rats and dogs respectively. There was no effect of hemodialysis on PK in a clinical study. Urine was the primary elimination route following a single dose. A lacteal excretion and placental transfer study in pregnant rats demonstrated findings in lacteal excretion sample, and limited (<0.3% of administered dose) penetration through the placenta.

Definitive toxicology studies, single- (mice and rats), and repeat-dose (rats and dogs) were conducted. For the single dose studies, exaggerated pharmacological effects such as increased red blood cells and enlarged spleen were observed. Key study findings for the repeat dose toxicity studies included drug-related deaths and unscheduled moribund sacrifices due to severe polycythemia or severe anemia (anti-EPO Ab-mediated effect). Histopathological findings in these animals included myocardial degeneration, necrosis in kidneys, liver, or ileum/cecum and erosion in glandular stomach. Mircera did not affect reproductive parameters in rats. Teratology findings in rats and rabbits included dose-related reduction in fetal weights, developmental delays (incomplete and no ossification), bone malformation and increased number of-resorbed conceptuses/litter in rabbits. Increased deaths and significant reduction in growth rate of F1 generation were observed during lactation and early post weaning period. The mutagenic or carcinogenic potential of Mircera was not evaluated.

Dr. Ouyang concluded that the preclinical package for Mircera was complete, and that the studies conducted support the safety and efficacy of Micera from preclinical pharmacology/toxicology perspectives. She recommends approval of the BLA.

I concur with Dr. Ouyang's recommendations.

Adebayo Lanionu, Ph.D.



5/14/07

Supervisory Pharmacologist



**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

BLA NUMBER: 125164

SERIAL NUMBER: 000

DATE RECEIVED BY CENTER: 19 April, 2006

PRODUCT: Pegserepoetin beta (Mircera)

INTENDED CLINICAL POPULATION: Patients with anemia associated with chronic renal failure including patients on dialysis and patients not on dialysis

SPONSOR: Hoffmann-La Roche Inc.

DOCUMENTS REVIEWED: Module 2 and 4

REVIEW DIVISION: Division of Medical Imaging and Hematology Products (DMIHP)

PHARM/TOX REVIEWER: Yanli Ouyang, MD, PhD, DABT

PHARM/TOX SUPERVISOR: Adebayo Lanionu, Ph.D.

DIVISION DIRECTOR: Rafel (Dwayne) Rieves, MD

PROJECT MANAGER: Florence O. Moore, M.S.

Date of review submission: May 14, 2007

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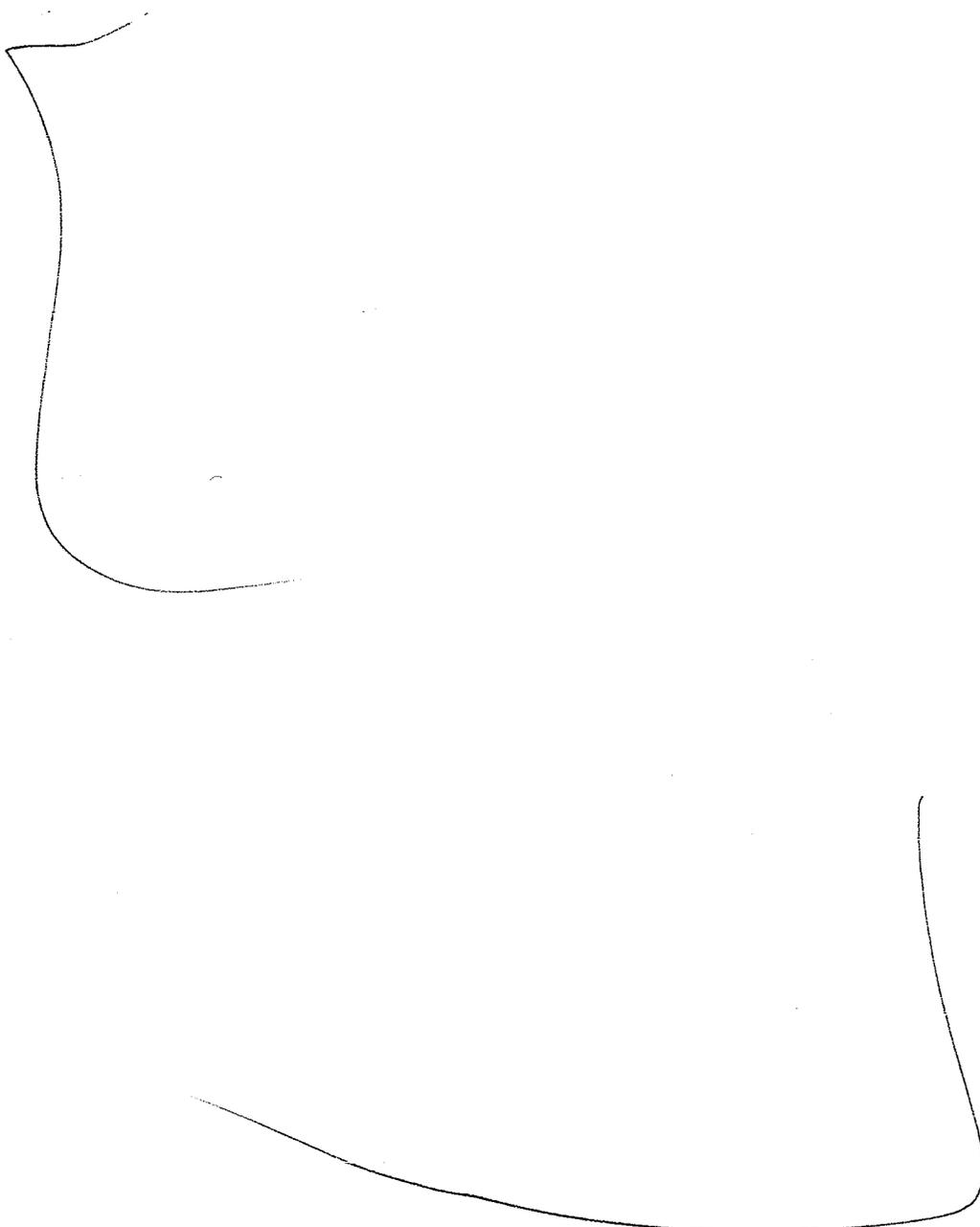
EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability: Approval.

B. Recommendation for nonclinical studies: N/A.

C. Recommendations on labeling:



II. Summary of Nonclinical Findings

A. Brief overview of nonclinical findings

Pharmacodynamics

RO0503821 is a pegalated erythropoietin receptor (EPO-R) activator. Compared to epoetin beta, RO0503821 shows a different activity profile at the receptor level characterized by a slower association to and faster dissociation from the receptor in vitro, decreased receptor-mediated elimination, and an increased in vivo erythropoiesis activity and half-life. These pharmacological properties are the foundation for an up to once monthly dosing regimen.

Safety Pharmacology

No significant drug related cardiovascular and respiratory effects were observed at doses up to 50 mcg/kg with a total cumulative dose up to 70 mcg/kg when compared to the controls in a dog study. No significant drug related neurological effects were observed at doses up to 3 mcg/kg in a rat 26 wk study.

ADME

Following subcutaneous injection, slow depot absorption with T_{max} ranged from 24h to 72h and lower systemic exposure than that of IV injection was noted. The bioavailability after SC dosing in rats was 31% and 45% in the 2.5 and 25 mcg/kg dose groups, respectively, and was 80% and 46% in the 3 and 10 mcg/kg dose groups in dogs, respectively.

¹⁴C-labeled RO0503821 was detected as radioactivity in almost all analyzed matrices in a rat study. The highest tissue radioactivity was found in the injection site (SC), lymph nodes, testis, blood, adrenal gland, and spleen. The radioactivity was also detected in cerebellum, cerebrum, and CSF at very low level (0-0.947 mcg equivalent/g). In addition, RO0503821 was found penetrating through the placenta at a level of <0.3% of administered dose and excreting into the milk.

General Toxicology

Five repeat dose toxicity studies were conducted, and study design and key findings are summarized below.

Summary of Repeat Dose Toxicity Study Design

Species	Routes	Doses (mcg/kg/dose)*	Interim Sacrifice (wk)	Terminal Sacrifice (wk)	Recovery Sacrifice (wk)
Dog	IV	1, 3, 10, and 30	4	13	8
Dog	SC	1, 3, 10, and 30	4	13	8
Rat	IV	1, 3, 10, and 30	4	13	8
Rat	SC	1, 3, 10, and 30	4	13	8
Rat	SC	0.3, 1, and 3	N/A	26	12

* QW, 30 mcg for 4 weeks only, prepared by the reviewer.

Key study findings:

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-mediated effect, no clear dose relationship)

Common abnormalities:

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

Body weights and food consumptions:

Reversible, dose-related reduction in body weights and food consumptions.

Ophthalmoscopy:

Reversible, drug-, and time- related hyperemia and dilated retinal blood vessels in dogs at Week 13 only (not at Week 4).

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;

- Decreased hematopoiesis in bone marrow, no clear dose relationship, partially reversible;
- Increased hematopoiesis in spleen and liver, at least partially reversible;
- Dose- and time-related erosions in the stomach in rats only (not in dogs);
- 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 SC;
- Tmax values from 16 to 87 hr post dosing following SC injection, indicating a relatively slow absorption process;
- Greater than dose-proportional increases in AUC and Cmax, in general between 1 and 10 mcg/kg/dose, but not 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 of 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 (0.3 or 1 mcg/kg/dose group);
- No clear dose relationship except for the dog IV study;
- 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 the 4 week rat SC study and 0.3 mcg/kg/dose based on the 26 week rat SC study. NOAELs could not be established based on other studies.

Carcinogenicity

RO0503821 did not induce a proliferative response in either the EPO-R positive cell lines HepG2 and K562 or the EPO-R negative cell line RT112. In addition, using a panel of human tissues, the in vitro binding of RO0503821 was observed only in target cells. The carcinogenic potential of Mircera was not evaluated in long-term animal studies.

Fertility and Early Embryonic Development

There was no remarkable drug-related-adverse-effect in reproductive parameters in a rat study, the reproductive NOAEL for RO0503821 in the male and female rats could be established at 50 mcg/kg/dose.

Teratology potential was evaluated in rats and rabbits by subcutaneous injection. The key study findings were listed below.

- Two unscheduled rabbit sacrifices in 20 mcg/kg group due to miscarriage and premature delivery, accompanied by late resorption in one rabbit;
- Dose-related reduction in body weights, body weight gains, and food consumptions in dams of both rats and rabbits;
- Dose-related reduction in fetal weights in both rats and rabbits;
- Dose-related developmental delays (incomplete and no ossification) for fetuses in both rats and rabbits;
- Dose-related increased percentage of resorbed conceptuses /litter in rabbits;
- Dose-related increased numbers of fetuses with alterations in the rabbits;
- Dose-related increase in incidence of hyoid with angulated alae, flat ribs in the rabbits;
- Three caudal vertebrae present in one fetus in the 50 mcg/kg/dose group resulting in a thread-like tail in the rat study;
- Two rabbits with skeletal malformations in the 50 mcg/kg/dose dose group (one with absent 1st digit metacarpal and phalanx on each forelimb resulting in absent plex and another with fused 4th and 5th cervical vertebrae centra).

NOAEL could not be established (less than 5 mcg/kg/dose for both maternal and fetal toxicities).

Prenatal and Postnatal Development

Developmental and perinatal/postnatal reproduction toxicity study was conducted in rats using subcutaneous administration. The key study findings were summarized below.

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 sacrifice 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 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.

B. Pharmacologic activity

Accelerated erythropoiesis

C. Nonclinical safety issues relevant to clinical use

Polycythemia-related toxicities

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 heart, kidney, liver, and stomach. Therefore, careful dose titrating and close Hb monitoring are critical to the safe use of RO0503821.

Neutralizing antibody formation-induced anemia

Severe anemia following repeat drug treatment occurred and resulted in unscheduled moribund sacrifices. Severe anemia was considered to result from RO0503821-induced neutralizing antibodies which neutralized not only RO0503821 but also endogenous EPO. Of interest, 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 although there was no direct evidence due to the deficiency of study design. Therefore, close monitoring of drug efficacy and antibody production is critical to the safe use of RO0503821.

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2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

BLA number: 125164

Review number: 001

Sequence number/date/type of submission: 000/19 April, 2006/standard

Information to sponsor: Yes () No (X)

Sponsor and/or agent: Hoffmann-La Roche Inc.

Manufacturer for drug substance: Hoffmann-La Roche Inc.

Reviewer name: Yanli Ouyang, MD, PhD, DABT

Division name: DMIHP

Review completion date: May 14, 2007

Drug:

Trade name: Mircera

Generic name: N/A

Code name: RO0503821

Chemical name: pegserepoetin beta

CAS registry number: N/A

Molecular formula/molecular weight: approximately 60,000 Daltons

Structure: See the figure below for the amino acid sequence and primary structure



Relevant INDs/NDAs/DMFs: BB-IND 10,158

Drug class: Long-lasting Erythropoietin Receptor Activators

Intended clinical population: Patients with anemia associated with chronic renal failure (CRF) including patients on dialysis and patients not on dialysis.

Clinical formulation: Injectable solutions of Mircera in vials and prefilled syringes are formulated in an aqueous solution containing sodium phosphate, sodium sulphate, mannitol, methionine, and poloxamer 188. The solution is clear, colorless to slightly yellowish and the pH is 6.2 ± 0.2 . Single use vials are available containing 50, 100, 200, 300, 400, 600, or 1000 mcg in 1mL solution of Mircera. Single use prefilled syringes are available containing 50, 75, 100, 150, 200, or 250 mcg in 0.3 mL solution of Mircera and 400, 600, or 800 mcg in 0.6 mL solution of Mircera.

Route of administration: intravenous (IV) or subcutaneous (SC)

Proposed Dosage:

Starting Dose

The recommended starting dose of Mircera for the treatment of anemia in CRF patients is 0.6 mcg/kg body weight administered as a single IV or SC injection once every two weeks.

Converting Dose

When converting from epoetin alfa or darbepoetin alfa, Mircera can be administered once monthly or, if desired, once every two weeks. The dose of Mircera, given as a single IV or SC injection, should be based on the total weekly epoetin or darbepoetin alfa dose at the time of conversion.

Conversion from Epoetin Alfa

Previous Weekly Epoetin Alfa Dose (Units/week)	Mircera Dose	
	Once Monthly (mcg/month)	Once Every Two Weeks (mcg/every two weeks)
<8000	120	60
8000-16000	200	100
>16000	360	180

Conversion from Darbepoetin Alfa

Previous Weekly Darbepoetin Alfa Dose (mcg/week)	Mircera Dose	
	Once Monthly (mcg/month)	Once Every Two Weeks (mcg/every two weeks)
<40	120	60
40-80	200	100
>80	360	180

Disclaimer: Tabular and graphical information are constructed by the sponsor unless cited otherwise.

Studies reviewed within this submission:

Study # Report #	Study Type	Species	Dose, mcg/kg
Study No. DDHA1001, HLR Study No. 07311, Report No. 1002864	General Hemodynamics and Respiration Rate	Anesthetized Beagle Dogs	0, 5, 15, and 50
Study No. 6131-304, HLR Study No. 07301, Report No. 1002691	13-week sc injection toxicity study with a 4-week interim sacrifice and a 8-week recovery phase	Beagle Dogs	0, 1, 3, 10, or 30, qw , 4w; 0, 1, 3, or 10, qw, 13w
Study No. 6131-303, HLR Study No. 07310, Report No. 1002693	13-week IV injection toxicity study with a 4-week interim sacrifice and an 8-week recovery period	Beagle Dogs	0, 1, 3, 10, or 30, qw , 4w; 0, 1, 3, or 10, qw 13w
HLR Study No. 07302, Report No. 1002576	13-week sc injection toxicity and toxicokinetic study with a 4-week interim sacrifice and an 8-week recovery period	Rats	0, 1, 3, 10, or 30, qw , 4w; 0, 1, 3, or 10, qw 13w
studies no. 6131-305, 6131-319. HLR study no. 07304, 07418. Report No. 1002692.	13-week IV injection toxicity study with a 4-week interim sacrifice and a 8-week recovery phase	Rats	0, 1, 3, 10, or 30, qw , 4w; 0, 1, 3, or 10, qw 13w
HLR Study No. 07437, Report No. 1003071	26 week sc injection toxicity and toxicokinetic study, followed by a 12-week recovery period.	Rats	0, 0.3, 1, or 3, qw , 26w
Study No. IM946, HLR Study No. 08392, Report No. 1015621	Ligand-Receptor binding study of	Normal human tissues	
_001 Report No. 1019698	Proliferation study \	HepG2, K562, RT112	
Roche Study No. 08140, Study No. 208-046 Report No. 1010684	Fertility and general reproduction toxicity study, SC	Rats	0, 5, 20, or 50, qw
08085, Report No. 1010681	Teratology and TK study, SC	Rats	0, 5, 20, or 50 on DGs 6, 9, 12, & 15
Study No. 208-044, HLR Study No. 08086, Report No. 1010682	Teratology and TK study, SC	New Zealand White rabbits	0, 5, 20, or 50 on DGs 6, 9, 12, 15, & 18
Study No. 208-047, HLR Study No. 08141, Report No.1016154	A developmental and perinatal/postnatal reproduction toxicity study, SC	Rats	0, 5, 20, or 50 on DGs 6, 13, & 20 & DLs 5, 12, & 19

* The table is constructed by the reviewer.

Studies not reviewed within this submission*:

Report #	Study Type	Species
1005700	PD	Mouse
1005701	PD: multiple dosing	Mouse
1005817	PD	5/6 nephrectomized rat
1002321	4-week IV PD and pilot tolerability study	Rat
1005816	Reticulocytes, i.v. or s.c.	Beagle dog
1002322	4-week SC PD and pilot tolerability study	Rat
1019702	Stimulation of UT-7 Cell Line	UT-7 cells
1019704	Stimulation of CD34+ bone marrow and cord blood cells	CD34+ cells
1019701	Consumption	UT-7 cells
1019700	Binding constants	UT-7 cells
1019699	Interaction analysis of RO0503821 or epoetin beta and the extracellular part of the erythropoietin receptor determined by surface plasmon resonance.	
1010329	Evaluation of the therapeutic equivalence of "optimized" versus "preliminary" Ro 50-3821 API and formulation for effects on reticulocytes	Mouse
1014495	Validation report for analysis of Ro 50-3821 in rabbit serum from study no. 24092 4	
1016434	Validation report for analysis of Ro 50-3821 in rat serum from study no.24092 2	
N-181656	Validation of a procedure to measure the concentrations of Ro 50-3821 in dog serum.	
1017234	Anti-RO0503821 Ab: Partial validation of an immunoassay method for the measurement of anti-RO0503821 antibodies in rat serum	
1003129	PK studies in rat and dog after single iv or sc injection	Rat and dog
1012588	In vivo stability and tissue localization of RO0503821 after single and multiple dose	Rat
1014641	Lacteal excretion and placental transfer of radioactivity, a single SC dose of 14C-RO0503821 and tissue distribution and excretion of radioactivity following administration of a single IV dose of 14C-RO0503821	Rat
1015803	Tissue distribution and excretion of 14C-RO0503821 following a single or multiple sc administration t	Rat
1012025	Evaluation of the PK equivalence of "optimized" versus "preliminary" Ro 50-3821 API and formulation	Rat
N-181639	A single-dose acute iv toxicity study	Mouse
N-181638	A single-dose acute IV toxicity study	Rat
1002694	An eight-week exploratory toxicity study of IV and SC administration followed by an eight-week recovery period	Dog
1009922	A four-week formulation comparability study, sc	Rat
1009783	A pilot teratology study, sc	Rat
1010100	A pilot teratology and serum concentration study, sc	Rabbit
1004870	A single-dose iv and sc injection site local tolerability study	Rabbit
1004874	A single dose sc injection site local tolerability study	Rabbit
1005338	A single dose iv and sc injection site tolerability study	Rabbit

* The table is constructed by the reviewer.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Pharmacodynamics

RO0503821 is a pegalated erythropoietin receptor (EPO-R) activator. Compared to epoetin beta, RO0503821 shows a different activity profile at the receptor level characterized by a slower association to and faster dissociation from the receptor in vitro, decreased receptor-mediated elimination, and an increased in vivo erythropoiesis activity and half-life. These pharmacological properties are the foundation for an up to once monthly dosing regimen.

Safety Pharmacology

Cardiovascular safety pharmacology study

Effects of RO0503821 on the ECG, general hemodynamics, and respiration rate were evaluated in anesthetized beagle dogs. The systemic exposure of RO0503821 as high as 1810 ng/mL was achieved by intravenous administration of increasing cumulative doses of 5, 15, and 50 mcg/kg of RO0503821 in 30-minute intervals with a total cumulative dose of 70 mcg/kg. There were no notable effects on any of the lead II ECG variables including QTcF (corrected according to Fredericia's formula) and QTcV (corrected according to Van de Water's formula), and hemodynamics and respiratory variables measured..

Neurological effects were evaluated in a 26 wk SC rat study and no remarkable drug-related effects were noted.

2.6.2.2 Primary pharmacodynamics

The brief summaries of key pharmacodynamic data were provided below based on the study reports. These study reports were not reviewed by the reviewer.

RO0503821 is a long-lasting EPO-R activator. RO0503821 differs from EPO through integration of an amide bond between either the N-terminal amino group or the ϵ -amino group of lysine, predominantly Lys52 and Lys45 and methoxy polyethylene glycol (PEG) butanoic acid. This results in a molecular weight of approximately 60,000 Daltons for RO0503821 and the PEG-moiety having an approximate molecular weight of 30,000 Daltons.

Binding affinity

The binding affinity of RO0503821 to the EPO-R was lower than that of epoetin beta. The binding affinity was approximately 100- and 50-fold lower in the competitive binding experiments with UT-7 cells and in the biochemical receptor interaction study

using the extracellular part of the EPO-R, respectively. This lower binding affinity was mainly driven by a greatly reduced association rate and a slightly faster dissociation rate of RO0503821 on the EPO-R.

In vitro cell proliferation

Approximately 10- and 40-fold higher concentration of RO0503821 than that of epoetin beta was found to be required to achieve an equal level of proliferation of UT-7 cells (a human acute myeloid leukemia cell line that expresses the EPO-R and is dependent on growth factors, e.g. EPO, for cell proliferation) and erythroid cell formation of CD34+ primary cells (from bone marrow or cord blood), respectively. Interestingly, epoetin beta disappeared from the medium of cell cultures of UT-7 cells in a time- and EPO-R-dependent manner while no statistically significant disappearance of RO0503821 could be measured. According to the sponsor, this reduced receptor mediated elimination, resulting in a longer half-life, likely contributed to high *in vivo* potency of RO0503821 (see *in vivo* section below) and long-lasting activation of the EPO-R.

In vivo cell proliferation

In contrast to *in vitro* pharmacodynamic studies mentioned above, RO0503821 was found to be a more potent stimulator of erythropoiesis than epoetin beta in *in vivo* pharmacodynamic studies using normal mice, dogs, rats, and 5/6 nephrectomized rats. Both the magnitude and the duration of the erythropoietic response elicited by RO0503821 were substantially increased, suggesting that it is feasible that RO0503821 could be used at a longer dosing interval. The notion was supported by the fact that RO0503821 stimulated erythropoiesis in a dose-dependent manner at either once every week, once every two week, or once every three week dosing regimens in mice.

The effect of RO0503821 was specific to erythrocytes. White blood cells and platelets were not significantly affected by administration of RO0503821 in both rats and mice.

The magnitude of RO0503821-stimulated erythropoiesis was affected by the route of administration in rats and dogs but not in mice. At the same dose levels, a greater degree of erythropoietic augmentation was achieved by IV administration than that by SC administration in dogs and rats while similar augmentation by both routes in mice.

Mechanism of action:

RO0503821 bound to EPO-R and subsequently stimulated erythropoiesis.

Drug activity related to proposed indication:

RO0503821-stimulated erythropoiesis was of a higher magnitude and longer duration than that of epoetin beta *in vivo*, suggesting that RO0503821 could be used at a longer dosing interval. The RO0503821-stimulated erythropoiesis was also confirmed in 5/6 nephrectomized rats.

2.6.2.3 Secondary pharmacodynamics (module 4. volume 1.1) N/A**2.6.2.4 Safety pharmacology**

Neurological effects: Not stand alone safety pharmacology study on neurological effects was reported. However, neurological examination was conducted once prior to initiation of treatment, and after 25 week treatment in a twenty-six week subcutaneous injection toxicity study in rats (Study no.: 07437). There were no remarkable drug-related neurological findings, according to the study report.

Cardiovascular effects:

Pulmonary effects:

Study title: Ro 50-3821/000: Effects of Ro 50-3821/000 on General Hemodynamics and Respiration Rate in Anesthetized Beagle Dogs

Key study findings: No significant Ro 50-3821/000-related cardiovascular and respiratory effects were observed at doses up to 50 mcg/kg with a total cumulative dose up to 70 mcg/kg when compared to the controls.

Study no.: Study No. DDHA1001, HLR Study No. 07311, Report No. 1002864

Volume #, and page #: Module 4, page 1-179

Conducting laboratory and location:

The serum drug concentration determination was conducted by the sponsor, the department of Non-Clinical Drug Safety at Hoffmann-La Roche Inc., Nutley, New Jersey

Date of study initiation: 14 February 2000

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: Ro 50-3821/000, Batch #, Bulk Drug: Lot Nos. 002109, 003119, and 004129; 10 mcg /mL, Lot No.L197869; 30 mcg g/mL, Lot No.L197919; 100 mcg/mL, Lot No.L197879.

Methods

Doses: 0 (10 mM phosphate buffer with 7.73 mg/mL NaCl, pH 7.0), 5, 15, and 50 mcg/kg (ascending doses in the same dogs of the drug group with 30 min interval)

Species/strain: Dog/Beagle

Number/sex/group or time point: 4/M/group

Route, formulation, volume, and infusion rate: intravenous bolus injection over approximately 30 seconds, dose volume of 0.5 mL/kg

Age: between 6 months and 12 months

Weight: 9.9-14.3 kg

The study was conducted in anesthetized, spontaneously respiring male dogs (the animals were first sedated with acetylpromazine 0.14-0.18 mg/kg i.m. then anesthetized by sodium pentobarbitone, induction dose: 16.3-21.2 mg/kg i.v., maintain dose: 2.25-7.89

mg/kg/h). Serum samples were collected at pre-dose (t-15), 1, and 30 minutes after the start of each administration for the analysis of serum concentrations of Ro 50-3821. Hemodynamics data and ECG were captured continuously, starting at least 15 minutes prior (t-15) to administration of the first dose. Data samples for each variable were taken at t-15 and t0 for baseline values and 1, 2, 5, 10, 15, 20, and 30 minutes after the start of each administration. The following parameters were recorded: standard ECG configurations of I, III, aVR, aVL, and aVF; lead II ECG variables (RR, PR, and QT intervals and QRS duration), heart rates, arterial blood pressures, mean femoral arterial blood flow, left ventricular pressure variables, cardiac output, respiration rate, arterial blood gases, base excess/deficit, standard bicarbonate, % O₂ saturation, and pH. Total peripheral resistance, femoral arterial conductance, stroke volume, QTcF (corrected according to Fredericia's formula), and QTcV (corrected according to Van de Water's formula) were also calculated.

Percent change from baseline (mean of t-15 and t0) was calculated for each parameter at each time point for both vehicle and Ro 50-3821/000 groups. For the evaluation of drug related effects, a comparison of percent change data was made between the vehicle and Ro 50-3821/000 groups at each individual time point using unpaired Student's t-test.

Results

TK: Serum concentrations were shown in Table 1. A time- and dose-dependent increase in mean serum concentration (from 104 to 1810 ng/mL) was noted by three cumulative single doses of 5, 15, and 50 mcg/kg administered to each dogs in a 30 minute-interval.

Table 1. Summary of Study Design, Serum Concentrations, and Key Findings

Dose group	Dose Administration	Time points (Minutes after each dosing)	Mean Serum Concentration (pg/mL)	Key Findings
Group A: Vehicle	First dose, 0.5 mL/kg	1 minute 30 minute	BLQ BLQ	No treatment related findings.
	Second dose, 0.5 mL/kg	1 minute 30 minute	BLQ BLQ	No treatment related findings.
	Third dose, 0.5 mL/kg	1 minute 30 minute	191 BLQ	No treatment related findings.
Group B: Ro 50-3821/000	First dose, 5 µg/kg	1 minute 30 minute	104000 363000	No treatment related findings.
	Second dose, 15 µg/kg	1 minute 30 minute	538000 1050000	No treatment related findings.
	Third dose, 50 µg/kg	1 minute 30 minute	1480000 1810000	No treatment related findings.

BLQ: Below the Limit of Quantitation, < 150 pg/mL

ECG morphology: No drug related abnormality in gross morphology or rhythm in ECG wave form analysis.

ECG variables: No changes in PR interval, RR interval, QRS duration, QT interval, QTcF or QTcV.

Hemodynamics: No changes in heart rate, blood pressures, mean femoral flow and femoral conductance, left ventricular pressure variables (diastolic, systolic, dP/dt_{max+},

dP/dt_{max}- and dP/dtP-1), cardiac output, total peripheral resistance, and femoral arterial conductance.

Respiratory variables: No changes in respiration rate, arterial blood gases (pO₂ or pCO₂), base excess/deficit, standard bicarbonate, % O₂ saturation, and pH.

Conclusion

The study report concluded that intravenous administration of increasing cumulative doses of 5, 15, and 50 mcg/kg of Ro 50-3821/000 in 30-minute intervals (total cumulative dose of 70 mcg/kg) in anesthetized beagle dogs achieved mean serum concentration of Ro 50-3821 as high as 1810 ng/mL, and had no notable effects on any of the cardiovascular or respiratory variables measured. ECG waveform analysis showed no abnormalities in gross morphology or rhythm that could be attributed to the administration of either test article or vehicle.

Reviewer's comments:

The study was not optimally designed because of the following study deficiencies. But the study was considered acceptable.

- Male only.
- Relative small sample size (4/male/group, 4/sex/group is preferred group size and could, with 80% chance, detect a 5% change (10% if 2/sex/group) in appropriately corrected QT.
- No dose confirmation data provided.
- Using separated control and drug groups (control dogs were given three doses vehicle and dogs in the drug group given three ascending doses), which resulted in some higher baseline values in the drug group and additional variables. Latin square study design with 2/dose/time, longer washout time, is preferred study design.
- Short (30 min) washout time, leading to dosing the animals before the complete recovery.

Renal effects: N/A

Gastrointestinal effects: N/A

Abuse liability: N/A

Other: N/A

Safety Pharmacology Summary

No significant Ro 50-3821/000-related cardiovascular and respiratory effects were observed at doses up to 50 mcg/kg with a total cumulative dose up to 70 mcg/kg when compared to the controls.

No significant Ro 50-3821/000-related neurological findings in a twenty-six week (once/week) subcutaneous injection toxicity study in rats at dose up to 3 mcg/kg, according to the study report.

2.6.2.5 Pharmacodynamic drug interactions: N/A

2.6.3 PHARMACOLOGY TABULATED SUMMARY: N/A

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

Synopsis of pharmacokinetics data are presented below based on the study reports. The study reports were not reviewed by the reviewer. Toxicokinetics data are presented in related toxicity studies.

2.6.4.1 Brief summary

RO0503821 was found to possess slower depot absorption after SC administration (Tmax 24-72h) and slower systemic clearance than epoetin beta. Compared to epoetin beta, Tmax after SC administration of RO0503821 was delayed by 12 hours in rats, and apparent elimination t1/2 was prolonged by approximately 2 folds in rats and 7-11 folds in dogs.

Higher systemic exposure was achieved by IV than by SC (eg. mean AUC 20845 in IV vs. 9220 in SC). The bioavailability after SC dosing in rats was 31% and 45% in the 2.5 and 25 mcg/kg dose groups, respectively, and was 80% and 46% in the 3 and 10 mcg/kg dose groups in dogs, respectively.

¹⁴C-RO0503821 distribution and excretion study (SC) revealed that radioactivity was detected in almost all analyzed matrices and the highest tissue radioactivity was found in the injection site, lymph nodes, testis, blood, adrenal gland (medulla), and spleen. The radioactivity (0-0.947 mcg equivalent/g) was also detected in cerebellum, cerebrum, and CSF. After a single dose, urine was the primary elimination (23%) pathway.

A lacteal excretion and placental transfer study with a single SC administration of ¹⁴C-RO0503821 in pregnant rats demonstrated limited (<0.3% of administered dose) penetration of RO0503821 through the placenta. ¹⁴C-RO0503821 was found in lacteal excretion samples.

2.6.4.2 Methods of Analysis

The PK samples were analyzed by an enzyme-linked-immunosorbent assay (ELISA). The pharmacokinetic characteristics of RO0503821 were evaluated by non-compartmental analysis.

2.6.4.3 Absorption

Pharmacokinetic studies were conducted after single IV and SC administrations of RO0503821 to rats at doses of 0.25, 2.5, or 25 mcg/kg and dogs at doses of 3, 7.5, or 10 mcg/kg.

Rat Study

PK parameters were shown in Table 2. Compared to epoetin beta, decreased absorption (after SC administration), systemic clearance, and volume of distribution were observed for RO0503821. Specifically, compared to epoetin beta, T_{max} after SC administration of RO0503821 was delayed by 12 hours, reflecting slower depot absorption. After a single IV injection, systemic clearance (Cl) of RO0503821 was only ~1/10 of the value obtained with epoetin beta, and the terminal phase volume of distribution (V_z) was ~1/5 of epoetin beta value, indicating limited tissue distribution. As a result of the profound reduction in systemic clearance, the apparent elimination $t_{1/2}$ of RO0503821 was prolonged by approximately 2-fold when compared to epoetin beta.

The bioavailability of RO0503821 after SC dosing was 31% and 45% in the 2.5 and 25 mcg/kg dose groups, respectively.

Table 2. PK Parameters of RO0503821 Following Single IV or SC Injection in Rats

Dose $\mu\text{g}/\text{kg}$	0.25 [#]	2.5	25
Intravenous Injection			
Cl (mL/hr/kg) (range)	3.7 (3.1-4.0)	1.5 (1.1-1.9)	1.3 (1.0-1.5)
V_z (mL/kg) (range)	78 (52-185)	59 (28-89)	51 (38-62)
$t_{1/2}$ (hr) (range)	18 (10-32)	25 (18-32)	27 (27-29)
AUC _{0-144hr} (ng•hr/mL) (range)	65 (60-65)	1761 (1233-2288)	18615 (16420-25096)
Subcutaneous Injection			
AUC _{0-144hr} (ng•hr/mL) (range)	8.5 ^a	547 (482-668)	8376 (7519-9518)
C_{max} (ng/mL) (range)	0.23 ^a	10 (9.2-12)	108 (102-132)
T_{max} (hr) (range)	24 ^a	24 (24-24)	24 (24-24)
Bioavailability (%) (range)	ND	31 (27-38)	45 (40-51)

#: Due to the extremely low serum concentrations, the PK parameters in 0.25 mcg/kg group could not be fully characterized in all animals.

ND: not determined

^a: mean data

Dog Study

PK parameters were shown in Table 3. Compared to epoetin beta, decreased systemic clearance and volume of distribution were also observed in dogs. Specifically, the apparent terminal $t_{1/2}$ was prolonged by a factor of ~7 to 11 and V_z was about 50% of epoetin beta value.

The bioavailability after SC dosing was 80 % and 46% in the 3 and 10 mcg/kg dose groups, respectively.

Table 3. PK Parameters of RO0503821 Following Single IV or SC Injection in Dogs

Dose ($\mu\text{g}/\text{kg}$)	3	7.5	10
Intravenous Injection			
Cl (mL/hr/kg) (range)	0.8 (0.3-1.1)	1.3 (1.2-1.7)	0.38 (0.34-0.52)
V_z (mL/kg) (range)	44 (37-52)	84 (57-86)	38 (32-56)
$t_{1/2}$ (hr) (range)	41 (29-86)	44 (23-49)	70 (62-77)
AUC _{0-168hr} (ng•hr/mL) (range)	3632 (2189-5624)	7158 (5780-7449) ^b	20845 (15476-24428)
Subcutaneous Injection			
AUC _{0-168hr} (ng•hr/mL) (range)	2911 (2324-3787)	--	9620 (7154-16579)
C _{max} (ng/mL) (range)	35 (28-40)	--	90 (64-136)
T _{max} (hr) (range)	48 (24-48)	--	48 (48-72)
Bioavailability (%) (range)	80 (64-104)	--	46 (34-80)

^bAUC_{0-144hr}

The reviewer comment:

- Higher systemic exposure achieved by IV than by SC (eg. mean AUC 20845 in IV vs. 9220 in SC in dogs)
- Slow absorption by SC (T_{max} 24-72h)

2.6.4.4 Distribution

Tissue Distribution

To assess the tissue distribution and excretion profiles, ¹⁴C-RO0503821 was administered to rats by a single or multiple SC injections at 0.67 mg/animal. Radioactivity was detected in almost all analyzed matrices except for esophageal contents. Due to the high dose, the majority of radioactivity remained at the injection site unabsorbed in both single and multiple dose groups. Besides the injection site, the highest tissue radioactivity was found in lymph nodes (a major transportation pathway for

proteins), testis (an indication of crossing the blood/testis barrier), blood, adrenal gland (medulla), and spleen. This profile was essentially consistent between the single and multiple dose groups. The ¹⁴C-RO0503821-derived radioactivity (0-0.947 mcg equivalent/g) was also detected in cerebellum, cerebrum, and CSF following the single and multiple dosing, as well as in medulla oblongata and spinal cord after multiple doses, suggesting that drug-derived radioactivity cross the blood-brain barrier, but at very low levels. Following single or multiple doses, with a few exceptions, the tissue: serum concentration ratios in most organs were less than one at all time points (up to 72h).

After a single dose, urine was the primary elimination (23% vs. 4% in feces) pathway.

Immunohistochemistry analysis with selected tissue slices demonstrated a co-localization between immunoreactive human EPO and ¹⁴C-RO0503821 radioactivity.

Lacteal Excretion and Placental Transfer

A lacteal excretion and placental transfer study was conducted in pregnant rats after a single SC administration of ¹⁴C-RO0503821. Only limited (<0.3% of administered dose) penetration of RO0503821 through the placenta was noted. ¹⁴C-RO0503821 was found in lacteal excretion samples. The ¹⁴C-RO0503821 activity reached its peak in milk at T_{max} and was approximately 10% of serum levels. However, RO0503821 via breast feeding is most likely degraded during passage through the digestive system owing to its protein nature; therefore, unlikely to produce pharmacological consequences in infants.

2.6.4.5 Metabolism: N/A

2.6.4.6 Excretion:

After a single dose, urine was the primary elimination (23% vs. 4% in feces) pathway. See the distribution section for more details.

2.6.4.7 Pharmacokinetic drug interactions: N/A

2.6.4.8 Other Pharmacokinetic Studies

Extensive clinical PK studies were conducted and key PK parameters and characteristics were summarized below.

Key PK Parameters

- Elimination Half-life in CKD patients:
 - IV administration: 134 h
 - SC administration: 139 h
- Half-life after IV administration 15 to 20 times longer compared to rhEPO
- Low clearance (~ 0.5 mL/h/kg)
- Small volume of distribution (~ 5L)
- Bioavailability of 50-60% (SC) in CKD patients

Key PK Characteristics

- No effect of multiple dosing on clearance, volume of distribution and bioavailability
- No major deviation from dose proportionality in PK
- No effect of hemodialysis on PK
- No clinically relevant effect of site of SC injection (abdomen, arm, thigh) on PK, PD, or local tolerability

2.6.4.9 Discussion and Conclusions

The systemic clearance of RO0503821 was slower than epoetin beta with the apparent elimination $t_{1/2}$ prolonged by approximately 2- and 7-11- folds in rats and dogs, respectively, when compared to epoetin beta. This property made the longer dosing interval feasible.

Following subcutaneous injection, slow depot absorption (T_{max} 24-72h) and lower systemic exposure (than that of IV injection, mean AUC 20845 in IV vs. 9220 in SC in dogs) was noted. The bioavailability after SC dosing in rats was 31% and 45% in the 2.5 and 25 mcg/kg dose groups, respectively, and was 80% and 46% in the 3 and 10 mcg/kg dose groups in dogs, respectively.

^{14}C -labeled RO0503821 was detected as radioactivity in almost all analyzed matrices and the highest tissue radioactivity was found in the injection site (SC), lymph nodes, testis, blood, adrenal gland, and spleen. The radioactivity was also detected in cerebellum, cerebrum, and CSF at very low level (0-0.947 mcg equivalent/g). In addition, RO0503821 was noted penetrating the placenta at the level of <0.3% of administered dose and excreting into the milk.

2.6.4.10 Tables and figures to include comparative TK summary

Toxicokinetic results from 13-week toxicity and toxicokinetic studies were summarized in Table 4. See repeat toxicity section for more details.

Table 4. TK Parameters of RO0503821 in the Thirteen-Week Rat and Dog Studies

Dose ($\mu\text{g}/\text{kg}/\text{dose}$)	AUC _{0-168hr} (ng•hr/mL)			C _{max} (ng/mL)		
	Day 0*	Day 21	Day 84	Day 0*	Day 21	Day 84
Rat Study, SC						
1	199	387	369	3.97	6.72	5.26
3	754	1200	1460	13.5	15.8	20.3
10	3290	4300	4180	61.5	59.2	71.6
30	9460	13300	N/A	153	178	N/A
	Day 1	Day 22	Day 85	Day 1	Day 22	Day 85
Rat Study, IV**						
1	623	543	680	20.4	20.7	21.9
3	2140	1740	1980	78.0	75.6	67.0
10	12700	9580	10400	416	452	422
30	43600	43000	N/A	1140	1740	N/A
Dog Study, SC						
1	361	396	293	8.11	7.49	3.71
3	2180	2970	869	27.3	34.9	9.36
10	10200	22300	84.0	98.0	187	0.93
30	38400	61400	N/A	380	561	N/A
Dog Study, IV						
1	798	1520	521	15.1	28.4	36.0
3	3130	6780	5550	54.6	96.4	95.0
10	17300	22900	13800	272	319	239
30	52100	32200	N/A	707	775	N/A

*: First day of dosing is designated as Day 0 in this study.

** : AUC_{0-144hr} in this study

N/A: not applicable

2.6.5 PHARMACOKINETICS TABULATED SUMMARY: N/A

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

The nonclinical safety of RO0503821 was evaluated in extensive toxicity studies. These studies included, but not limited to, two single-dose acute toxicity studies in mice and rats, four 13-week toxicity studies in rats and dogs, one 26-week toxicity study in rats, one reproductive study in rats, two teratology studies in rats and rabbits, and one prenatal and postnatal developmental study in rats. The key study findings were summarized below.

General toxicology

Single-Dose Toxicity Studies

Single-dose toxicity studies were conducted in rats and mice at doses up to 750 mcg/kg administered as IV bolus injection. Exaggerated pharmacology effects such as increased

red blood cell parameters and enlarged spleen were observed at the end of the 2-week observation period while no adverse clinical signs were noted.

Repeat-Dose Toxicity Studies

Five repeat dose toxicity studies were conducted and reviewed. The study designs (Table 5) and key findings were summarized below.

Table 5. Summary of Study Design of Repeat Dose Toxicity Studies

Species	Routes	Doses (mcg/kg/dose)*	Interim Sacrifice (wk)	Terminal Sacrifice (wk)	Recovery Sacrifice (wk)
Dog	IV	1, 3, 10, and 30	4	13	8
Dog	SC	1, 3, 10, and 30	4	13	8
Rat	IV	1, 3, 10, and 30	4	13	8
Rat	SC	1, 3, 10, and 30	4	13	8
Rat	SC	0.3, 1, and 3	N/A	26	12

* QW, 30 mcg for 4 weeks only, prepared by the reviewer.

Key study findings:

Mortality:

Drug-related deaths and unscheduled moribund sacrifices due to:

- Severe polycythemia (exaggerated erythropoiesis-expected pharmacology effect, dose-related) and secondary lesions
- Severe anemia (ant-EPO Ab-mediated 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 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 in rats only (1/24 in the 1 mcg/kg group and 8/28 in the 3 mcg/kg group 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 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.

Genetic toxicology: N/A

Carcinogenicity:

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.

The cell membrane binding pattern, which is considered a pharmacologically and toxicologically relevant binding to extracellular domain of EPO-R, was only seen in the hematological progenitor cells in the bone marrow, the intended target cells for RO0503821. Overall, the tissue binding profile of RO0503821 was comparable to that of epoetin beta, and no aberrant tissue binding pattern was identified.

Reproductive toxicology:

Fertility and early embryonic development

Fertility was evaluated in rats by subcutaneous injection and key study findings were summarized below.

- 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 RO0503821 in the male and female rats could be established at 50 mcg/kg/dose.

Teratology potential was evaluated in rats and rabbits by subcutaneous injection. The key study findings were summarized below.

- Two unscheduled rabbit sacrifice in 20 mcg/kg group due to miscarriage and premature delivery, accompanied by late resorption in one rabbit;

- Dose-related reduction in body weights, body weight gains, and food consumptions in dams of both rats and rabbits;
- Dose-related reduction in fetal weights in both rats and rabbits;
- Dose-related developmental delays (incomplete and no ossification) for fetuses in both rats and rabbits;
- Dose-related increased percentage of resorbed conceptuses /litter in rabbits (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 increased numbers of fetuses with alterations in the rabbits (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 in the rabbits;
- Three caudal vertebrae present in one fetus in the 50 mcg/kg/dose group resulting in a thread-like tail in the rat study;
- Two rabbits with skeletal malformations in the 50 mcg/kg/dose dose group (one with absent 1st digit metacarpal and phalanx on each forelimb resulting in absent plex and another with fused 4th and 5th cervical vertebrae centra).

NOAEL could not be established (less than 5 mcg/kg/dose for both maternal and fetal toxicities).

Prenatal and postnatal development

Perinatal and postnatal developmental toxicity was evaluated in rats by subcutaneous route and key study findings were summarized below.

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 sacrifice (pale liver) in the 20 mcg/kg/dose group and three deaths in the 50 mcg/kg/dose groups during the first week postweaning.
- 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.

2.6.6.2 Single-dose toxicity

Single-dose toxicity studies were conducted in rats and mice at doses up to 750 mcg/kg administered as IV bolus injections. Exaggerated pharmacology effects such as increased red blood cell parameters and enlarged spleen were observed at the end of the 2-week observation period while no adverse clinical signs were noted.

2.6.6.3 Repeat-dose toxicity

Five repeat dose toxicity studies (A-D) were conducted and reviewed separately below with more details for 26 week rat toxicity study. The study design was summarized in Table 5.

A.

Study title: Ro 50-3821/000: A 13-Week Subcutaneous Injection Toxicity Study with a 4-Week Interim Sacrifice and a 8-Week Recovery Phase in Dogs

Key study findings:Mortality:

- One unscheduled sacrifice in 10 mcg/kg/dose TK group on Day 83 with severe polycythemia (Hct = 66.7%), body tremors, sternal recumbence, moderate ataxia, low vocalization (intermittently), red conjunctivae of the eyes, and red gums.

Clinical signs:

- Dose- and time-related red skin (gums or ears) and red conjunctivae. These signs remained prevalent through the recovery phase in dogs given 3 or 10 mcg/kg/dose.
- Pale skin (gums) in dogs given 1, 3, or 10 mcg/kg/dose during Week 13 of the treatment phase and continued during the recovery period for one male and one female given 3 mcg/kg/dose.

Body weights and food consumptions:

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

Ophthalmoscopy:

- Drug-related hyperemia in dogs given 1, 3, or 10 mcg/kg/dose at Week 13 and remained so in one of two males given 1, 3, or 10 mcg/kg/dose at Week 21.
- Drug-related dilated retinal blood vessels for dogs given 10 mcg/kg/dose at Week 13.

Hematology:

- Dose- and time-related aberrations in erythrocyte parameters.

Histopathology:

- Increased hematopoiesis in bone marrow and spleen in females given 3, 10, or 30 mcg/kg/dose and males given 1, 3, 10, or 30 mcg/kg/dose at Weeks 4 and 13 sacrifice, no dose-dependent trend in the severity but only minimal or slight erythroid and myeloid hyperplasia in males (not in females) receiving 1 mcg/kg/dose while slight to moderately severe erythroid, myeloid, and megakaryocytic hyperplasia in both males and females receiving 3 or 10 mcg/kg/dose at Week 13. At least partially reversible.
- Bone marrow hypoplasia noted as early as at Week 4 sacrifice (1/6 and 2/6 in 10 and 30 mcg/kg/dose groups, respectively) and higher incidence at Week 13 (1/6 and 3/6 in 3 and 10 mcg/kg/dose groups, respectively) and recovery [1/6, 2/6, and 2/6 in 1, 3, and 10 mcg/kg/dose groups, respectively) sacrifices. Only erythroid hypoplasia was observed at Week 4 sacrifice while erythroid, myeloid, and megakaryocytic hypoplasia were seen at Week 13 sacrifice. Erythroid hypoplasia correlated with decreased or no absolute reticulocyte counts.
- Moderate myelofibrosis of the bone marrow in 1/4 dogs receiving 10 mcg/kg/dose at recovery sacrifice.
- Minimally to moderately increased renal tubular basophilia in 3 dogs (1M2F), accompanied by slightly or moderately increased thrombosis of glomerular capillaries in 2 dogs (1M1F) in 10 mcg/kg/dose group at Week 13 sacrifice. These animals were polycythemic at Week 13. Minimal to moderate segmental glomerular sclerosis in all males in 1, 3, or 10 mcg/kg/dose groups while in 1/2 or 2/2 females in 3 or 10 mcg/kg/dose group, respectively, accompanied by minimal or slight interstitial fibrosis in all but one (in 3 mcg/kg/dose group) males in all drug groups while in females in 10 mcg/kg/dose group only at recovery sacrifice.

Doses: 0 (vehicle), 1, 3, 10, or 30 mcg/kg/dose, once weekly for 4 wks (4 doses) for interim sacrifice; 0 (vehicle), 1, 3, or 10 mcg/kg/dose, once weekly for 13 wks (13 doses on Days 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, and 85) for terminal sacrifice with an 8-week recovery period.

Table 6. Summary of Study Design

Group	No. of Animals		Dose Level ^a (mcg/kg/dose)	Dose
	Male	Female		Concentration (mcg/mL/dose)
Toxicity Animals				
1 (Control) ^b	8 ^c	8 ^c	0	0
2 (Low)	8 ^c	8 ^c	1	10
3 (Mid-low)	8 ^c	8 ^c	3	30
4 (Mid-high)	8 ^c	8 ^c	10	100
5 (High)	3 ^c	3 ^c	30	300
Toxicokinetic Animals				
6 (Low)	2	2	1	10
7 (Mid-low)	2	2	3	30
8 (Mid-high)	2	2	10	100
9 (High)	2	2	30	300

^a The dose volume was 0.1 mL/kg/dose.

^b The control animals received the vehicle only.

^c Three animals/sex/group were sacrificed after 4 weeks of treatment (interim sacrifice); three animals/sex/group in Groups 1 through 4 were sacrificed after 13 weeks of treatment (terminal sacrifice); and two animals/sex/group in Groups 1 through 4 were sacrificed after at least 13 weeks of treatment followed by 8 weeks without treatment (recovery sacrifice).

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) and results of re-testing at the end of the study were _____ mcg/mL _____, of claim, respectively). The stability (at least 4 months) of these solutions was confirmed 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: Dog/beagle

Number/sex/group or time point: 3/sex/group/time point

Route, formulation, volume, and infusion rate: subcutaneous (four dose sites, rotated each week), dose volume: 0.1 mL/kg.

Satellite groups used for toxicokinetics or recovery: 2/sex/group for TK or recovery.

Age: approximately 6 to 8.5 months

Weight: 7.3 to 12.4 kg

Sampling times: See other related sections.

Unique study design or methodology (if any): N/A

Observations and times:

Mortality: Twice daily

Clinical signs: Twice daily for morbidity, once daily cageside observations for abnormal findings, once weekly and on the day of necropsy for detailed observations

Body weights: Twice before initiation of treatment, on the first day of treatment, and weekly thereafter.

Food consumption: Weekly beginning 1 week prior to initiation of treatment and weekly during treatment and recovery phases.

Ophthalmoscopy: Pre-dose, and weeks 4, 13, and 21 (recovery).

ECG: Pre-dose, and weeks 4, 13, and 21 (recovery).

Hematology: Twice prior to initiation of treatment, weekly during the study, fast overnight.

Clinical chemistry: Twice prior to dose and at near the end of Weeks 4, 13, and 21, fast overnight.

Urinalysis: Twice prior to treatment and at near the end of Weeks 4, 13, and 21 (approximately 16 hours during the overnight fasting).

Gross pathology: 3/sex/group on Days 30 (interim sacrifice) or 93 (terminal sacrifice), all remaining dogs on Day 150 (recovery sacrifice) [except for a Group 8 female on Day 83 (Week 12)].

Organ weights: 3/sex/group on Days 30 (interim sacrifice) or 93 (terminal sacrifice), all remaining dogs on Day 150 (recovery sacrifice). Also see histopath table (Table 14).

Histopathology: 3/sex/group on Days 30 (interim sacrifice) or 93 (terminal sacrifice), all remaining dogs on Day 150 (recovery sacrifice). 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 high-dose groups; gross lesions were examined 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 the low- and mid-dose groups.

Adequate Battery: yes (X), no ()

Peer review: yes (), no (X)

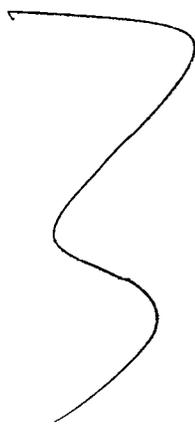
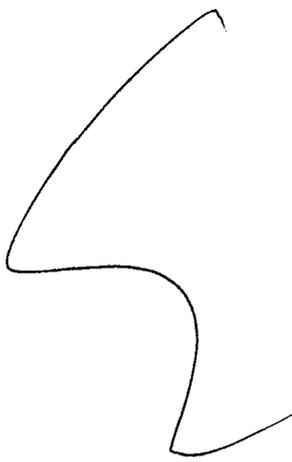
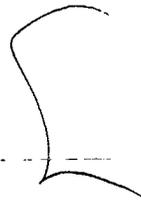
Toxicokinetic: On days 1, 22, and 85 at 0 (pre-dose), approximately 1, 3, 5, 12, 16, 24, 36, 48, 60, 72, 96, 120, 144, and 168 hours post dose (except that a blood sample was collected from a Group 8 female sacrificed in moribund condition on Day 83).

The serum samples for Ro 50-3821 concentrations were analyzed by a validated ELISA method, which detects free Ro 50-3821 only. The lower limit of quantitation was 150 pg/mL for Ro 50-3821. The calibration range of the standard curve for Ro 50-3821 was 150-3000 pg/mL. Any experimental samples below the limit of quantitation were annotated as BLQ. The % deviation allowed for the lowest Standard and QC sample was 25% and 20% for all other concentrations. From a single analytical run, no more than 1/3 of the Standards could be deactivated (Standards having a % deviation greater than defined above). For QC's, no more than 1/3 of the samples could be outside the above limits.

Watson (Version 6.1.1.03), a Hoffmann-La Roche, Inc. (HLR) validated laboratory information system, was used to estimate toxicokinetic parameters. For each dose group, the toxicokinetic parameters were estimated based on the individual (n=2/sex/group) concentration-time profile and a mean value was depicted. Samples with concentrations reported as <BLQ were set to zero. The toxicokinetic parameters estimated were AUC_{0-168hr}, dose normalized AUC (AUC_{0-168hr}/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 and C_{max} values. A difference between parameters is claimed if the value being compared exceeded ±30% of the corresponding comparison number.

Antibodies: Serum samples for anti-EPO Ab determinations were collected from all toxicity and toxicokinetic dogs pre-dose and after 4, 8, and 13 weeks of treatment, and after 4 and 8 weeks of recovery. However, antibody assays were performed for the samples collected after 4 and 13 weeks only.

A:  ELISA  was used to analyze anti-EPO Ab.



1 Page(s) Withheld

 X § 552(b)(4) Trade Secret / Confidential

 § 552(b)(4) Draft Labeling

 § 552(b)(5) Deliberative Process

 1 pharma

Results

Mortality: One female (H05099) in toxicokinetic 10 mcg/kg/dose group was sacrificed in moribund condition on Day 83 (Week 12). Clinical signs such as body tremors, sternal recumbence, moderate ataxia, low vocalization (intermittently), red conjunctivae of the eyes, and red gums were noted prior to sacrifice. Hematology revealed severe polycythemia (Hct = 66.7%). A necropsy performed on this dog did not reveal any drug-related macroscopic findings.

All other animals survived to their scheduled sacrifice.

Clinical signs:

Dose- and time-related red skin (gums or ears) and red conjunctivae: Over the first 4 weeks of the study, red conjunctivae was noted in one male given 30 mcg/kg/dose only. Red skin (gums or ears) and red conjunctivae were generally noted in animals given 1, 3, or 10 mcg/kg/dose in the later weeks of the study and remained prevalent through the recovery phase in animals given 3 or 10 mcg/kg/dose.

Pale skin (gums) was first observed in dogs given 1, 3, or 10 mcg/kg/dose during Week 13 of the treatment phase and continued during the recovery period for one male and one female given 3 mcg/kg/dose.

Generally, the clinical observations of red skin, gums, and conjunctiva were associated with high hematocrits (polycythemia) while pale skin and gums with low hematocrits and positive anti-EPO-Ab.

Body weights: Dose-related reduction in body weight: over the first 5 weeks of the treatment period, body weight decreases were statistically significant for males given 10 or 30 mcg/kg/dose and for females given 10 mcg/kg/dose. The decrease in body weight gains continued for females receiving 10 mcg/kg/dose through the remainder of the treatment period. The reduction in body weight corresponded to mildly decreased food consumption during Weeks 1 through 4 for females receiving 30 mcg/kg/dose and during Weeks 1 through 14 for females receiving 10 mcg/kg/dose. However, the reductions were of small magnitude and were not considered toxicologically significant, according to the study report.

Food consumption: Mild reductions in food consumption were noted in the 10 and 30 mcg/kg/dose groups. However, the reductions were of small magnitude and were not considered toxicologically significant, according to the study report.

Ophthalmoscopy: No drug-related changes were noted at the examination after 4 weeks of treatment. At Week 13, drug-related hyperemia was noted in animals given 1, 3, or 10 mcg/kg/dose. In addition, drug-related dilated retinal blood vessels were noted for animals given

10 mcg/kg/dose. At Week 21, hyperemia was noted in one of two males given 1, 3, or 10 mcg/kg/dose.

ECG and BP: No drug-related changes in electrocardiograph parameters or blood pressure.

Hematology:

Dose- and time-related aberrations in erythrocyte parameters: During the first several weeks of the study, higher red blood cell count, hemoglobin, hematocrit, and absolute reticulocyte count for males and females at all dose levels; lower mean corpuscular volume and mean corpuscular hemoglobin for males and females given 3, 10, or 30 mcg/kg/dose; and lower mean corpuscular hemoglobin concentration for males and females given 10 or 30 mcg/kg/dose. The erythrocyte effects among animals given the lowest dose took longer to develop than those for animals in the other dose groups. After approximately 4 to 6 weeks of treatment, several animals (generally one or two in each drug group) stopped producing circulating reticulocytes. Red blood cell counts, hemoglobin concentrations, and hematocrits for these animals dropped slowly until many had values that were below those of the control animals suggesting the antibody may also have affected native EPO. Because the development of resistance was relatively inconsistent, the erythrocyte data were quite variable for treated animals.

Mildly higher platelet count during the first few weeks of the study, primarily for animals given 3, 10, or 30 mcg/kg/dose.

After discontinuation of treatment, both polycythemic and anemic animals tended to recover although complete recovery was not achieved by the end of the eight-week recovery period.

Clinical chemistry:

Slightly decreased glucose and slightly increased activated partial thromboplastin time, total protein, albumin, calcium, inorganic phosphorus, sodium, potassium, chloride, and aspartate aminotransferase were noted in drug groups. With the exception of higher activated partial thromboplastin time, these minor differences were most apparent at Week 4 and generally affected animals given 3, 10, or 30 mcg/kg/dose. According to the study report, most, if not all, of these effects were likely artifactual changes associated with the high red blood cell mass and did not reflect direct toxic effects of the drug.

At week 4, lower serum iron and slightly higher total and unbound iron binding capacity were noted in the 3, 10, and 30 mcg/kg/dose groups. These changes in iron were seen in some dogs from each dose group at the end of the treatment period but reversed during the recovery period.

Urinalysis: No remarkable drug-related changes.

Gross pathology:

4-Week Interim Sacrifice

Enlarged spleen in one dog given 30 mcg/kg/dose, correlating with increased extramedullary hematopoiesis.

13-Week Terminal Sacrifice

Diffusely light marrow of the sternum and femur in one male and decreased marrow in the sternum in one female in 10 mcg/kg/dose group, correlating with decreased number of cells in the marrow.

Recovery Sacrifice

Thickening at the injection sites in three dogs at 10 mcg/kg/dose group (no changes microscopically).

Organ weights: No remarkable drug related changes.

Histopathology: See Tables 7-10.

Table 7. Summary of Major Histopathologic Findings

Dose (mcg/kg)	4-wk Sacrifice				13-wk Sacrifice			Recovery Sacrifice		
	1	3	10	30	1	3	10	1	3	10
N	3M3F	3M3F	3M3F	3M3F	3M3F	3M3F	3M3F	2M2F	2M2F	2M2F
Hyperplasia										
Bone marrow	0	3M1F	3M3F	3M2F	1M	2M2F	1M2F	0	1F	0
Erythroid		3M1F*	3M2F	2M1F	1M	2M2F	1M2F		1F	
Myeloid		2M1F	3M3F	2M2F	1M	1F	0		0	
Megakaryocytic		1M1F	3M2F	2M2F	0	1M	0		0	
Spleen	2M0F	1M3F	3M2F	1M1F	1M1F	2M1F	3M3F	2M	1F	0
Liver	0	0	0	0	0	1M	0	0	0	0
Adrenal cortex	0	1F	0	0	0	0	0	0	0	0
Hypocellular Bone Marrow	0	0	0	0	0	1M1F	2M1F	1F	1M1F	1M1F
Hypoplasia										
Bone marrow	0	0	1F	1M1F	0	1M	2M1F	1F	1M1F	1M1F
Erythroid	0	0	1F	1M1F	0	1M	2M1F	1F	1M1F	1M 1F
Myeloid	0	0	0	0	0	1M	1F	0	0	0
Megakaryocytic	0	0	0	0	0	1M	2M1F	0	0	0
Myelofibrosis	0	0	0	0	0	0	0	0	0	1F
KIDNEYS										
Tubular basophilia	0	0	0	0	0	0	1M2F	0	0	1F
Thrombosis of glomerular capillaries	0	0	0	0	0	0	1M1F	0	0	0
Segmental glomerular sclerosis	0	0	0	0	0	0	0	2M	2M1F	2M2F
Interstitial fibrosis	0	0	0	0	0	0	0	2M	1M	2M2F

* Another dog had an increased proportion of erythroid elements in the femur marrow without an increase in the overall number of cells in the marrow. Prepared by the reviewer based on the submission.

4-Week Interim Sacrifice (Tables 7 and 8)

Increased hematopoiesis in femur marrow, sternum marrow, and spleen in females given 3, 10, or 30 mcg/kg/dose and males given 1, 3, 10, or 30 mcg/kg/dose, some with hyperplasia of all three hematopoietic elements (erythroid, myeloid, and megakaryocytic) while others with erythroid or myeloid response only, no dose-dependent trend in the severity.

Slight extramedullary hematopoiesis (erythroid) in the adrenal cortex of one female in the 3 mcg/kg/dose group.

Table 8. Incidence and Severity of Drug-Related Microscopic Findings (Interim Sacrifice)

Number examined	Ro 50-3821/000 mcg/kg/dose										
	Males					Females					
	0	1	3	10	30	0	1	3	10	30	
Marrow, Sternum											
Hyperplasia, Myeloid											
	Slight	0	0	0	1	0	0	0	1	0	1
	Moderate	0	0	1	0	0	0	0	0	0	0
Hyperplasia, Erythroid											
	Slight	0	0	0	0	0	0	1	2	0	
	Moderate	0	0	1	2	2	0	0	0	0	
Hypoplasia, Erythroid											
	Slight	0	0	0	0	1	0	0	0	0	
	Moderate	0	0	0	0	0	0	0	1	1	
Marrow, Femur											
Hyperplasia, Erythroid											
	Minimal	0	0	1	0	0	0	0	0	0	
	Slight	0	0	1	0	1	0	0	0	1	
	Moderate	0	0	1	1	0	0	0	1	1	
	Moderately-severe	0	0	0	2	1	0	0	0	0	
Hyperplasia, Megakaryocytic											
	Minimal	0	0	0	1	0	0	1	1	1	
	Slight	0	0	1	1	1	0	0	0	1	
	Moderate	0	0	0	1	1	0	0	0	0	
Hyperplasia, Myeloid											
	Minimal	0	0	0	2	0	0	1	0	0	
	Slight	0	0	2	0	1	0	0	0	2	
	Moderate	0	0	0	1	1	0	0	0	1	
Hypoplasia, Erythroid											
	Moderate	0	0	0	0	1	0	0	0	1	
	Moderately-severe	0	0	0	0	0	0	0	0	1	
Spleen											
Hyperplasia, Erythroid											
	Minimal	0	1	0	1	0	0	2	0	0	
	Slight	0	1	1	1	1	0	1	1	0	
	Moderate	0	0	0	1	0	0	0	1	1	
Siderofibrosis											
	Moderate	0	0	0	0	1	0	0	0	0	
Hyperplasia, Myeloid											
	Slight	0	0	0	0	1	0	0	0	0	
Hyperplasia, Megakaryocytic											
	Minimal	0	1	1	0	0	0	0	0	0	
	Slight	0	0	0	0	1	0	0	0	2	
Fibrosis, Capsular											
	Slight	0	1	0	0	0	0	0	0	0	
	Moderate	0	1	0	0	0	0	0	0	0	

Erythroid hypoplasia of the femur and sternum marrow in one male receiving 30 mcg/kg/dose and one female each at 10 and 30 mcg/kg/dose groups, correlating with decreased absolute reticulocyte counts.

13-Week Terminal Sacrifice (Tables 7 and 9)

Hyperplasia of the hematopoietic elements in the bone marrow in some males at all doses and in two females each receiving 3 (Nos. H05076 and H05077) and 10 (Nos. H05089 and H05094) mcg/kg/dose. Only minimal or slight erythroid and myeloid hyperplasia was noted in males (not in females) receiving 1 mcg/kg/dose while slight to moderately severe hyperplasia of all three elements in both males and females receiving 3 or 10 mcg/kg/dose.

Minimal to moderate splenic erythroid, myeloid, or megakaryocytic hyperplasia in some dogs from all treated groups [1M and 1F at 1 mcg/kg/dose, 1F at 3 mcg/kg/dose, 3M and 2F with erythroid and megakaryocytic hyperplasia and another female with hyperplasia of erythroid, myeloid, and megakaryocytic cells at 10 mcg/kg/dose group].

Slight extramedullary hematopoiesis in the liver of one male dog in the 3 mcg/kg/dose group.

Moderate or moderately severe hypocellularity of the bone marrow in two dogs in 3 mcg/kg/dose group and three dogs in 10 mcg/kg/dose group. The animals with erythroid hypoplasia had no circulating reticulocytes.

Increased renal tubular basophilia in 3 dogs (1 male and 2 females, slight or moderate), accompanied by increased thrombosis of glomerular capillaries in 2 dogs (slight or moderate) in 10 mcg/kg/dose group. These animals were polycythemic at Week 13. The findings were regarded as a secondary effect of the drug resulting from hemodynamic effects of a high hematocrit by the study report.

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Table 9. Incidence and Severity of Drug-Related Microscopic Findings (Terminal Sacrifice)

Number Examined		Ro 50-3821/000 mcg/kg/dose							
		Males				Females			
		0	1	3	10	0	1	3	10
		3	3	3	3	3	3	3	3
Marrow, Sternum									
Hyperplasia, myeloid	Slight	0	1	0	0	0	0	0	0
Hyperplasia, erythroid	Minimal	0	1	0	0	0	0	0	0
	Slight	0	0	2	1	0	0	1	0
	Moderate	0	0	0	0	0	0	0	2
Hyperplasia, megakaryocytic	Moderate	0	0	1	0	0	0	0	0
Hypocellular marrow	Moderately Severe	0	0	1	2	0	0	0	1
Hypoplasia, myeloid	Slight	0	0	0	0	0	0	0	1
	Moderate	0	0	1	0	0	0	0	0
Hypoplasia, erythroid	Moderately Severe	0	0	1	2	0	0	0	1
Hypoplasia, megakaryocytic	Moderate	0	0	1	2	0	0	0	0
Marrow, femur									
Hyperplasia, myeloid	Minimal	0	1	0	0	0	0	1	0
Hyperplasia, erythroid	Minimal	0	1	1	0	0	0	1	0
	Slight	0	0	0	0	0	0	1	0
	Moderate	0	0	1	1	0	0	0	0
	Moderately Severe	0	0	0	0	0	0	0	2
Hypocellular marrow	Moderate	0	0	1	1	0	0	0	0
	Moderately Severe	0	0	0	1	0	0	1	1
Hypoplasia, myeloid	Moderate	0	0	0	0	0	0	0	1
Hypoplasia, erythroid	Moderate	0	0	1	0	0	0	0	0
	Moderately Severe	0	0	0	2	0	0	0	1
Hypoplasia, megakaryocytic	Slight	0	0	0	1	0	0	0	0
	Moderate	0	0	1	1	0	0	0	1
Liver									
Extramedullary Hematopoiesis	Slight	0	0	1	0	0	0	0	0
Spleen									
Hyperplasia, myeloid	Slight	0	0	0	0	0	0	0	1
	Moderate	0	0	2	0	0	0	0	0
Hyperplasia, erythroid	Minimal	0	0	0	1	0	0	1	0
	Slight	0	0	0	0	0	0	0	1
	Moderate	0	1	0	2	0	1	0	2
	Moderately Severe	0	0	1	0	0	0	0	0
Hyperplasia, megakaryocytic	Minimal	0	0	1	0	0	0	0	0
	Slight	0	1	0	0	0	0	0	2
	Moderate	0	0	0	3	0	1	1	1
	Moderately Severe	0	0	1	0	0	0	0	0
Kidney									
Basophilia, tubular	Minimal	0	0	0	1	0	0	0	0
	Slight	0	0	0	0	0	0	0	1
	Moderate	0	0	0	0	0	0	0	1
Thrombus, glomerular	Slight	0	0	0	1	0	0	0	0
	Moderate	0	0	0	0	0	0	0	1

Recovery Sacrifice (Tables 7 and 10)

Erythroid hypoplasia and/or hypocellular bone marrow in five animals [1F (moderate), 1M1F (moderate or moderately severe), and 1M1F (slight or moderate) in 1, 3, and 10 mcg/kg/dose groups, respectively]. The animals exhibiting evidence of erythroid hypoplasia were typically animals that had developed resistance to the drug by the end of treatment (i.e., they had few or no circulating reticulocytes at Week 13).

Moderate myelofibrosis of the femur marrow in one of two females receiving 10 mcg/kg/dose.

Table 10. Incidence and Severity of Drug-Related Microscopic Findings (Recovery Sacrifice)

Number Examined		Ro 50-3821/000 mcg/kg/dose							
		Males				Females			
		0	1	3	10	0	1	3	10
		2	2	2	2	2	2	2	2
Marrow, Femur									
	Hyperplasia, Erythroid							1	0
	Hypocellular, marrow	Moderate	0	0	0	0	0	0	1
		Slight	0	0	0	0	0	0	1
	Hypoplasia, Erythroid	Moderate	0	0	0	1	0	1	0
		Moderate	0	0	0	0	0	0	1
Marrow, Sternum									
	Hyperplasia, Erythroid							1	0
	Hypocellular, marrow	Moderate	0	0	0	0	0	0	1
		Slight	0	0	0	0	0	0	0
	Hypoplasia, Erythroid	Moderate	0	0	1	1	0	1	1
		Slight	0	0	0	0	0	0	1
		Moderate	0	0	1	1	0	1	0
	Moderately-severe	0	0	0	0	0	0	1	0
Kidney									
	Basophilia, tubular							0	1
	Sclerosis, segmental, glomerular	Minimal	0	0	0	0	0	0	0
		Slight	0	1	1	1	0	0	1
		Moderate	0	0	0	1	0	0	0
	Fibrosis, Interstitial	Minimal	0	2	1	0	0	0	0
		Slight	0	0	0	2	0	0	2
Liver									
	Pigment, Kupffer Cell	Minimal	0	0	0	0	0	1	0
		Slight	0	1	1	1	0	0	0
		Moderate	0	0	0	1	0	0	0
Spleen									
	Hyperplasia, Erythroid	Slight	0	1	0	0	0	0	1
		Moderate	0	1	0	0	0	0	0

In males, the hyperplastic effect on erythroid, myeloid, and megakaryocytic marrow elements was no longer as evident as it was earlier. However, the two males receiving 1 mcg/kg/dose had slight to moderate erythroid hyperplasia in the spleen.

In females, one animal in 3 mcg/kg/dose had moderate erythroid hyperplasia in the marrow (femur and sternum) and slight erythroid hyperplasia in the spleen.

Minimal to moderate segmental glomerular sclerosis in all males in 1, 3, or 10 mcg/kg/dose groups while in all two females in 10 mcg/kg/dose group, one in 3 mcg/kg/dose group and none in 1 mcg/kg/dose group.

Minimal or slight interstitial fibrosis (localized to areas near glomeruli and included thickening of the exterior surface of the Bowman's capsule) in all but one (in 3 mcg/kg/dose group) males in drug groups while in females in 10 mcg/kg/dose group only.

The glomerular sclerosis and interstitial fibrosis were judged to be secondary sequelae of glomerular thrombi and tubular basophilia observed at the terminal sacrifice. It is important to note that thrombi and basophilia were much less common and restricted to the highest dose group while sclerosis and interstitial fibrosis affected all dose groups. According to the study report, the likely reason for the inconsistency is that thrombosis and basophilia are transient events and are often difficult to observe when present at very low levels. Sclerosis and interstitial fibrosis reflect cumulative changes and, as such, indicate the sum of the more difficult to observe transient changes.

Reviewer's comments:

Bone marrow hypoplasia was noted as early as at 4-week sacrifice (1/6 and 2/6 in 10 and 30 mcg/kg/dose groups, respectively, Tables 7-10). The incidence increased at 13 wk sacrifice (1/6 and 3/6 in 3 and 10 mcg/kg/dose groups, respectively) and the hypoplasia remained at recovery sacrifice. Only erythroid hypoplasia was noted at 4 wk sacrifice while erythroid, myeloid, and megakaryocytic hypoplasia at 13 wk sacrifice. Erythroid hypoplasia was correlated with decreased absolute reticulocyte counts.

Toxicokinetics:

The TK parameters were shown at Table 11. Due to the limited number of animals (2/sex/group) used and large inter-subject variability, the toxicokinetic results of this study should be interpreted with caution.

Table 11. Summary of Toxicokinetic Parameters for Ro 50-3821

Day 1

Dose ($\mu\text{g}/\text{kg}/\text{dose}$)	Parameter	Units	Male	Female	Overall
1	AUC	$\text{ng}^*\text{Hours}/\text{mL}$	100	621	361
	AUC/Dose	$\text{ng}^*\text{Hours}/\text{mL}/\mu\text{g}/\text{kg}$	100	621	361
	C _{max}	ng/mL	2.63	13.6	8.11
	T _{max}	Hours	60.0	60.0	60.0
3	AUC	$\text{ng}^*\text{Hours}/\text{mL}$	2770	1590	2180
	AUC/Dose	$\text{ng}^*\text{Hours}/\text{mL}/\mu\text{g}/\text{kg}$	920	530	725
	C _{max}	ng/mL	31.2	23.4	27.3
	T _{max}	Hours	48.0	54.0	51.0
10	AUC	$\text{ng}^*\text{Hours}/\text{mL}$	9070	11400	10200
	AUC/Dose	$\text{ng}^*\text{Hours}/\text{mL}/\mu\text{g}/\text{kg}$	907	1140	1020
	C _{max}	ng/mL	90.3	106	98.0
	T _{max}	Hours	84.0	84.0	84.0
30	AUC	$\text{ng}^*\text{Hours}/\text{mL}$	30500	46300	38400
	AUC/Dose	$\text{ng}^*\text{Hours}/\text{mL}/\mu\text{g}/\text{kg}$	1020	1540	1280
	C _{max}	ng/mL	268	493	380
	T _{max}	Hours	66.0	54.0	60.0

Day 22

Dose ($\mu\text{g}/\text{kg}/\text{dose}$)	Parameter	Units	Male	Female	Overall
1	AUC	$\text{ng}^*\text{Hours}/\text{mL}$	135	657	396
	AUC/Dose	$\text{ng}^*\text{Hours}/\text{mL}/\mu\text{g}/\text{kg}$	135	657	396
	C _{max}	ng/mL	4.50	10.5	7.49
	T _{max}	Hours	78.0	96.0	87.0
3	AUC	$\text{ng}^*\text{Hours}/\text{mL}$	3660	2290	2970
	AUC/Dose	$\text{ng}^*\text{Hours}/\text{mL}/\mu\text{g}/\text{kg}$	1220	762	992
	C _{max}	ng/mL	43.6	26.2	34.9
	T _{max}	Hours	60.0	42.0	51.0
10	AUC	$\text{ng}^*\text{Hours}/\text{mL}$	32400	12200	22300
	AUC/Dose	$\text{ng}^*\text{Hours}/\text{mL}/\mu\text{g}/\text{kg}$	3240	1220	2230
	C _{max}	ng/mL	249	126	187
	T _{max}	Hours	84.0	24.5	54.3
30	AUC	$\text{ng}^*\text{Hours}/\text{mL}$	79600	43200	61400
	AUC/Dose	$\text{ng}^*\text{Hours}/\text{mL}/\mu\text{g}/\text{kg}$	2650	1440	2040
	C _{max}	ng/mL	674	449	561
	T _{max}	Hours	42.0	24.0	33.0

Day 85

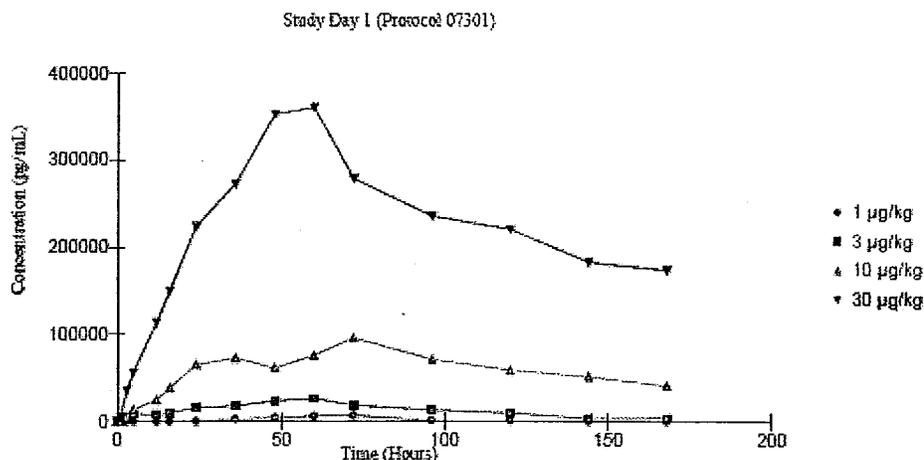
Dose ($\mu\text{g}/\text{kg}/\text{dose}$)	Parameter	Units	Male	Female	Overall
1	AUC	$\text{ng}^*\text{Hours}/\text{mL}$	221	366	293
	AUC/Dose	$\text{ng}^*\text{Hours}/\text{mL}/\mu\text{g}/\text{kg}$	221	366	293
	Cmax	ng/mL	2.39	5.03	3.71
	Tmax	Hours	66.0	78.0	72.0
3	AUC	$\text{ng}^*\text{Hours}/\text{mL}$	404	1330	869
	AUC/Dose	$\text{ng}^*\text{Hours}/\text{mL}/\mu\text{g}/\text{kg}$	135	445	290
	Cmax	ng/mL	4.06	14.7	9.36
	Tmax	Hours	48.0	48.0	48.0
10	AUC	$\text{ng}^*\text{Hours}/\text{mL}$	103	46.6 ^a	84.0
	AUC/Dose	$\text{ng}^*\text{Hours}/\text{mL}/\mu\text{g}/\text{kg}$	10.3	4.66 ^a	8.40
	Cmax	ng/mL	1.11	0.563 ^a	0.927
	Tmax	Hours	18.0	48 ^a	28.0

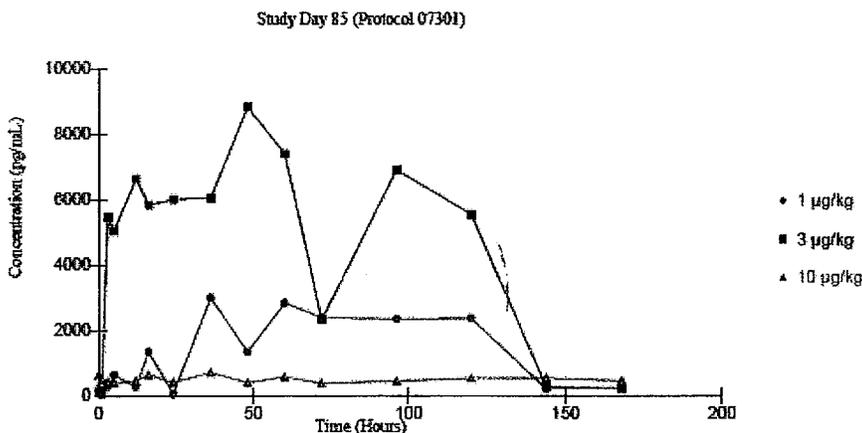
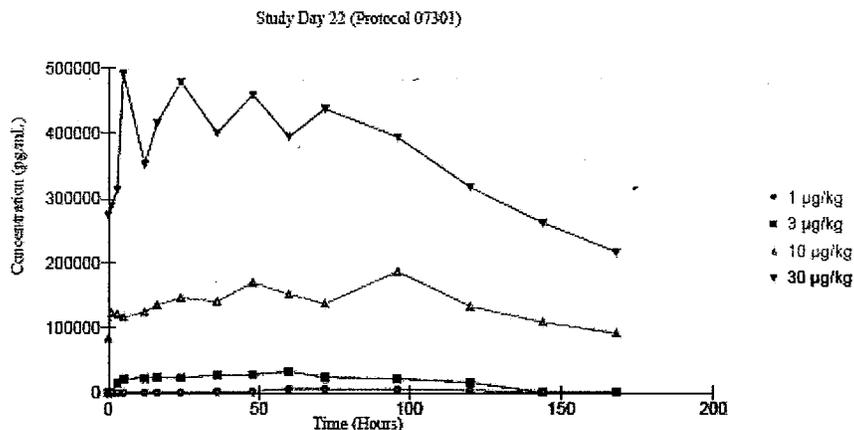
^a: n=1

Concentrations vs. Time Profiles

The concentration-time profiles were shown in Figure 1. The Tmax values varied from 28 to 87 hours indicating a prolonged absorption process of Ro 50-3821/000. On Day 22, the concentration versus time profile fluctuated for about 96 hours post dose due to the inter-subject variability. On Day 85, the profile in the highest dose group (10 mcg/kg/dose) was lower than that of the 1 and 3 mcg/kg/dose groups. For the dose of 3 mcg/kg/dose, only one dog (H05043) showed a reasonable drug level () at 72 hours post-dose on Day 85 resulting in an exceptional low mean value.

Figure 1. Mean Serum Concentration Versus Time Profile of Ro 50-3821





Dose vs. Exposure

The dose-proportional factor, calculated as a fold increase of exposure (AUC_{0-168hr} and C_{max}) at each dose level compared to the exposure at the lowest dose (1 mcg/kg/dose) were shown in the Table 12.

Table 12. Overall Dose Proportionality Factor in Dogs after 13-week SC Dosing

Dose (µg/kg/dose)	Theoretical Increases in Exposure (fold)	Observed Increases in Exposure (fold)	
		AUC _{0-168hr}	C _{max}
Day 1			
1	--	--	--
3	3.0	6.04	3.37
10	10	28.3	12.1
30	30	106	46.9
Day 22			
1	--	--	--
3	3.0	7.50	4.66
10	10	56.3	25.0
30	30	155	74.9
Day 85			
1	--	--	--
3	3.0	2.97	2.52
10	10	0.29	0.25

Greater than dose-proportional increases in AUC_{0-168h} and C_{max} were found at all doses on Days 1 and 22. Accumulation was noted on Day 22 at doses of 3, 10, and 30 mcg/kg/dose. On Day 85, approximately dose-proportional (3 mcg/kg/dose) or significantly less than dose-proportional (10 mcg/kg/dose) increases in AUC and C_{max} were observed and drug accumulation were no longer present. The exposures on Day 85 were decreased at all doses compared to exposures on Days 1 and 22. The cause of these phenomena is not clear. However, according to the study report, the bioanalytical assay used in this study detected free Ro 50-3821 only, it can be suggested that the presence of anti-EPO-Ab may have reduced free Ro 50-3821 by forming antibody complexes.

Gender Differences

Although females in the 1 mcg/kg/dose group showed higher exposure than males, there was no clear gender effect.

Antibody Analysis:

Anti-EPO Ab was detected in some drug-treated dogs after 4 weeks of treatment (Table 13). At Week 4, 1/20, 11/20, 10/20, and 9/10 dogs were positive for anti-EPO Ab in the 1, 3, 10, and 30 mcg/kg/dose groups, respectively. At Week 13, 4/14, 9/14, and 6/13 dogs were positive for anti-EPO Ab in the 1, 3, and 10 mcg/kg/dose groups, respectively. Time-dependent increase was noted in 1 mcg/kg/dose group (1/20 at 4wk vs. 4/14 at 13 wk) while this time-dependency was no longer obvious with increasing doses. Trend of dose-dependent increase was also noted although no significant difference was noted between 3 and 10 mcg/kg/dose groups. In general, the dogs that were Ab positive at Week 4 remained positive at Week 13 except for 1 dog (H05006) at 30 mcg/kg/dose group. No analysis was conducted for the samples from recovery dogs.

Table 13. Summary of Incidence of Anti-EPO Ab*

Dose (mcg/kg)	4 wk Sacrifice				13 wk Sacrifice			
	Male		Female		Male		Female	
	+	±	+	±	+	±	+	±
0	0/8	1/8	0/8	0/8	0/5	0/5	0/5	0/5
1	1/10	2/10	0/10	1/10	2/7	0/7	2/7	0/7
3	7/10	0/10	4/10	1/10	5/7	0/7	4/7	1/7
10	6/10	0/10	4/10	0/10	4/7	0/7	2/6**	0/6
30	4/5	0/5	5/5	0/5	1/2	0/2	1/2	1/2

* + Positive, ± Ambiguous, No. of positive or ambiguous results/No. of samples assayed.

**One animal was sacrificed prior to scheduled sampling.

Other:

Table 14. Histopathology inventory

Study	07301	07310	07302	07304	07437
Species	Dog	Dog	Rat	Rat	Rat
Adrenals	X*	X*	X*	X*	X*
Aorta	X	X	X	X	X
Bone Marrow smear	X	X	X ^a	X ^a	X ^a
Bone (femur)	X	X	X ^{ab}	X ^{ab}	X ^{ab}
Brain	X*	X*	X ^{*a}	X ^{*a}	X ^{*a}
Cecum	X	X	X	X	X
Cervix	X*	X*	X	X	X
Colon	X	X	X	X	X
Duodenum	X	X	X	X	X
Epididymis	X*	X*	X	X	X
Esophagus	X	X	X	X	X
Eye	X	X	X	X	X
Fallopian tube	N	N	N	N	N
Gall bladder	X*	X*	N	N	N
Gross lesions	X	X	X	X	X
Harderian gland	N	N	N	N	N
Heart	X*	X*	X ^{*a}	X ^{*a}	X ^{*a}
Ileum	X	X	X	X	X
Injection site	X	X	X ^a	X ^a	X ^a
Jejunum	X	X	X	X	X
Kidneys	X*	X*	X ^{*a}	X ^{*a}	X ^{*a}
Lachrymal gland	X	X	X	X	X
Larynx	N	N	N	N	N
Liver	X*	X*	X ^{*a}	X ^{*a}	X ^{*a}
Lungs	X*	X*	X ^a	X ^a	X ^a
Lymph nodes, cervical	N	N	N	N	N
Lymph nodes mandibular	X	X	X ^a	X ^a	X ^a
Lymph nodes, mesenteric	X	X	X ^a	X ^a	X ^a
Mammary Gland	X	X	X	X	X
Nasal cavity	N	N	N	N	N
Optic nerves	N	N	N	N	N
Ovaries	X*	X*	X*	X*	X*
Pancreas	X	X	X	X	X
Parathyroid	X*	X*	X	X	X

Peripheral nerve	N	N	N	N	N
Pharynx	N	N	N	N	N
Pituitary	X*	X*	X	X	X
Prostate	X*	X*	X	X	X
Rectum	X	X	X	X	X
Salivary gland	X	X	X	X	X
Sciatic nerve	X	X	X	X	X
Seminal vesicles	N	N	X	X	X
Skeletal muscle	X	X	X	X	X
Skin	X	X	X	X	X
Spinal cord	X	X	X	X	X
Spleen	X*	X*	X ^{*a}	X ^{*a}	X ^{*a}
Sternum	X	X	X ^a	X ^a	X ^a
Stomach	X	X	X ^a	X ^a	X ^a
Testes	X*	X*	X ^{*a}	X ^{*a}	X ^{*a}
Thymus	X*	X*	X ^a	X ^a	X ^a
Thyroid	X*	X*	X	X	X
Tongue	X	X	X	X	X
Trachea	X	X	X	X	X
Urinary bladder	X	X	X	X	X
Uterus	X*	X*	X	X	X
Vagina	X	X	X	X	X
Zymbal gland	N	N	N	N	N

X, histopathology performed

N, histopathology not performed

*, organ weight obtained

^a, target organ

^b, Bone (proximal femur and sternum with marrow)

Conclusion:

According to the study report, the drug-related findings were considered to be exaggerated pharmacological effects or secondary to exaggerated pharmacological effects. NOAEL could not be established due to drug effects at all dose levels.

Reviewer comments:

The most prominent findings were dose- and time-dependent effects on erythrocyte parameters in a manner consistent with markedly accelerated erythropoiesis (a known pharmacological effect of the drug), functional iron deficiency, and the development of resistance to the pharmacological effect of the drug, probably as a result of neutralizing antibody production. After approximately 4 to 6 weeks of treatment, several animals (generally one or two in each group treated with Ro 50-3821/000) stopped producing circulating reticulocytes. Red blood cell counts, hemoglobin concentrations, and hematocrits for these animals dropped slowly until many had values that were below those of the control animals suggesting the antibody may also have affected native EPO. Because the development of resistance was relatively inconsistent, the erythrocyte data were quite variable for treated animals. The group given 3 mcg/kg/dose had the most animals (three males

and two females) that appeared to develop resistance antibody. None of the animals selected following recovery period were anemic.

Drug-related microscopic observations at the interim sacrifice were restricted to these tissues (bone marrow and spleen) in which hematopoiesis normally occurs, with the exception of one animal with extramedullary hematopoiesis in the adrenal cortex. In females given 3, 10, or 30 mcg/kg/dose and males given 1, 3, 10, or 30 mcg/kg/dose, there was increased hematopoiesis; however, all animals within a group were not affected equally. In addition, some animals had increased numbers of all hematopoietic elements while others had only erythroid or myeloid responses. Erythroid hypoplasia of the bone marrow and decreased absolute reticulocyte counts were noted in one male receiving 30 mcg/kg/dose and one female each receiving 10 or 30 mcg/kg/dose.

At the terminal sacrifice, hyperplasia of bone marrow elements was noted in some animals in drug groups while hypoplasia in other animals, often in the same dose groups. Whether or not the neutralizing antibody was produced might be the reason for the observed differences. The animals with erythroid hypoplasia had no circulating reticulocytes. In the kidneys of one male and two females in 10 mcg/kg/dose group, there was increased tubular basophilia and thrombosis of glomerular capillaries. This was regarded as a secondary effect of the drug resulting from hemodynamic effects of a high hematocrit. These three animals were polycythemic at Week 13.

At the recovery sacrifice, all males receiving the drug (1, 3, or 10 mcg/kg/dose) had minimal to moderate segmental glomerular sclerosis; all but one of them (a male given 3 mcg/kg/dose) also had minimal or slight interstitial fibrosis. Similar lesions occurred to females in 3 and 10 mcg/kg/dose groups only. The effects of the drug on the kidneys were not reversible. The glomerular thrombi and tubular basophilia observed at the terminal sacrifice appeared to mature into glomerular sclerosis and interstitial fibrosis.

NOAEL could not be established due to drug effects at all dose levels.

Deficiencies:

The following deficiencies were identified which will have negative impact on quality and interpretation of data.

Ab analysis:

1. Only qualitative analysis was conducted, which made the correlation of erythrocyte parameters with anti-EPO Ab production rather difficult if not impossible.
2. The earliest time point was on Day 28, which failed to provide the early kinetics of Ab production.
3. No analysis for recovery animals, which failed to provide the reversibility information regarding Ab production.
4. Non GLP.

TK

Two dogs per time point and large inter-subject variability resulted in unreliable TK data.

B.

Study title: Ro 50-3821/000: 13-Week Intravenous Injection Toxicity Study with a 4-Week Interim Sacrifice and an 8-Week Recovery Phase in Dogs

Key study findings:Clinical signs:

- Color aberrations (red or pale) in skin and gum during late treatment (from Week 12) and recovery periods.

Ophthalmoscopy:

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

Hematology:

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

Organ weights:

- Increased absolute and relative spleen weights for animals given 30 mcg/kg/dose at 4 week sacrifice and for animals given 1, 3, or 10 mcg/kg/dose at 13 week sacrifice compared to the controls and remained increased, but to a lesser degree, at recovery sacrifice compared to the terminal sacrifice.

Histopathology:*Four-Week Interim Sacrifice*

- Erythroid and megakaryocytic hyperplasia of the bone marrow in majority of animals in all drug groups (5/6, 6/6, 5/6, and 4/6 at 1, 3, 10, and 30 mcg/kg/dose, respectively); Dose-related severity in general.
- Erythroid hypoplasia of the bone marrow for one female given 10 mcg/kg/dose and one male and one female given 30 mcg/kg/dose; no circulating reticulocytes for these two females.
- Moderate myelofibrosis of the femur for two males and one female at 30 mcg/kg/dose group.
- Increased erythroid and megakaryocytic hematopoiesis of the spleen for three animals (3/6) given 30 mcg/kg/dose.

Thirteen-Week Terminal Sacrifice

- Erythroid and megakaryocytic hyperplasia of the bone marrow for animals given 1 (3/6) or 3 (4/6) mcg/kg/dose.
- Erythroid hypoplasia of the bone marrow for animals given 1 (2/6), 3 (2/6), or 10 (6/6) mcg/kg/dose; No circulating reticulocytes in these animals with erythroid hypoplasia; The erythroid hypoplasia of the femur and sternum correlated with the pale marrow cavities observed grossly.
- Erythroid and megakaryocytic hematopoiesis of the spleen for animals given 10 mcg/kg/dose (4/6) and one female given 1 mcg/kg/dose.

Recovery Sacrifice

- Erythroid hyperplasia of the bone marrow for animals given 1 (2/4), 3 (2/4), or 10 (3/4) mcg/kg/dose, accompanied by myeloid hyperplasia in 3 (1/4) or 10 (2/4) mcg/kg/dose groups and megakaryocytic hyperplasia in one female given 10 mcg/kg/dose.
- Erythroid hypoplasia of the bone marrow for animals given 3 (2/4) or 10 (1/4) mcg/kg/dose.
- Hematopoiesis in spleen of two animals each in 1, 3, or 10 mcg/kg/dose groups, accompanied by increased pigment.
- Hepatic hematopoiesis in two animals given 10 mcg/kg/dose and Kupffer cell pigmentation for animals given 3 (1/4) or 10 (2/4) mcg/kg/dose.

Antibody Analysis:

- Dose- and time-dependent increase in incidence of Anti-EPO-Ab (positive: 2/20, 9/20, 14/20, and 8/10 in the 1, 3, 10, and 30 mcg/kg/dose groups, respectively, at Week 4 vs. 9/14, 8/14, and 12/14 in the 1, 3, and 10 mcg/kg/dose groups, respectively, at Week 13). The dogs positive at Week 4 remained positive at Week 13.

NOAEL could not be established.

Study no: HLR Study No. 07310, _____ No. 6131-303, Report No. 1002693

Volume #, and page #: Module 4 Volume 1.8, page 1-1194

Conducting laboratory and location: _____

Bioanalytical assays and Toxicokinetic calculations in the Department of Non-Clinical Drug Safety, Hoffmann-La Roche Inc., 340 Kingsland Street, Nutley, NJ. Anti-EPO-Ab assays by _____

Date of study initiation: February 1, 2000

Route, formulation, volume, and infusion rate: bolus intravenous injection into a cephalic vein (dose site rotated each week), dose volume: 0.1 mL/kg.

Satellite groups used for toxicokinetics or recovery: 2/sex/group for TK or recovery (8-week).

Age: approximately 6 to 9 months

Weight: 5.7 to 11.8 kg

Sampling times: See other related sections.

Unique study design or methodology (if any): N/A

Observations and times:

Mortality: Twice daily

Clinical signs: Twice daily for morbidity, once daily cageside observations for abnormal findings, once weekly and on the day of necropsy detailed observations

Body weights: Twice before initiation of treatment, on the first day of treatment, and weekly thereafter.

Food consumption: Weekly beginning 1 week prior to initiation of treatment and weekly during treatment and recovery phases.

Ophthalmoscopy: Pre-dose, and weeks 4, 13, and 21 (recovery).

ECG: Pre-dose, and during weeks 4, 13, and 21 (recovery). Electrocardiographic measurements including heart rate, P, PR, QT, and QTc (the Van de Water formula).

Hematology: Twice prior to initiation of treatment, weekly during the study, fast overnight.

Clinical chemistry: Twice prior to dose and at near the end of Weeks 4, 13, and 21, fast overnight.

Urinalysis: Twice prior to treatment and at near the end of Weeks 4, 13, and 21 (approximately 16 hours during the overnight fasting).

Gross pathology: 3/sex/group on Days 30 (interim sacrifice) or 93 (terminal sacrifice), all remaining dogs on Day 150 (recovery sacrifice).

Organ weights: 3/sex/group on Days 30 (interim sacrifice) or 93 (terminal sacrifice), all remaining dogs on Day 150 (recovery sacrifice). Also see histopath table (Table 14).

Histopathology: 3/sex/group on Days 30 (interim sacrifice) or 93 (terminal sacrifice), all remaining dogs on Day 150 (recovery sacrifice). Target organs and tissues as outlined in the histopath table (Table 14), were examined from all interim-, terminal-, and recovery-sacrificed animals.

Adequate Battery: yes (x), no ()

Peer review: yes (x), no ()

Toxicokinetic: On days 1, 22, and 85 at 0 (pre-dose), approximately 5 and 15 minutes, and 1, 3, 5, 12, 24, 48, 72, 96, 120, 144, and 168 hours. More details were described in 13 wk SC dog study above.

Antibodies: Serum samples for anti-EPO Ab determinations were collected from all toxicity and toxicokinetic dogs pre-dose and after 4, 8, and 13 weeks of treatment, and after 4 and 8 weeks of recovery. However, antibody assays were performed for the samples collected after 4 and 13 weeks only.

A ELISA analyze anti-EPO Ab. More details were described in 13 wk SC dog study above.

Results

Mortality:

All animals survived to their scheduled sacrifice.

Clinical signs:

No drug-related clinical signs over the first 4 weeks of the study.

Red or pale skin and gums and red conjunctivae were observed over the remaining weeks of the treatment period. Red skin and gums and red conjunctivae were generally observed in animals given 1, 3, or 10 mcg/kg/dose during Weeks 12 and 13. The highest incidence of these observations was noted in animals given 1 or 3 mcg/kg/dose and remained prevalent through the recovery phase. Pale skin and gums were first observed in females given 10 mcg/kg/dose during Weeks 13 and 14 and then noted in males given 10 mcg/kg/dose during Weeks 17 through 21.

Generally, the clinical observations of red skin, gums and conjunctivae were associated with high hematocrits (polycythemia) while pale skin and gums with low hematocrits (anemia).

Body weights:

Dose- and time-related trend in reduction of body weight gains: the mean body weight gains over the first 4 weeks of treatment were 0.5 and 0.6 kg for males and females in control group, respectively, and 0.2 and 0.0 kg for males and females in 30 mcg/kg/dose group, respectively.

Food consumption:

Dose- and time-related trend in reduction of food consumption, but within the normal range, according to the study report.

Ophthalmoscopy:

Dose- and time-related increase in incidence of hyperemia and dilated retinal blood vessels was noted. Although no drug-related ophthalmic lesions were noted at the Week 4 examination, hyperemia and dilated retinal blood vessels were noted in animals given 1, 3, or 10 mcg/kg/dose at Week 13. The incidences of these findings were highest in animals given 1 or 3 mcg/kg. Generally, hyperemia and dilated retinal blood vessels correlated with high hematocrits. At Week 21, all animals appeared normal.

ECG and BP: No drug-related changes in ECG parameters or blood pressure.

Hematology:

Erythrocyte parameters

As expected, the most prominent findings affected were erythrocyte parameters and were consistent with markedly accelerated erythropoiesis, functional iron deficiency, and the development of resistance to the pharmacological effect of drug, probably as a result of neutralizing antibody production.

During the first several weeks of the study, the erythrocyte findings were higher red blood cell count, hemoglobin, hematocrit, and absolute reticulocyte count for males and females at all dose levels; lower mean corpuscular volume and mean corpuscular hemoglobin for males and females at all dose levels; and lower mean corpuscular hemoglobin concentration for males and females given 3, 10, or 30 mcg/kg/dose. The effects on red blood cell count, hemoglobin, hematocrit, and absolute reticulocyte count were observed as early as Week 1 for animals at all dose levels except for animals at 1 mcg/kg/dose group. The effects on red blood cell size and hemoglobin content took longer to be manifested. By Weeks 4 and 5, the erythrocyte effects did not exhibit a clear relationship to dose,

After approximately 4 weeks of treatment, several animals, particularly these given 10 or 30 mcg/kg/dose, stopped producing circulating reticulocytes. Red blood cell counts, hemoglobin concentrations, and hematocrits for these animals dropped slowly until many had values that were below these of the control animals suggesting the antibody may also have affected native EPO. The groups given 1 or 3 mcg/kg/dose were much less likely to develop resistance. Following cessation of treatment, anemic animals eventually began producing circulating reticulocytes in an appropriate manner, and polycythemic animals that had not developed resistance stopped producing circulating reticulocytes, also in an appropriate manner. At Week 21, the most notably anemic animal was a female that had been given 10 mcg/kg/dose; however, its hematocrit at Week 21 (27.9%) was considerably higher than it had been at Week 16 (14.9%).

Platelet

During the first few weeks of the study, increased platelet count, primarily for animals given 3, 10, or 30 mcg/kg/dose, was noted. Markedly high platelet counts were observed for several animals when their mean corpuscular volumes reached a level at which the small red blood cells interfered with the ability of the analyzer to count platelets. Therefore, this markedly high platelet counts could be artifactual, according to the study report.

Clinical chemistry:

Some changes such as changes in glucose and activated partial thromboplastin time were noted in drug groups. According to the study report, most, if not all, of these effects were likely artifactual changes associated with the high red blood cell mass and did not reflect direct toxic effects of the drug,

Urinalysis: No drug-related changes.

Gross pathology:

Four-Week Interim Sacrifice

No drug-related macroscopic findings.

Thirteen-Week Terminal Sacrifice

Diffusely light bone marrow of the femur and pale connective tissue in one female given 10 mcg/kg/dose, and diffusely light bone marrow of the sternum in three females given 10 mcg/kg/dose.

Focal to diffuse reddening of the gastrointestinal tract in one female given 1 mcg/kg/dose and one male and two females given 3 mcg/kg/dose.

Recovery Sacrifice

No drug-related macroscopic findings.

Organ weights:

Increased absolute and relative spleen weights for animals given 30 mcg/kg/dose at 4 week sacrifice and for animals given 1, 3, or 10 mcg/kg/dose at 13 week sacrifice compared to the controls and remained increased, but to a lesser degree, at recovery sacrifice compared to the terminal sacrifice.

No other drug-related changes for absolute or relative organ weights.

Histopathology: The major histopathology findings were summarized at Tables 16-19 and described below.

Table 16. Summary of Major Histopathologic Findings*

Dose (mcg/kg)	4-wk Sacrifice				13-wk Sacrifice			Recovery Sacrifice		
	1	3	10	30	1	3	10	1	3	10
N	3M3F	3M3F	3M3F	3M3F	3M3F	3M3F	3M3F	2M2F	2M2F	2M2F
Hyperplasia										
Bone marrow	2M3F	3M3F	3M2F	2M2F	1M2F	1M3F	0	2F	1M1F	2M1F
Erythroid	2M3F	3M3F	3M2F	2M2F	1M2F	1M3F		2F	1M1F	2M1F
Myeloid	1M	1M	1M	0	0	1F		0	1M	1M1F
Megakaryocytic	1F	2M2F	3M	2M2F	1M2F	3F		0	0	1F
Spleen	0	0	0	1M2F	1F	0	1M3F	2F	1M1F	1M1F
Liver	0	0	0	0	0	0	0	0	0	1M1F
Hypoplasia										
Bone marrow	0	0	1F	1M1F	1M1F	2M	3M3F	0	1M1F	1F
Erythroid			1F	1M1F	1M1F	2M	3M3F		1M1F	1F
Myeloid			0	0	0	0	0		0	0
Megakaryocytic			0	0	0	0	0		0	0
Myelofibrosis	0	0	0	2M1F	0	0	0	0	0	0

*Prepared by the reviewer based on the submission.

Four-Week Interim Sacrifice: Tables 16 and 17

Drug-related microscopic findings were limited to the bone marrow and spleen.

Erythroid and megakaryocytic hyperplasia of the bone marrow in animals at all drug groups (5/6, 6/6, 5/6, and 4/6 at 1, 3, 10, and 30 mcg/kg/dose, respectively); Dose-related severity, in general.

Erythroid hypoplasia of the bone marrow for one female given 10 mcg/kg/dose and one male and one female given 30 mcg/kg/dose; no circulating reticulocytes for the two females.

Myelofibrosis of the bone marrow for two males and one female at 30 mcg/kg/dose group.

Increased erythroid and megakaryocytic hematopoiesis of the spleen for three animals (3/6) given 30 mcg/kg/dose.

Table 16. Interim Sacrifice: Incidence and Severity of Drug-Related Microscopic Findings

		Ro 50-3821/000 mcg/kg/dose									
		Males					Females				
		0	1	3	10	30	0	1	3	10	30
Number examined		3	3	3	3	3	3	3	3	3	3
Marrow, Sternum											
Hyperplasia, Erythroid											
	Minimal	0	1	0	0	0	0	1	0	0	0
	Slight	0	1	1	2	1	0	2	2	2	2
	Moderate	0	0	1	0	1	0	0	1	0	0
Hyperplasia, Myeloid											
	Minimal	0	1	0	0	0	0	0	0	0	0
	Slight	0	0	1	1	0	0	0	0	0	0
Hyperplasia, Megakaryocytic											
	Minimal	0	0	1	1	1	0	0	1	0	0
	Slight	0	0	0	0	0	0	0	0	0	1
Hypoplasia, Erythroid											
	Minimal	0	0	0	0	0	0	0	0	0	1
	Slight	0	0	0	0	0	0	0	0	1	0
	Moderate	0	0	0	0	1	0	0	0	0	0
Myelofibrosis											
	Minimal	0	0	0	0	1	0	0	0	0	1
Marrow, Femur											
Hyperplasia, Erythroid											
	Minimal	0	1	0	1	0	0	3	0	1	0
	Slight	0	1	2	0	0	0	0	1	1	1
	Moderate	0	0	1	2	1	0	0	0	0	1
	Moderately-severe	0	0	0	0	1	0	0	2	0	0
Hyperplasia, Megakaryocytic											
	Minimal	0	0	2	3	2	0	1	1	0	2
	Slight	0	0	0	0	0	0	0	1	0	0
Hypoplasia, Erythroid											
	Minimal	0	0	0	0	0	0	0	0	0	1
	Slight	0	0	0	0	0	0	0	0	1	0
	Moderate	0	0	0	0	1	0	0	0	0	0
Myelofibrosis											
	Moderate	0	0	0	0	2	0	0	0	0	1
Spleen											
Hematopoiesis, Erythroid											
	Minimal	0	0	0	0	0	0	0	0	0	2
	Slight	0	0	0	0	1	0	0	0	0	0
Hematopoiesis, Increased, Extramedullary, Megakaryocytic											
	Minimal	0	0	0	0	1	0	0	0	0	1

Thirteen-Week Terminal Sacrifice; Tables 16 and 18.

Drug-related microscopic findings were limited to the bone marrow, spleen, and gastrointestinal tracts.

Erythroid and megakaryocytic hyperplasia of the bone marrow for animals given 1 (3/6) or 3 (4/6) mcg/kg/dose.

Erythroid hypoplasia of the bone marrow for animals given 1 (2/6), 3 (2/6), or 10 (6/6) mcg/kg/dose; No circulating reticulocytes in these animals; Correlated with the pale marrow cavities observed grossly.

Erythroid and megakaryocytic hematopoiesis, and increases in pigment of the spleen for animals mainly given 10 mcg/kg/dose (4/6) and one female given 1 mcg/kg/dose.

Congestion (only observed in animals that were polycythemic) and edema of the gastrointestinal tracts, correlating with the reddening of the gastrointestinal tracts.

Table 18. Terminal Sacrifice: Incidence and Severity of Drug-Related Microscopic Findings

		Ro 50-3821/000 mcg/kg/dose							
		Males				Females			
		0	1	3	10	0	1	3	10
Number examined		3	3	3	3	3	3	3	3
Marrow, Femur									
Hyperplasia, Erythroid									
	Slight	0	0	0	0	0	1	0	0
	Moderate	0	0	1	0	0	1	0	0
	Moderately severe	0	1	0	0	0	0	3	0
Hyperplasia, Megakaryocytic									
	Minimal	0	1	0	0	0	2	3	0
Hypoplasia, Erythroid									
	Minimal	0	0	0	1	0	0	0	1
	Slight	0	1	2	2	0	1	0	2
Marrow, Sternum									
Hyperplasia, Erythroid									
	Minimal	0	1	0	0	0	0	0	0
	Slight	0	0	0	0	0	2	0	0
	Moderate	0	0	1	0	0	0	2	0
Hyperplasia, Myeloid									
	Moderate	0	0	0	0	0	0	1	0
Hypoplasia, Erythroid									
	Minimal	0	0	1	1	0	0	0	0
	Slight	0	1	1	0	0	0	0	0
	Moderate	0	0	0	1	0	0	0	0
	Moderately-severe	0	0	0	1	0	1	0	3
Spleen									
Hematopoiesis, Erythroid									
	Minimal	0	0	0	0	0	0	0	2
Hematopoiesis, Increased, Extramedullary, Megakaryocytic									
	Minimal	0	0	0	1	0	1	0	1
	Slight	0	0	0	0	0	0	0	2
Pigment, Increased									
	Minimal	0	0	0	1	0	1	0	1
	Slight	0	0	0	0	0	0	0	1

Recovery Sacrifice: Tables 16 and 19

Drug-related microscopic findings were limited to the bone marrow, spleen, and liver.

Erythroid hyperplasia of the bone marrow for animals given 1 (2/4), 3 (2/4), or 10 (3/4) mcg/kg/dose, accompanied by myeloid hyperplasia at 3 (1/4) or 10 (2/4) mcg/kg/dose groups and megakaryocytic hyperplasia in one female given 10 mcg/kg/dose.

Table 19. Recovery Sacrifice: Incidence and Severity of Drug-Related Microscopic Findings

		Ro 50-3821/000 mcg/kg/dose									
		Males					Females				
		0	1	3	10	30	0	1	3	10	30
Number examined		2	2	2	2	0	2	2	2	2	0
Marrow, Sternum											
Hyperplasia, Erythroid											
	Minimal	0	0	0	0	0	0	0	0	1	0
	Slight	0	0	0	0	0	0	1	0	0	0
	Moderate	0	0	1	1	0	0	1	1	0	0
Hyperplasia, Myeloid											
	Minimal	0	0	0	0	0	0	0	0	1	0
	Moderate	0	0	1	1	0	0	0	0	0	0
Hyperplasia, Megakaryocytic											
	Minimal	0	0	0	0	0	0	0	0	1	0
Hypoplasia, Erythroid											
	Minimal	0	0	1	0	0	0	0	0	1	0
	Slight	0	0	0	0	0	0	0	1	0	0
Marrow, Femur											
Hyperplasia, Erythroid											
	Minimal	0	0	0	1	0	0	0	1	0	0
	Slight	0	0	1	0	0	0	2	0	1	0
	Moderate	0	0	0	1	0	0	0	0	0	0
Hyperplasia, Myeloid											
	Minimal	0	0	0	1	0	0	0	0	1	0
Hypoplasia, Erythroid											
	Minimal	0	0	0	0	0	0	0	0	1	0
	Moderate	0	0	1	0	0	0	0	1	0	0
Spleen											
Hematopoiesis, Erythroid											
	Minimal	0	0	0	1	0	0	2	1	1	0
	Slight	0	0	1	0	0	0	0	0	0	0
Hematopoiesis, Increased, Extramedullary, Megakaryocytic											
	Minimal	0	0	1	1	0	0	0	0	1	0
Pigment, Increased											
	Minimal	0	0	1	1	0	0	2	2	1	0
	Slight	0	1	0	0	0	0	0	0	0	0
Liver											
Hematopoiesis, Extramedullary											
	Minimal	0	0	0	1	0	0	0	0	1	0
Pigment, Kupffer Cell											
	Minimal	0	0	0	0	0	0	0	0	1	0
	Slight	0	0	1	1	0	0	0	0	0	0

Erythroid hypoplasia of the bone marrow for animals given 3 (2/4) or 10 (1/4) mcg/kg/dose.

Erythroid hematopoiesis in spleen for animals given 1 or 3 mcg/kg/dose; megakaryocytic hematopoiesis for animals given 3 (1/4) or 10 (2/4) mcg/kg/dose; and increases in pigment for animals given 1, 3, or 10 mcg/kg/dose.

Hepatic hematopoiesis for two animals given 10 mcg/kg/dose and Kupffer cell pigmentation for animals given 3 (1/4) or 10 (2/4) mcg/kg/dose.

Reviewer's comments

In general, dose- and time-related increased incidence in bone marrow hypoplasia was observed (Tables 16-19). The bone marrow hypoplasia remained at recovery sacrifice.

Toxicokinetics:

In general, greater than dose-proportional increases in AUC and Cmax were found on all study days at all doses. However, on Day 85, the absolute AUC values at all doses were generally lower than that on Day 22 and/or Day 1. The cause of reduced exposures on Day 85 is not clear. According to the study report, the bioanalytical assay used in this study measured free Ro 50-3821 only, therefore, the presence of anti-EPO-Ab seen in toxicokinetic animals at week 4 and week 13 may have reduced free Ro 50-3821 by forming antibody complexes. Accumulation was observed mainly on Day 22 at two lower doses (1 and 3 mcg/kg/dose). There were no gender differences in exposure.

The TK parameters were shown at Table 20. Due to the limited number of animals (2/sex/group) used and large inter-subject variability, the toxicokinetic results of this study should be interpreted with caution.

Table 20. Summary of Toxicokinetic Parameters for Ro 50-3821

Day 1					
Dose ($\mu\text{g}/\text{kg}/\text{dose}$)	Parameter	Units	Male	Female	Overall
1	AUC	ng*Hours/mL	824	772	798
	AUC/Dose	ng*Hours/mL/ $\mu\text{g}/\text{kg}$	824	772	798
	Cmax	ng/mL	16.1	14.1	15.1
3	AUC	ng*Hours/mL	2630	3630	3130
	AUC/Dose	ng*Hours/mL/ $\mu\text{g}/\text{kg}$	877	1210	1040
	Cmax	ng/mL	48.5	60.7	54.6
10	AUC	ng*Hours/mL	16400	18200	17300
	AUC/Dose	ng*Hours/mL/ $\mu\text{g}/\text{kg}$	1640	1820	1730
	Cmax	ng/mL	247	297	272
30	AUC	ng*Hours/mL	50800	53500	52100
	AUC/Dose	ng*Hours/mL/ $\mu\text{g}/\text{kg}$	1700	1790	1740
	Cmax	ng/mL	575	840	707

Day 22

Dose ($\mu\text{g}/\text{kg}/\text{dose}$)	Parameter	Units	Male	Female	Overall
1	AUC	ng*Hours/mL	1300	1750	1520
	AUC/Dose	ng*Hours/mL/ $\mu\text{g}/\text{kg}$	1300	1750	1520
	C _{max}	ng/mL	25.4	31.4	28.4
3	AUC	ng*Hours/mL	4950	8610	6780
	AUC/Dose	ng*Hours/mL/ $\mu\text{g}/\text{kg}$	1650	2870	2260
	C _{max}	ng/mL	75.9	117	96.4
10	AUC	ng*Hours/mL	23100	22700	22900
	AUC/Dose	ng*Hours/mL/ $\mu\text{g}/\text{kg}$	2310	2270	2290
	C _{max}	ng/mL	284	354	319
30	AUC	ng*Hours/mL	51200	13300	32200
	AUC/Dose	ng*Hours/mL/ $\mu\text{g}/\text{kg}$	1710	443	1070
	C _{max}	ng/mL	1110	439	775

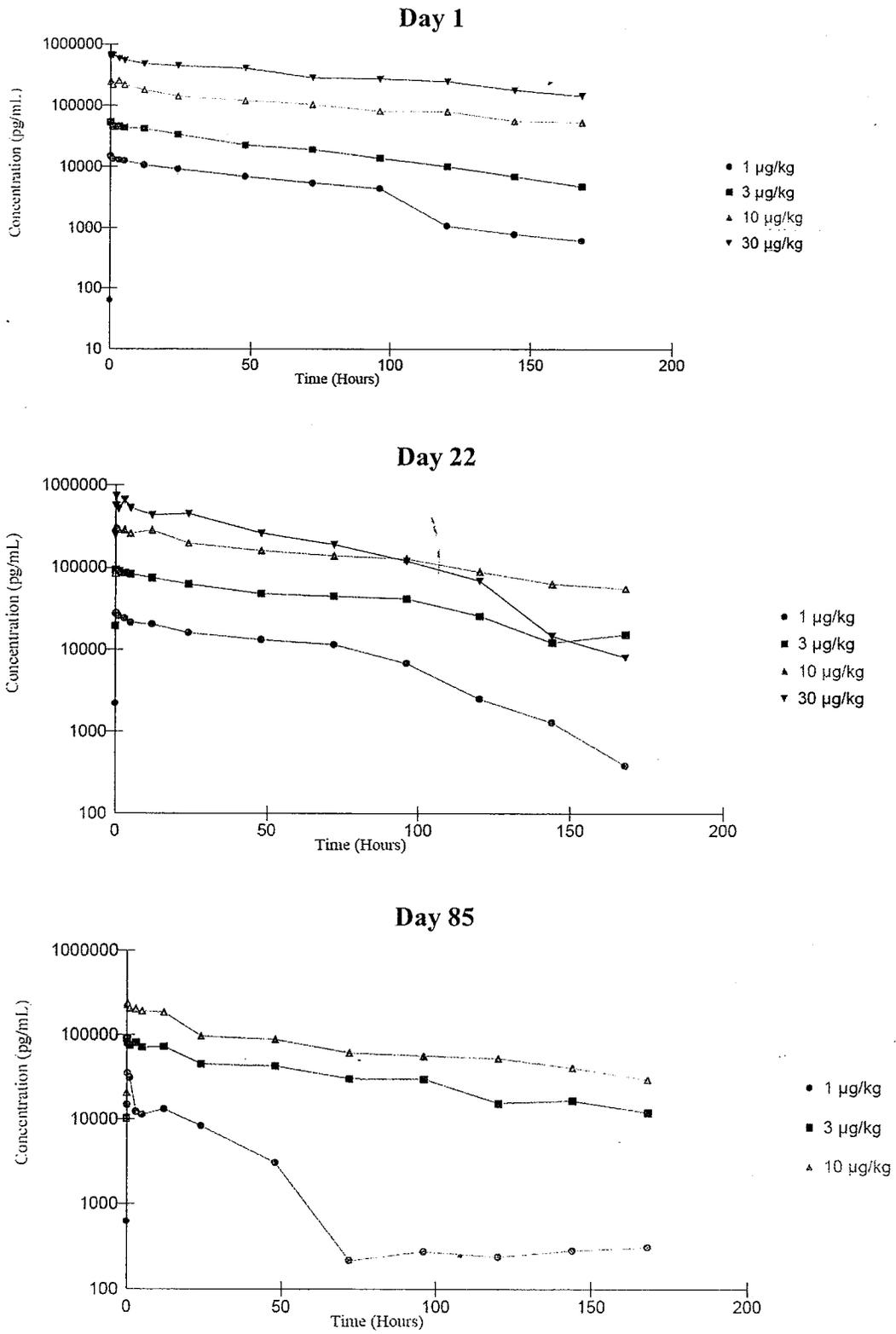
Day 85

Dose ($\mu\text{g}/\text{kg}/\text{dose}$)	Parameter	Units	Male	Female	Overall
1	AUC	ng*Hours/mL	122	921	521
	AUC/Dose	ng*Hours/mL/ $\mu\text{g}/\text{kg}$	122	921	521
	C _{max}	ng/mL	8.24	63.8	36.0
3	AUC	ng*Hours/mL	4040	7060	5550
	AUC/Dose	ng*Hours/mL/ $\mu\text{g}/\text{kg}$	1350	2360	1850
	C _{max}	ng/mL	78.9	111	95.0
10	AUC	ng*Hours/mL	15800	11800	13800
	AUC/Dose	ng*Hours/mL/ $\mu\text{g}/\text{kg}$	1580	1180	1380
	C _{max}	ng/mL	262	216	239

Concentrations vs. Time Profiles

The concentration versus time profiles were shown in Figure 2.

Figure 2. Mean Serum Concentration Versus Time Profile of Ro 50-3821



Dose vs. Exposure

A small amount (239 pg/ml) of Ro 50-3821 was detected from one vehicle sample. According to the study report, because this was an isolated event, the observation was likely due to contamination.

The dose-proportional factor, calculated as a fold increase of exposure (AUC_{0-168hr} and C_{max}) at each dose level compared to the exposure at the lowest dose (1 mcg/kg/dose) were shown in the Table 21 .

Table 21. Overall Dose Proportionality Factor of in Dogs after 13-week IV Dosing

Dose (µg/kg/dose)	Theoretical Increases in Exposure (fold)	Observed Increases in Exposure (fold)	
		AUC _{0-168hr}	C _{max}
Day 1			
1	--	--	--
3	3.0	3.9	3.6
10	10	21.7	18.0
30	30	65.4	46.8
Day 22			
1	--	--	--
3	3.0	4.5	3.4
10	10	15.1	11.2
30	30	21.2	27.3
Day 85			
1	--	--	--
3	3.0	10.7	2.6
10	10	26.5	6.64

Changes During Repeated Administration

Changes in drug exposure following repeated administration were assessed by calculating the repeated dose factor [which is the ratio of AUC_{0-168hr} or C_{max} found on the day after repeated administration (Days 22 or 85) to the AUC_{0-168hr} or C_{max} obtained on the first day of dosing (Day 1)]. As shown below, accumulation was mainly obtained on Day 22 at the two lower doses (1 and 3 mcg/kg/dose). There was no clear trend of accumulation on Day 85.

Table 22. Overall Repeated Dose Factor of Ro 50-3821 in Dogs after 13W IV Dosing

Dose (µg/kg/dose)	AUC _{day22} /AUC _{day1}	AUC _{day85} /AUC _{day1}
	1	1.9
3	2.2	1.8
10	1.3	0.80
30	0.62	N/A
	C _{max,day22} /C _{max,day1}	C _{max,day85} /C _{max,day1}
1	1.9	2.4
3	1.8	1.7
10	1.2	0.88
30	1.1	N/A

Gender Differences

No clear trend of gender effect.

Antibody Analysis:

Dose- and time-dependent increase in incidence of anti-EPO-Ab were noted (Table 23). At Week 4, 2/20, 9/20, 14/20, and 8/10 dogs were positive for antibodies in the 1, 3, 10, and 30 mcg/kg/dose groups, respectively. At Week 13, 9/14, 8/14, and 12/14 dogs were positive for antibodies in the 1, 3, and 10 mcg/kg/dose groups, respectively. At Week 4, 2/20 dogs were positive in 1 mcg/kg/dose groups while at Week 13, 9/14 dogs were positive. The dogs were positive at Week 4 remained positive at Week 13. No analysis was conducted for the samples from recovery dogs.

Table 23. Summary of Incidence of Anti-EPO Ab IV*

Dose (mcg/kg)	4 wk Sacrifice				13 wk Sacrifice			
	Male		Female		Male		Female	
	+	±	+	±	+	±	+	±
0	1/10	0/10	0/10	0/10	0/7	0/7	0/7	0/7
1	0/10	0/10	2/10	1/10	5/7	0/7	4/7	0/7
3	5/10	1/10	4/10	0/10	4/7	0/7	4/7	1/7
10	8/10	0/10	6/10	0/10	6/7	0/7	6/7	0/7
30	4/5	0/5	4/5	0/5	2/2	0/2	2/2	0/2

* + Positive, ±Ambiguous, No. of positive or ambiguous results/No. of samples assayed; Prepared by the reviewer based on the submission.

Conclusion:

According to the study report, the adverse effects that were observed in this study represented exaggerated pharmacological activity (markedly accelerated erythropoiesis) of the drug. Exaggerated pharmacological effects were manifested as clinical observations, hematology changes, and via microscopic changes in the bone marrow, spleen, and gastrointestinal tract. These changes were well correlated with polycythemic or anemic condition of animals; therefore these observations were considered secondary to exaggerated pharmacological effects or a consequence of neutralizing antibody formation and the subsequent neutralization of exogenous and endogenous EPO.

Reviewer comments:

The reviewer agreed with the conclusion above.

The same deficiencies were identified as described in the study above.

C.

Study title: Ro 50-3821/000: A Thirteen-Week Subcutaneous (Injection) Toxicity and Toxicokinetic Study in Rats With a 4-Week Interim Sacrifice, Followed By An Eight-Week Recovery Period

Key study findings:

Mortality:

- Two females with severe anemia (Hct: 7 or 11%, respectively) in 1 mcg/kg/dose group and one female with severe polycythemia (Hct = 63.3%) in 10 mcg/kg/dose group were sacrificed unscheduled on Days 80, 84, and 64, respectively. Interestingly, following microscopic findings were noted in all three animals: mononuclear cell infiltrate and myocardial degeneration in the heart; alveolar macrophages and/or perivascular infiltration in the lung; focal necrosis in the liver (with elevated total bilirubin and ALT or AST).

Body weights and food consumption:

- Decreased mean body weight and food consumption for males in 10 (89% and 91% of controls on Day 91, respectively) and 30 mcg/kg/dose (92% and 90% of controls on Day 28, respectively), reversible.

Hematology:

- Dose-related, in general, increases in erythrocyte counts, hemoglobin, hematocrit, red cell distribution width, and reticulocytes, at least partially reversible;
- Increased incidences of anisocytosis and polychromasia, at least partially reversible;
- Appearance of Howell-Jolly bodies, at least partially reversible.

Clinical chemistry:

- Dose- and time-related increases in aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (TB), urea nitrogen (BUN), creatinine, and potassium, reversible.

Organ weights:

- Moderate to marked, dose-, and time-related increases in absolute and relative spleen weights, at least partially reversible.

Gross pathology:

- Reversible, dose- and time-related enlargement of the spleen.
- Reversible, dose- and time-related erosions in the glandular stomach.

Histopathology:

- Increased hematopoiesis in the bone marrow of rats in 1, 3, 10, and 30 mcg/kg/dose groups with an increase in mainly erythroid series in males while mainly granulocytic series in the females in the 10 mcg/kg/dose group, reversible.
- Increased production of nucleated erythrocytes in the red pulp of the spleen in 1, 3, 10, and 30 mcg/kg/dose groups, reversible.
- Extramedullary erythropoiesis in the livers of rats in 3, 10, and 30 mcg/kg/dose groups, primarily in males and to a lesser extent in females after 4 week treatment only, reversible.
- Congestion (hyperemia) of spleen, liver, adrenal glands, kidneys, lungs, and stomach in 1, 3, 10, and 30 mcg/kg/dose groups, at least partially reversible.
- Ulceration and/or erosions of the mucosal surface in the stomach, mainly the glandular region in rats given 1, 3, 10, or 30 mcg/kg/dose, at least partially reversible.

Toxicokinetics:

- Tmax achieved at 24 – 36 hour post dosing indicating a relatively slow absorption process following subcutaneous injection.
- Generally, greater than dose-proportional increases in systemic exposure.
- No gender differences.

Antibody

- Anti-EPO Ab was detected in a few animals (1-3) at all dose groups, correlating with anemia.

NOAEL at the 4-week interim evaluation was 1 mcg/kg/dose, while the NOAEL after 13 weeks of treatment could not be established.

Study no.: 07302, Report No. 1002576

Volume #, and page #: Module 4 Volume 1.8, page 1-1709

Conducting laboratory and location: Department of Non-Clinical Drug Safety, Hoffmann-La Roche Inc., Nutley, NJ. The TK samples were analyzed by _____

Date of study initiation: N/A, study report date: August 17, 2001

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 Nos. 002109, 003119, 004129; Test solutions: 1 mcg/mL, Lot No. L197849-01, 3 mcg/mL, Lot No. L197859-01, 10 mcg/mL, Lot No. L197869-01, 30 mcg/mL, Lot No. L197919-01, purity, N/A

Observations and times:

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

Clinical signs: at least once daily to record mortality and morbidity during the treatment and recovery periods; at least twice daily, before and after dosing during the treatment period; once weekly to record clinical signs during the treatment and recovery periods.

Neurologic examination: Once prior to initiation of treatment, after 4 and 13 doses.

Body weights: Pre-dose (Day -4), once weekly during the treatment and recovery periods beginning on Day 0, and just prior to sacrifice (terminal body weight).

Food consumption: Once weekly during the treatment and recovery periods beginning on Day 0.

Ophthalmoscopy: Once prior to initiation of treatment, after 4 and 13 doses.

ECG: Not performed.

Hematology: Weekly for one-half of each group on alternating weeks during the treatment and recovery periods.

Clinical chemistry: After 5 and 14 doses, and after 8 weeks of recovery.

Urinalysis: After 5 and 14 doses, and after 8 weeks of recovery.

Gross pathology: After 5 and 14 doses, and after 8 weeks of recovery.

Organ weights: After 5 and 14 doses, and after 8 weeks of recovery. Also see histopath table (Table 14).

Histopathology: After 5 and 14 doses, and after 8 weeks of recovery. A histopathologic evaluation was conducted on all protocol-designated organs and tissues from two humane-sacrificed, all interim-sacrificed animals in the control, 10, and 30 mcg/kg/dose groups, and all terminal and recovery-sacrificed animals in the control and 10 mcg/kg/dose groups; Target organs and tissues as outlined in the histopath table (+ stomach, Table 14), were examined from all interim-, terminal-, and recovery-sacrificed animals in 1 and 3 mcg/kg/dose groups.

Adequate Battery: yes (X), no ()

Peer review: yes (), no (X)

Toxicokinetic: Blood samples were collected prior to dosing (0 hour), 30 minutes, and 1, 3, 5, 8, 12, 16, 24, 36, 48, 72, 96, 120, 144, and 168 hours after dosing on Days 0, 21, and 84 of the treatment period by a validated ELISA assay procedure.

Antibodies: Development of anti-EPO Ab was determined after 4 and 13 weeks of treatment, and 8 weeks of recovery using _____ ELISA _____

See the dog study above for more details.

Results**Mortality:**

Two females in 1 mcg/kg/dose were unscheduled sacrificed on Days 80 (157F) and 84 (161F) due to severe anemia as evidenced by marked decreases in erythrocyte counts, hemoglobin, and hematocrit (2.05 MI/mcL, 3.9 g/dL, and 11.0%, respectively, on Day 80 for 157F; 1.45 MI/mcL, 2.6 g/dL, 7.0 %, respectively, on Day 83 for 161F), moderate hypochromic, and mildly to moderately pale ears, limbs, tail, organs, and the carcass. Unfortunately, anti-EPO Ab data were not available for both rats.

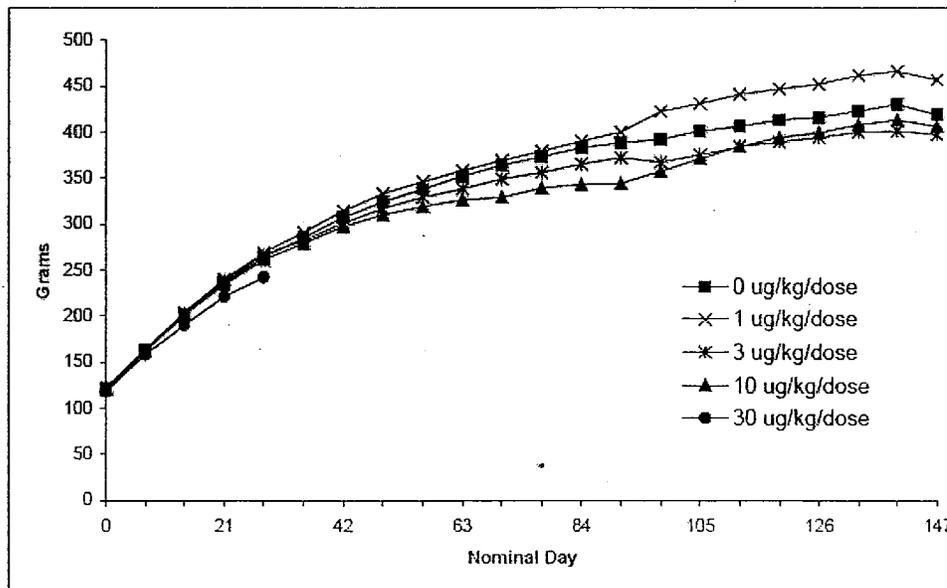
In addition, the followings were noted: slight (157F) or moderate (161F) focal necrosis in the liver with elevated ALT, total bilirubin, and triglyceride levels, slight mononuclear cell infiltrate in the heart, and slight bilateral plasmacytosis in the mandibular lymph nodes. Minimal osseous metaplasia and alveolar macrophage accumulation in the lung were noted for rat 157F. Slight myocardial degeneration in the heart, slight pervascular infiltration in the lung, slight pigmentation in the liver, slight bilateral basophilic tubules in the kidneys, and minimal hemosiderosis in the spleen were noted for rat 161F.

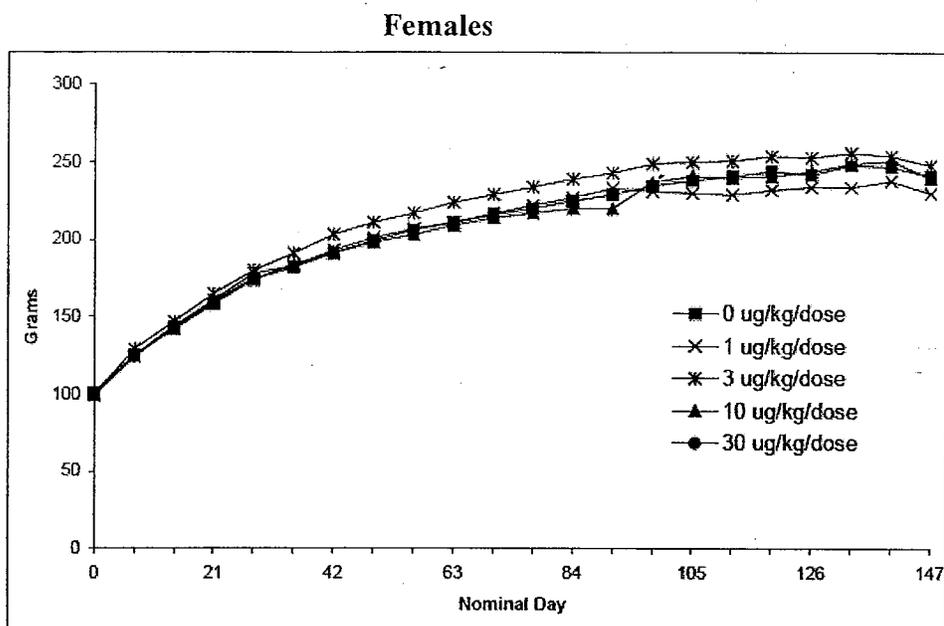
In addition, one female rat (202F) in the 10 mcg/kg/dose group was humane-sacrificed on Day 64 based on decreases in body weight and food consumption, and clinical signs, according to the study report. This animal had a severe polycythemia (Hct = 63.3% on Day 64) and moderate anisocytosis and few polychromasia. Severe rupture of the left eye, severe keratitis, moderate hemorrhage, and mild corneal opacity of the right eye were also noted. Microscopic findings included but not limited to slight lymphocytic infiltration and moderate chronic inflammation in the lacrimal/harderian glands; moderate erythrophagocytosis and plasmacytosis in the mandibular lymph nodes; mild to severe congestion of the heart, spleen, kidneys, liver, adrenal glands, ovaries, and lymph nodes; slight mononuclear cell infiltrate and myocardial degeneration in the heart; minimal alveolar macrophages in the lung; slight focal necrosis in the liver (with elevated AST and total bilirubin); moderate extramedullary hematopoiesis in the spleen that was consistent with the macroscopic finding of an enlarged spleen; slight increased granulopoiesis in bone marrow; and moderate fibroplasia and hyperostosis in the bone.

Clinical signs: No remarkable drug related findings.

Body weights: Decreased mean body weight for males in 10 (89% of controls on Day 91, the last day of dosing for this group) and 30 mcg/kg/dose (92% of controls on Day 28, the last day of dosing for this group). The mean BW for 10 mcg/kg/dose group was comparable to controls after eight weeks of recovery.

**Figure 3. Weekly Mean Body Weights
Males**





Food consumption: Decreased mean food consumption for males in 10 (91% of controls on Day 91, the last day of dosing for this group) and 30 mcg/kg/dose (90% of controls on Day 28, the last day of dosing for this group). The mean FC for 10 mcg/kg/dose group was comparable to controls after eight weeks of recovery.

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

Ophthalmoscopy: No remarkable drug related findings.

ECG: Not performed.

Hematology:

Erythrocyte parameters

Dose-related, in general, increases in erythrocyte counts, hemoglobin, hematocrit, red cell distribution width, and reticulocytes. Reticulocyte count increase tended to diminish over the course of the four- or thirteen-week treatment periods.

Platelet counts

Slight increase in platelet counts in 30 mcg/kg/dose group at the end of the four-week treatment period.

Decreases in platelet counts in 1, 3, or 10 mcg/kg/dose groups at the end of the thirteen week treatment period. The decreases in platelet counts were considered artifactual changes associated with increased erythropoiesis. The changes observed in the platelet parameters reversed to normal by the end of the eight-week recovery period.

Red blood cell morphology

Dose- and time-related increases in the incidence and severity (few to numerous) of anisocytosis and polychromasia in rats given 1, 3, 10, or 30 mcg/kg/dose. Polychromasia and/or anisocytosis were noted in most rats given 30 mcg/kg/dose even after one dose, and the severity increased over the four-week treatment period. Although there were a few animals in the 3 and 10 mcg/kg/dose groups with a few erythrocytes that had anisocytosis or polychromasia at the end of the recovery period, the incidence and severity of these findings decreased during the recovery period and were considered to be reversible, by the study report.

Dose- and time-related increases in the incidence of Howell-Jolly bodies. Howell-Jolly bodies were observed predominantly in the 10 and 30 mcg/kg/dose groups during the first four weeks of treatment, and in a few to most animals in the 1, 3, and 10 mcg/kg/dose groups after 13 week treatment. The incidence in the treated groups was not remarkably different from the control group, and the change was considered to be reversible by the end of the recovery period.

Coagulation parameters

The coagulation parameters PT and APTT were increased in rats in the 10 and 30 mcg/kg/dose groups following 4-wk treatment and in rats in the 3 and 10 mcg/kg/dose groups following 13-wk treatment. At the end of the eight-week recovery period, PT and APTT in drug groups were comparable to that in controls. According to the study report, increased PT and APTT times were likely due to relatively higher plasma concentrations of sodium citrate (the anticoagulant used for the blood sample collection) in the whole blood samples resulting from decreased plasma volume and increased red blood cell mass. Therefore, these effects were likely artifactual changes and were considered to be unrelated to the direct effects of Ro 50-3821/001, according to the study report.

Clinical chemistry:

Dose- and time- related increases in aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (TB), urea nitrogen (BUN), creatinine, and potassium (Table 26, male data as an example), reversible.

Urinalysis:

Because urine samples were not available from many animals at necropsy, urinalysis parameters could not be adequately evaluated for several male and female groups following four or thirteen weeks of treatment. However, a review of the individual data did not suggest that there were any effects of treatment with Ro 50-3821/000, according to the study report.

Table 26. Summary of Clinical Chemistry (Mean, Male)

Groups (mcg/kg/dose)	0	1	3	10	30
AST (IU/L)					
Day 28	98	93	107	101	149**
Day 92	91	93	140**	174**	N/A
ALP (IU/L)					
Day 28	133	152	189**	165	167
Day 92	73	73	85	105**	
TB (mg/dL)					
Day 28	0.10	0.10	0.11	0.12*	0.13**
Day 92	0.10	0.11	0.16**	0.19**	N/A
BUN (mg/dL)					
Day 28	15.7	15.1	15.4	16.5	17.4
Day 92	15.6	15.9	18.7**	19.7**	N/A
Creatinine (mg/dL)					
Day 28	0.2	0.2	0.2	0.2	0.2
Day 92	0.2	0.2	0.3**	0.3**	N/A
Potassium (mMol/L)					
Day 28	4.7	4.6	4.6	5.0	5.4**
Day 92	4.6	4.5	5.5**	5.4**	N/A

* p<0.05, ** p<0.01, prepared by the reviewer based on the submission.

Gross pathology:

Four-Week Interim Sacrifice

Dose-related enlargement of the spleen in 3, 10, and 30 mcg/kg/dose groups, corresponding to increases in spleen weight.

Erosions in the glandular stomach in the 10 and 30 mcg/kg/dose groups.

Thirteen-Week Terminal Sacrifice

Dose-related enlargement of the spleen in the 3 and 10 mcg/kg/dose groups.

Erosions in the glandular stomach in the 3 and 10 mcg/kg/dose groups and reddish colored foci in the glandular region of the stomach in 1, 3, and 10 mcg/kg/dose groups.

Recovery Sacrifice

No drug-related macroscopic findings at the recovery sacrifice except for reddish foci in the glandular stomach in one female given 10 mcg/kg/dose.

Organ weights: Moderate to marked, dose-related increases in absolute and relative spleen weights in rats given 3, 10, and 30 mcg/kg/dose after four weeks of treatment and in rats given 1, 3, and 10 mcg/kg/dose after thirteen weeks of treatment; These changes tended to reverse to normal in all treatment groups after eight weeks of recovery, but the

absolute and relative spleen weights in 10 mcg/kg/dose rats remained slightly higher than those of the controls.

Histopathology:

Four-Week Interim Sacrifice

Target organs: spleen and bone marrow (femur and sternum)

Increased production of nucleated erythrocytes in the red pulp of the spleen and in the bone marrow of rats in 1, 3, 10, and 30 mcg/kg/dose groups.

Extramedullary erythropoiesis in the livers of rats in 3, 10, and 30 mcg/kg/dose groups, primarily in males and to a lesser extent in females.

Congestion (hyperemia) of the liver, adrenal glands and kidneys in 1, 3, 10, and 30 mcg/kg/dose groups, characterized by excessive accumulation of red blood cells in the effected organ resulting in the dilatation of the lumen of the blood vessels of that organ.

Ulcerations or necrosis of the lamina propria in one male in 10 mcg/kg/dose group, and one male and one female in 30 mcg/kg/dose group.

Serocellular crust correlated with the scabs present at the injection sites in 2/8 males in the 30 mcg/kg/dose group and in 1/8 males in the 1 mcg/kg/dose group.

Thirteen-Week Terminal Sacrifice

Extramedullary erythroid hematopoiesis with the nucleated red blood cells completely filled the red pulp in the spleen of rats in the 1, 3, and 10 mcg/kg/dose groups.

Congestion (hyperemia) in various tissues (e.g., liver, kidneys, lungs, stomach, and adrenal glands) in the 1, 3, and 10 mcg/kg/dose groups.

Ulceration and/or moderate to massive erosions of the mucosal surface in the stomach, mainly the glandular region in rats in the 1, 3, and 10 mcg/kg/dose groups.

Hemorrhage and congestion in the mucosa of the stomach in some animals in the 1, 3, and 10 mcg/kg/dose groups.

Increased hematopoiesis in the bone marrow of the femur and sternum in the 10 mcg/kg/dose groups with an increase in the mainly erythroid series in males while an increase in the mainly granulocytic series in the females.

Increase in the granulocytic series in the females given 3 mcg/kg/dose. This increase in granulocytes is probably due to the depletion of the erythroid series, according to the study report.

Recovery Sacrifice

Slight erosions of stomach in one female given 10 mcg/kg/dose.

Increased hemosiderin pigment in the red pulp of the spleens, resulted from increased erythropoiesis observed at the terminal sacrifice,

Congestion in liver, kidneys, lungs, stomach remained but with lower incidence and to a lesser degree than that at the terminal sacrifice.

Toxicokinetics:

Toxicokinetic parameters and concentrations vs. time profiles were presented in Table 27 and Figure 4. Generally, greater than dose-proportional increases in systemic exposure ($AUC_{(0-168h)}$ and C_{max}) of Ro 50-3821 were observed for most dose groups at all three study intervals. The $AUC_{(0-168h)}$ at all dose levels on Days 21 and 84 were 1.3-1.9 fold higher than that on Day 0 indicating an accumulation of drug after repeated dose. No gender differences were seen in AUC and C_{max} . Generally, T_{max} was achieved at 24 – 36 hour post dosing, indicating a relatively slow absorption process following subcutaneous injection of Ro 50-3821/000.

Table 27. Toxicokinetic Parameters for Ro 50-3821

Day 0

Dose (ug/kg/dose)	Parameter	Units	Male	Female	Overall
1	AUC0-168h	ng*Hours/mL	190	207	199
	AUC/Dose	ng*Hours/mL/ μ g/kg	190	207	199
	C_{max}	ng/mL	3.95	4.31	3.97
	T_{max}	Hours	36	24	24
3	AUC0-168h	ng*Hours/mL	759	750	754
	AUC/Dose	ng*Hours/mL/ μ g/kg	253	250	251
	C_{max}	ng/mL	13.6	13.4	13.5
	T_{max}	Hours	24	24	24
10	AUC0-168h	ng*Hours/mL	2950	3620	3290
	AUC/Dose	ng*Hours/mL/ μ g/kg	295	362	329
	C_{max}	ng/mL	52.0	71.0	61.5
	T_{max}	Hours	24	24	24
30	AUC0-168h	ng*Hours/mL	7820	11100	9460
	AUC/Dose	ng*Hours/mL/ μ g/kg	261	370	315
	C_{max}	ng/mL	143	183	153
	T_{max}	Hours	24	36	24

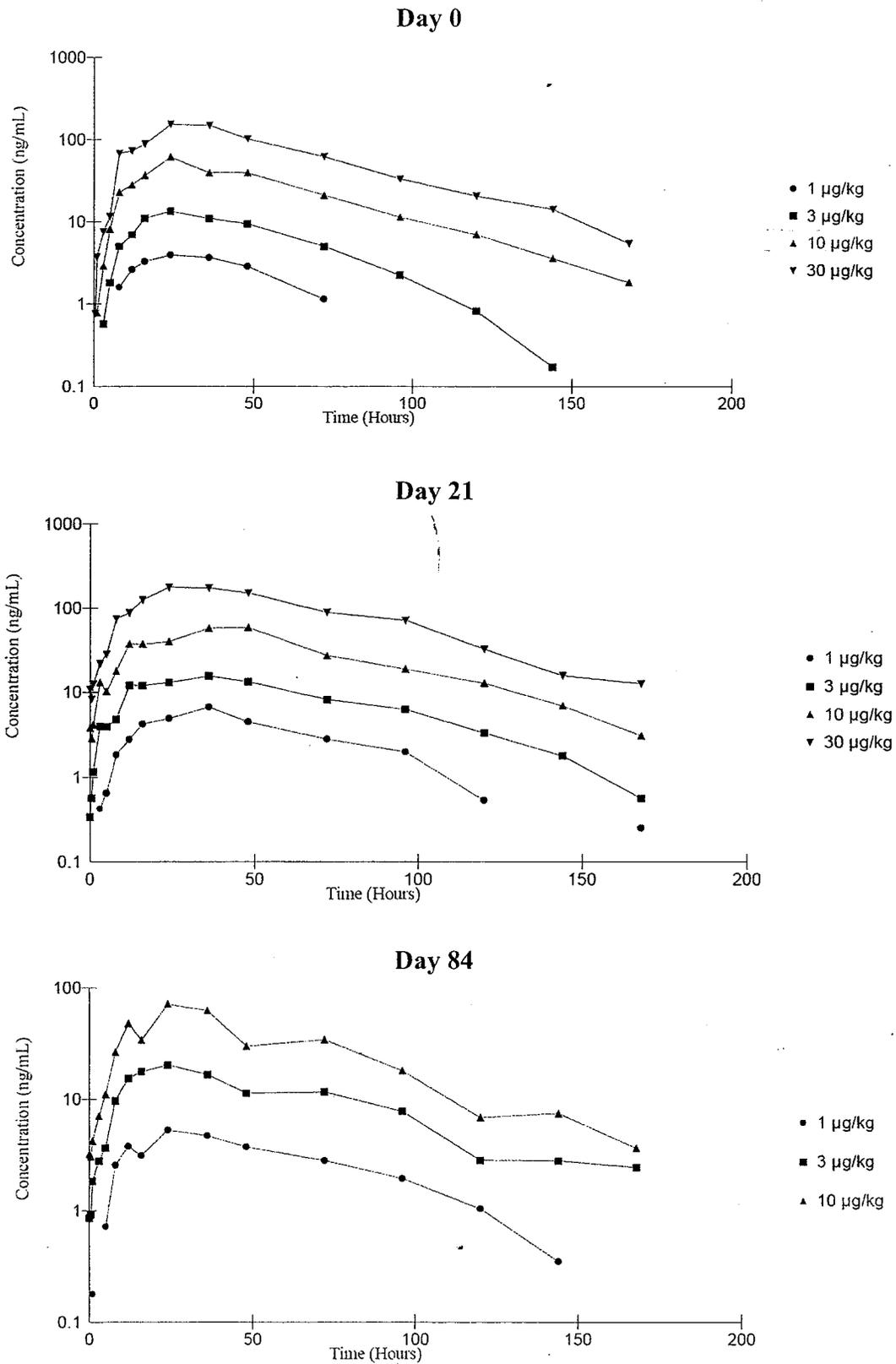
Day 21

Dose (ug/kg/dose)	Parameter	Units	Male	Female	Overall
1	AUC0-168h	ng*Hours/mL	479	297	387
	AUC/Dose	ng*Hours/mL/ μ g/kg	479	297	387
	Cmax	ng/mL	9.81	4.71	6.72
	Tmax	Hours	36	24	36
3	AUC0-168h	ng*Hours/mL	1190	1210	1200
	AUC/Dose	ng*Hours/mL/ μ g/kg	397	403	400
	Cmax	ng/mL	13.8	17.7	15.8
	Tmax	Hours	36	36	36
10	AUC0-168h	ng*Hours/mL	4890	3710	4300
	AUC/Dose	ng*Hours/mL/ μ g/kg	489	371	430
	Cmax	ng/mL	78.4	55.8	59.2
	Tmax	Hours	48	36	48
30	AUC0-168h	ng*Hours/mL	12500	14100	13300
	AUC/Dose	ng*Hours/mL/ μ g/kg	417	470	443
	Cmax	ng/mL	162	241	178
	Tmax	Hours	36	24	24

Day 84

Dose (ug/kg/dose)	Parameter	Units	Male	Female	Overall
1	AUC0-168h	ng*Hours/mL	377	358	369
	AUC/Dose	ng*Hours/mL/ μ g/kg	377	358	369
	Cmax	ng/mL	5.47	5.87	5.26
	Tmax	Hours	36	24	24
3	AUC0-168h	ng*Hours/mL	1420	1480	1460
	AUC/Dose	ng*Hours/mL/ μ g/kg	473	493	487
	Cmax	ng/mL	21.5	23.6	20.3
	Tmax	Hours	16	24	24
10	AUC0-168h	ng*Hours/mL	4730	3550	4180
	AUC/Dose	ng*Hours/mL/ μ g/kg	473	355	418
	Cmax	ng/mL	96.7	83.1	71.6
	Tmax	Hours	36	24	24

Figure 4. Mean Serum Concentration Versus Time Profile of Ro 50-3821/000



Antibody Analysis:

Anti-EPO Ab was detected after 4 weeks of treatment (the earliest time point) (Table 28). There was no clear dose relationship in incidence of Ab positive in general. Of interest, higher incidence of Ab positive was noted in recovery sample in 10 mcg/kg/dose group.

During the treatment period, the presence of anti-EPO Ab was associated with marked decreases in RBC, HGB and HCT levels in these rats.

Two rats that tested positive for anti-EPO Ab at the end of the recovery period, exhibited drug- and time-related increases in erythropoiesis over the first seven to eight weeks of the treatment period followed by time-related decreases. Near the end of the treatment period, RBC, HGB and HCT levels were lower than those measured near the beginning of the treatment period. Over the course of the recovery period the levels of these parameters reversed to normal.

Table 28. Summary of Incidence of Anti-EPO Ab*

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

* + Positive, ± Ambiguous, No. of positive or ambiguous results/No. of samples assayed.

^a no tube for one rat

^b no tubes for four rats

^c no tubes for five rats

N/A: no rats available

Prepared by the reviewer based on the submission.

Other: N/A

Conclusion:

According to the study report, T_{max} was achieved at 24 – 36 hour post dosing indicating a relatively slow absorption process following subcutaneous injection. Generally greater than dose-proportional increases in AUC and C_{max} were found at most doses on all three study days. Drug-related changes in erythrocyte parameters (including increased RBC, HCT, HGB, and reticulocytes), and increased hematopoiesis in bone marrow, spleen, and liver were attributed to the pharmacological activity of Ro 50-3821/000. Serum chemistry changes included increases in aspartate aminotransferase, alkaline phosphatase, and total bilirubin. Hyperemia (congestion) in various organs was noted at all dose levels, and erosions and ulceration noted in the glandular mucosa of the stomach. Anti-EPO Ab was

detected in a few animals (1-3) at all dose groups, which correlated with anemia. NOAEL at the 4-week interim evaluation was 1 mcg/kg/dose, while the NOAEL after 13 weeks of treatment was not established based on gross findings of stomach erosions in two rats at the 1 mcg/kg/dose group.

Reviewer's comments

The reviewer agreed with the conclusion above.

D.

Study title: Ro 50-3821/000: A 13-Week Intravenous Injection Toxicity Study with a 4-Week Interim Sacrifice and a 8-Week Recovery Phase in Rats

Key study findings:

Mortality:

- Seven rats in 10 mcg/kg/dose group died or were unscheduled sacrificed morbidly between Days 36 and 95 of treatment. Five rats from the toxicity group (no data available for 2 rats from TK study) suffered from severe polycythemia. Kidney necrosis, ileum/cecum necrosis, brain and spinal cord hemorrhage, and stomach erosion were considered as causes of deaths. Drug-related.

Hematology:

- Dose- and time-related aberrations in erythrocyte parameters.

Gross pathology:

- Enlarged spleens mainly in 10 and 30 mcg/kg/dose groups, reversible.
- Dose-related red or dark foci and/or erosions in the glandular mucosa of the stomach in males given 3, 10, and 30 mcg/kg/dose and females given 1, 3, 10, and 30 mcg/kg/dose, partially reversible.
- Kidney lesions with mottled, light focus/area, and/or irregular shape in males given 3 and 10 mcg/kg/dose and females given 3 mcg/kg/dose.

Histopathology:

- Dose- and time-related increase in hematopoiesis of the bone marrow and spleen, reversible.
- Dose- and time-related ossification of the bone marrow, reversible.
- Dose- and time-related congestion, hemorrhage, and/or erosion of the glandular mucosa of the stomach, partially reversible.
- Ischemic kidney necrosis in one male in 10 mcg/kg/dose group and two males and one female in 3 mcg/kg/dose group.

- Valvular inflammation and/or thrombosis in the heart in two rats in the 10 mcg/kg/dose group.

Toxicokinetics:

- Greater than dose-proportional increases in AUC and Cmax at all doses.
- No accumulation after repeated dosing.
- No gender effect.

Antibody Analysis:

- Anti-EPO Ab detected after 4 weeks of treatment (the earliest time point)
- No clear dose relationship in incidence of positive Ab.
- Time-dependent trend in incidence of Ab development 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.

NOAEL could not be established.

Study no.: Studies No. 6131-305, 6131-319, HLR Study No. 07304, 07418, Report No. 1002692

Volume #, and page #: Module 4 Volume 1.8, page 1-1769

Conducting laboratory and location:

Male reproductive capacity evaluated by
Bioanalytical assays and Toxicokinetic calculations by the
Department of Non-Clinical Drug Safety, Hoffmann-La Roche Inc., 340 Kingsland
Street, Nutley, NJ. Anti-EPO Ab assays by

Date of study initiation: 17 February 2000

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

QA report: yes (X) no ()

Drug, lot #, and % purity: Ro 50-3821/000, Lot Nos. L197849 (1 mcg/mL), L197859 (3 mcg/mL), L197869 (10 mcg/mL), and L197919 (30 mcg/mL), purity,

Methods

Doses: 0 (vehicle: 10 mM sodium/potassium phosphate buffer, pH 7.0, with 7.73 mg/mL NaCl, Group 1), 1 (Group 2), 3 (Group 3), 10 (Group 4), or 30 (Group 5) mcg/kg/dose, once weekly for 4 wks (4 doses) for interim sacrifice or 0 (Group 1), 1 (Group 2), 3 (Group 4), or 10 (Group 4) mcg/kg/dose, once weekly for 13 wks (13 doses) for terminal sacrifice.

Table 29. Summary of Study Design

Group	No. of Animals		Dose Level ^a (mcg/kg/dose)	Dose Concentration (mcg/mL)
	Male	Female		
Toxicity Animals				
1 (Control) ^b	26 ^c	26 ^c	0	0
2 (Low)	26 ^c	26 ^c	1	1
3 (Mid-low)	26 ^c	26 ^c	3	3
4 (Mid-high)	26 ^c	26 ^c	10	10
5 (High)	8 ^c	8 ^c	30	30
Toxicokinetic Animals				
6 (Control) ^b	12	12	0	0
7 (Low)	12	12	1	1
8 (Mid-low)	12	12	3	3
9 (Mid-high)	12	12	10	10
10 (High)	12	12	30	30

^a The dose volume: 1 mL/kg/dose.

^b The control animals received the vehicle.

^c Eight animals/sex/group were sacrificed after at least 4 weeks of treatment (interim sacrifice); 10 animals/sex/group in Groups 1 through 4 were sacrificed after at least 13 weeks of treatment (terminal sacrifice); and eight animals/sex/group in Groups 1 through 4 were sacrificed after at least 13 weeks of treatment followed by 8 weeks without treatment (recovery sacrifice). The number of animals at each interval was based on survival.

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

Number/sex/group or time point: 8 or 10/sex/group

Route, formulation, volume, and infusion rate: bolus intravenous injection into a lateral tail vein, dose volume: 1 mL/kg/dose.

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

Age: 69 to 75 day old

Weight: 234 to 309 grams for males and 165 to 228 grams for females

Sampling times: See other related sections.

Unique study design or methodology (if any): including male reproductive assessment.

Observations and times:

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

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

Body weights: Pre-dose (Day -4), once weekly during the treatment and recovery periods beginning on Day 0, and just prior to sacrifice (terminal body weight).

Food consumption: Once weekly during the treatment and recovery periods beginning on Day 0.

Ophthalmoscopy: Once prior to initiation of treatment, after 4 and 13 doses and at Week 21.

ECG: Not performed.

Hematology: Weekly for one-half of each group on alternating weeks during the treatment and recovery periods.

Clinical chemistry: After 4 and 13 doses, and after 8 weeks of recovery, fasted overnight.

Urinalysis: After 4 and 13 doses, and after 8 weeks of recovery (for approximately 16 hours)

Gross pathology: On Days 30, 96, and 154.

Organ weights: On Days 30, 96, and 154. Also see histopath table (Table 14).

Histopathology: On Days 30, 96, and 154. A histopathologic evaluation was conducted on all protocol-designated organs and tissues from two humane-sacrificed, all interim-sacrificed animals in the control, 10, and 30 mcg/kg/dose groups, and all terminal and recovery-sacrificed animals in the control and 10 mcg/kg/dose groups; Target organs and tissues as outlined in the histopath table (+ stomach, Table 14), were examined from all interim-, terminal-, and recovery-sacrificed animals in 1 and 3 mcg/kg/dose groups.

Adequate Battery: yes (X), no ()

Peer review: yes (), no (X)

Male Reproductive Assessment: On Day 30, right vas deferens for motility assessment, right epididymis (the caudal section) for total sperm count, sperm morphology assessed with two slides of sperm (a minimum of 200 sperm cells/animal) for each male.

Toxicokinetic: Blood was collected at pre-dose, approximately 5 and 20 minutes, and 1, 4, 10, 24, 48, 72, 96, 120, and 144 hours postdose on Days 1, 22, and 85 and analyzed by a validated ELISA assay procedure.

Antibodies: Development of anti-EPO Ab was determined after 4 and 13 weeks of treatment, and 8 weeks of recovery using a _____ ELISA with _____

_____. See related section aforementioned for more details.

Results

Mortality:

Nine animals either died or were sacrificed due to morbidity during treatment and were listed in Table 30.

Table 30. Summary of Information of Died or Sacrificed Rats

Group/sex	Dose level (mcg/kg/dose)	Animal number	Day of death	Type of death
1/F	0	C51134	23	Sacrificed
2/F	1	C51162	117	Died on test
4/M	10	C51021	86	Died on test
4/M	10	C51023	63	Died on test
4/F	10	C51206	36	Sacrificed
4/F	10	C51210	44	Sacrificed
4/F	10	C51216	95	Died on test
9/F	10	C51261	45	Died on test
9/F	10	C51272	81	Sacrificed

Seven rats in 10 mcg/kg/dose died or were sacrificed due to morbidity between Days 36 and 95 of treatment. Hypoactivity, irregular respiration, few feces, and hunched or thin appearance were noted in some animals prior to deaths or sacrifices. These deaths or sacrifices were considered to be drug related by the study report. Key findings from 5 rats (data unavailable for two toxicokinetic rats) were summarized in Table 31. All five rats from the toxicity group suffered from severe polycythemia. Kidney necrosis, ileum/cecum necrosis, brain and spinal cord hemorrhage, and stomach erosion were considered causes of deaths, according to the pathologist report. Significant increased urea nitrogen and creatinine values in three rats (the information available for these three rats only) were indicative of renal failure, probably resulted from kidney lesions.

Chronic progressive nephropathy was the cause of deaths for two rats in control and 1 mcg/kg/dose groups, according to the pathologist report.

Table 31. Summary of Key Findings for Died or Unscheduled Sacrificed Rats*

Groups (mcg/kg/dose)	0	1	10	10	10	10	10
Animal #	C51134	C51162	C51021	C51023	C51206	C51210	C51216
Kidney necrosis (infarction)	-	-	3	5 (cd)	3 (cd)	2	4 (cd)
Ileum/cecum -Ischemic necrosis -inflammation	-	A	5 (cd) 4	A	A	-	A
Heart valvular -inflammation -thrombus	-	-	-	2 +	-	-	+
Ch progressive nephropathy	5 (cd)	5 (cd)	--	-	-	-	-
Brain&spinal cord hemorrhage	-	-	-	4 (cd)	-	-	-
Lymphocytic depletion-spleen	2	3	3	-	4	3	2
Lymphocytic depletion-thymus	4	4	3	-	3	3	1
Adrenal cortex hemorrhage	1	-	2	-	3	-	-
Stomach mucosa erosion	-	-	-	2	4	5 (cd)	-
Increased BM hematopoiesis	-	2	3	4	3	3	3
HCT (%)**	29.8	13.8	68.8	67.4	71.4	66.2	72.7
Urea nitrogen (mg/dL)	207	N/A	N/A	N/A	370	159	N/A
Creatinine (mg/dL)	2.8	N/A	N/A	N/A	5.8	2.0	N/A

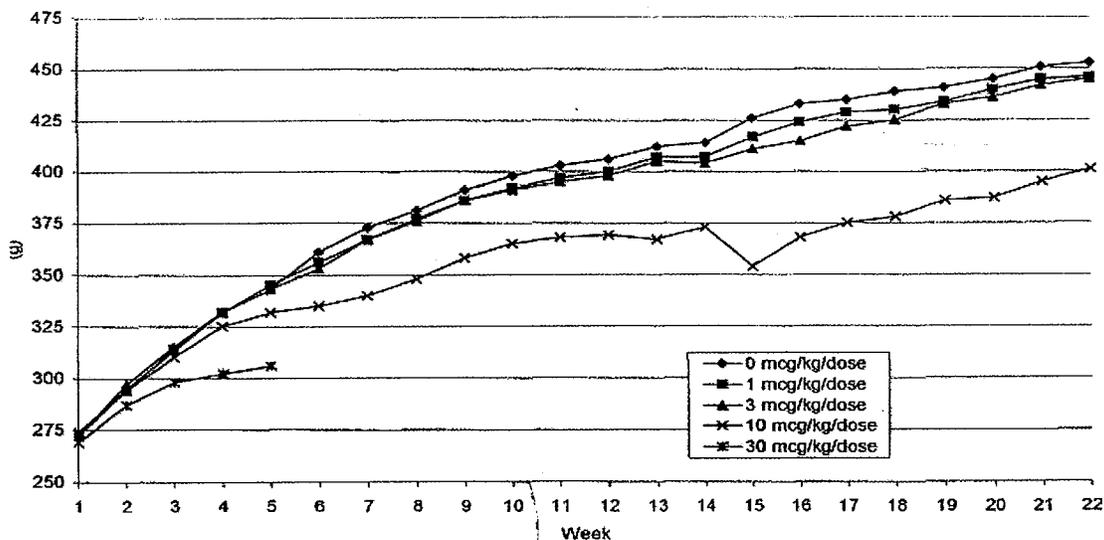
* Prepared by the reviewer based on the submission; +: present, -: not present, 1: minimal, 2: slight, 3: moderate, 4: moderately severe, 5: severe, A: autolytic, cd: cause of death, N/A: not available; data unavailable for two toxicokinetic rats

** the latest value available prior to death or sacrifice

Clinical signs: Blue skin on the paws, red ears and nasal discharge, or pale eyes, limbs, and body that were cold to touch. These signs were primarily observed during Weeks 4 through 14 and were most frequently noted for animals given 10 mcg/kg/dose. Some of these clinical signs including pale eyes, limbs, and body, or blue paws continued to be noted during the recovery period (Weeks 15 through 22). Generally, the clinical observation of blue skin was associated with animals having high hematocrits while the observation of pale eyes and body associated with animals having low hematocrits.

Body weights: Figure 5. Body weights were decreased in males treated with 10 or 30 mcg/kg/dose.

Figure 5. Weekly Mean Body Weights (Males)



Food consumption: Food consumption was decreased in males and females treated with 10 or 30 mcg/kg/dose. The decreases observed for males were statistically significant and probably led to decreased body weights. These changes reversed during the recovery period. The decreases observed in the females were slight and sporadic and did not affect body weight and were not considered adverse, according to the study report.

Ophthalmoscopy: No drug-related findings after 4 week treatment. At the Week 13 examination, ocular vascular engorgement in both eyes was noted for 7/18, 9/18, and 11/16 males given 1, 3, or 10 mcg/kg/dose, respectively, and 4/18, 12/17, and 13/16 females given 1, 3, or 10 mcg/kg/dose, respectively. Pale ocular vasculature in both eyes was noted for 2/18 and 5/16 males given 3 or 10 mcg/kg/dose, respectively, and 1/17 and 1/16 females given 3 or 10 mcg/kg/dose, respectively. Ocular vascular engorgement and pale vasculature were considered to be drug-related by the study report. The ocular vascular engorgement correlated with animals having high hematocrits and ocular pale vasculature correlated with animals having low hematocrits.

At the Week 21 examination, drug-related observations noted earlier had been resolved.

EKG: Not performed.

Hematology:

Markedly accelerated erythropoiesis and a functional iron deficiency in all drug groups. During the first week of the study, erythrocyte counts, hemoglobin, hematocrit, and reticulocyte counts increased markedly while mean corpuscular volume, mean

corpuscular hemoglobin, and mean corpuscular hemoglobin concentration decreased. These changes were dose- and time-related in the early phase of the study. As the study continued, the erythrocyte effects tended to vary widely among animals within each group, probably due to different degree of antibody formation among individual animals although actual antibody titers were not determined. In some rats, erythrocyte counts, hemoglobin, hematocrit, and reticulocyte counts began to fall from their peak values indicating that antibodies may have also affected the native EPO. After discontinuation of treatment, both anemic and polycythemic animals recovered and were similar to controls by the end of the recovery period.

Clinical chemistry:

The following changes were noted.

- Slight (<2 fold over controls) but dose-related increase in AST at Week 5 sacrifice.
- Increase in alkaline phosphatase in females at Weeks 5 and 13 (dose-related) sacrifice, may be related to the bone changes, reversible.

Urinalysis: No remarkable drug-related changes

Male Reproductive Assessment: No remarkable drug-related effect on mean percent sperm motility, total sperm count, and sperm morphology.

Gross pathology:

4-Week Interim Sacrifice

Enlarged spleens in rats receiving 10 or 30 mcg/kg/dose.

Red or dark foci and/or erosions in the glandular mucosa of the stomach in both males and females receiving 10 or 30 mcg/kg/dose and females receiving 3 mcg/kg/dose.

13-Week Terminal Sacrifice

Enlarged spleens in rats receiving 10 mcg/kg/dose and one male receiving 3 mcg/kg/dose.

Red, dark or light foci and/or diffuse reddening of the glandular mucosa of the stomach in males given 3 or 10 mcg/kg/dose and in females in all dose groups. The frequency of incidences was dose-related.

Kidney lesions of mottled, light focus/area, and/or irregular shape in males given 3 or 10 mcg/kg/dose and females given 3 mcg/kg/dose.

Recovery Sacrifice

Red, dark foci and/or erosion/ulceration of the glandular stomach in one or two females from each dose group including one control female.

Organ weights:

4-Week Interim Sacrifice

Increased absolute and relative spleen weights in males given 10 or 30 mcg/kg/dose and females given 3, 10, or 30 mcg/kg/dose, corresponding to extramedullary hematopoiesis in the spleen.

13-Week Terminal Sacrifice

Increased absolute and relative spleen weights in males given 10 mcg/kg/dose and females given 3 or 10 mcg/kg/dose; increased relative spleen weight in males given 3 mcg/kg/dose.

Histopathology:

4-Week Interim Sacrifice

Target organs: spleen, bone marrow, bone (femur), glandular stomach, and thymus.

Dose-related extramedullary hematopoiesis in the spleen (Table 32).

Dose-related increased hematopoiesis of the bone marrow (Table 32).

Table 32. Summary of Incidence of Increased Hematopoiesis*

Dose (mcg/kg)	4-wk Sacrifice				13-wk Sacrifice			Recovery Sacrifice		
	1	3	10	30	1	3	10	1	3	10
N	8M8F	8M8F	8M8F	8M8F	10M10F	10M10F	10M9F	8M7F	8M8F	6M6F
Increased Hematopoiesis										
Bone marrow	4M7F	7M8F	8M8F	8M8F	8M5F	8M8F	9M8F	0	0	0
Spleen	2F	3M6F	6M6F	8M6F	0	4M3F	6M9F	0	0	0

* Prepared by the reviewer based on the submission.

Ossification of the bone marrow (increased trabecular bone formation in the metaphysis of the femur) in 2 males in the 30 mcg/kg/dose group.

Congestion, hemorrhage, and/or erosion of the glandular mucosa of the stomach of females given 3, 10, or 30 mcg/kg/dose.

Lymphoid depletion of the thymus in 2 males and 2 females given 30 mcg/kg/dose.

13-Week Terminal Sacrifice

Dose-related increased hematopoiesis (erythropoiesis) of the bone marrow (Table 32).

Ossification of the bone marrow in some rats receiving 3 or 10 mcg/kg/dose.

Congestion, hemorrhage, and/or erosion of the glandular mucosa of the stomach in rats of all drug groups.

Dose-related increased extramedullary hepatopoiesis in the spleen (Table 32).

Pigment in spleens of a few rats in each drug group.

Ischemic kidney necrosis in one male in 10 mcg/kg/dose group and two males and one female in 3 mcg/kg/dose group.

Valvular inflammation and/or thrombosis in the heart in one male and one female in the 10 mcg/kg/dose group; Vascular inflammation in the pancreas of one female and one male in 10 mcg/kg/dose group accompanied by thrombosis for the female; These lesions were considered to be secondary to drug related vascular occlusion or polycythemia by the study report.

Recovery Sacrifice

In the glandular stomach, congestion of the mucosa in two females each receiving 1 or 3 mcg/kg/dose, hemorrhage in one female receiving 3 mcg/kg/dose group, and erosion of the mucosa in two females receiving 10 mcg/kg/dose.

Pigment deposition in the spleen of some animals in each dose group.

Toxicokinetics:

Toxicokinetic parameters were presented in Table 33, concentrations vs. time profiles in Figure 6, and the dose-proportional factor in Table 34.

Greater than dose-proportional increases in AUC and C_{max} were found at all doses on all three study days. This result suggested that possible interference in serum Ro 50-3821 analysis by anti-EPO Ab formation was, if any, minimal, according to the study report. There was essentially no accumulation after repeated dosing. In general, no gender effect was observed.

Compared to the exposures obtained from subcutaneous injection study (described above), the AUC levels from the intravenous injection study are much greater.

Table 33. Toxicokinetic Parameters for Ro 50-3821

Day 1

Dose µg/kg/dose (study#)	Parameter	Units	Male	Female	Overall
1 (07304)	AUC	ng*Hours/mL	652	596	623
	AUC/Dose	ng*Hours/mL/µg/kg	652	596	623
	Cmax	ng/mL	21.0	19.9	20.4
3 (07304)	AUC	ng*Hours/mL	2210	2080	2140
	AUC/Dose	ng*Hours/mL/µg/kg	737	693	713
	Cmax	ng/mL	76.5	80.0	78.0
10 (07304)	AUC	ng*Hours/mL	11500	12600	12000
	AUC/Dose	ng*Hours/mL/µg/kg	1150	1260	1200
	Cmax	ng/mL	356	371	354
10 (07418)	AUC	ng*Hours/mL	13300	13700	13400
	AUC/Dose	ng*Hours/mL/µg/kg	1330	1370	1340
	Cmax	ng/mL	356	597	477
30 (07418)	AUC	ng*Hours/mL	48000	39100	43600
	AUC/Dose	ng*Hours/mL/µg/kg	1600	1300	1450
	Cmax	ng/mL	1370	964	1140

Day 22

Dose mg/kg/dose	Parameter	Units	Male	Female	Overall
1	AUC	ng*Hours/mL	515	569	543
	AUC/Dose	ng*Hours/mL/µg/kg	515	569	543
	Cmax	ng/mL	21.5	19.9	20.7
3	AUC	ng*Hours/mL	1960	1550	1740
	AUC/Dose	ng*Hours/mL/µg/kg	653	517	580
	Cmax	ng/mL	100	57.1	75.6
10	AUC	ng*Hours/mL	11500	8160	9580
	AUC/Dose	ng*Hours/mL/µg/kg	1150	816	958
	Cmax	ng/mL	460	475	452
30	AUC	ng*Hours/mL	47500	38400	43000
	AUC/Dose	ng*Hours/mL/µg/kg	1580	1280	1430
	Cmax	ng/mL	1710	1770	1740

Day 85

Dose mg/kg/dose	Parameter	Units	Male	Female	Overall
1	AUC	ng*Hours/mL	755	603	680
	AUC/Dose	ng*Hours/mL/µg/kg	755	603	680
	Cmax	ng/mL	23.0	21.5	21.9
3	AUC	ng*Hours/mL	2200	1760	1980
	AUC/Dose	ng*Hours/mL/µg/kg	733	587	660
	Cmax	ng/mL	68.4	64.8	67.0
10	AUC	ng*Hours/mL	11000	9680	10400
	AUC/Dose	ng*Hours/mL/µg/kg	1100	968	1040
	Cmax	ng/mL	459	495	422

Figure 6. Mean Serum Concentration Versus Time Profile of Ro 50-3821/000

