

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

204819Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO

NDA: 204819

Submission date: 2/8/2013

Drug: riociguat

Applicant: Bayer Healthcare

Indication: Treatment of patients with chronic thromboembolic pulmonary hypertension and pulmonary arterial hypertension

Reviewing Division: Division of Cardiovascular and Renal Products

Discussion:

The sponsor has provided information to support calling riociguat a “soluble guanylate cyclase stimulator”. They assert that there are at least two modes of action for increasing the activity of soluble guanylate cyclase (i.e., heme-dependent and heme-independent) and that these two modes are known by the terms soluble guanylate cyclase stimulator and soluble guanylate cyclase activator, respectively. Both modes of action result in an increase in the conversion of GTP to cGMP by soluble guanylate cyclase. The two modes of action may be expected to have a similar therapeutic effect although some of the biological or toxic effects may differ. Therefore, there may be some value in differentiating these modes of action. If the term stimulator is considered sufficiently clinically meaningful to distinguish from the other term activator, then an appropriate established pharmacologic class for riociguat could be “soluble guanylate cyclase (sGC) stimulator”.

Riociguat was tested in 2 year carcinogenicity studies in rats and mice using the dietary route of administration. Both studies were found to be acceptable by the executive carcinogenicity assessment committee. No drug-related neoplasms were noted in either study.

Adverse findings in embryofetal development studies in rats and rabbits raised concern about the use of riociguat in pregnant women. Riociguat produced a significant increase in cardiac malformations such as ventricular septal defects in rats. Post implantation loss and resorptions were increased at high doses in both rats and rabbits. Evidence of incomplete ossification was also noted in rats. The teratogenic effects of riociguat appeared to occur at exposures that were within an order of magnitude of the anticipated human exposure.

The pharmacology toxicology review noted that the nonclinical studies suggested adverse bone effects from riociguat. The bone effects were suggested in the embryofetal studies and from standard toxicology studies. Effects on bone metabolism by riociguat appears to be consistent with published information suggesting that sGC modulates osteoclast/osteoblast balance. The reviewer

noted that this may raise some concern about the use of riociguat in pediatric subjects. This concern is being considered by the division as possible future pediatric trials are discussed.

Conclusions:

The pharmacology/toxicology reviewer conducted a thorough evaluation of the nonclinical information submitted in support of this NDA. I agree that this NDA may be approved for the above indication. Contraindicating use of riociguat in pregnant women appears appropriate given the effects observed on fetal development. It appears that no additional nonclinical studies are needed at this time but that it may be appropriate to include precautions and monitoring for potential adverse bone effects in any future pediatric clinical trial. I have provided comments on labeling to the division separately.

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/s/

PAUL C BROWN
09/20/2013

Memo to the File

Application: NDA 204819
Supporting documents: 038
Applicant's letter date: September 19, 2013
CDER stamp date: September 19, 2013
Product: Adempas®(riociguat)
Indication: Chronic thromboembolic pulmonary hypertension and pulmonary arterial hypertension
Applicant: Bayer Healthcare
Review Division: Cardiovascular and Renal Products
Reviewer: Elizabeth Hausner, D.V.M.
Supervisor/Team Leader: Thomas Papoian, Ph.D.
Division Director: Norman Stockbridge, M.D., Ph.D.
Project Manager: Ed Fromm, Pharm D., M.B.A.

The current material was submitted in response to a question pertaining to section *13.1 Carcinogenicity* of the label. The reviewer calculated the human to animal exposure margins based on total drug in the plasma, with no correction for protein binding. The sponsor calculated the exposure margins based on free (unbound) drug in the plasma. The sponsor's correction for protein binding resulted in larger exposure margin between animals and humans than the reviewer's use of total drug. The following information request was therefore sent:

FDA REQUEST

To resolve the difference in exposure ratios for the Carcinogenicity section of the label, we need some information from you:

1. Section 2.6.7 Toxicology Tabulated Summary

Please explain the difference in AUC values for the mouse carcinogenicity study from page 86 to the summary table on page 15.

2. Please provide the human unbound exposure of riociguat that you used to calculate the exposure ratios for the label.

Using the numbers provided in the response, the reviewer calculated the exposures for the carcinogenicity studies, summarized in the table below.

Human exposure module 2.7.2, section 2.6.2				
AUC ₍₀₋₂₄₎ µg.hr/l	Percent free	Unbound exposure	CDER Margin of Exposure	Sponsor's margin of ex.
4161	4.97%	207		
Mouse maximum exposure			Ratio of mouse to human	
AUC 6622	20.1%	1331	6.4	
Rat maximum exposure			Ratio of rat to human	
9099	15.7%	1428	6.9	

The reviewer's calculations match the numbers in the sponsor's table immediately below. What doesn't match is the exposure ratios that the sponsor has put in the label. The label paragraph is on the next page. The highlighted numbers are those that should be in the label since we are accepting the premise of correcting for protein binding.

Table 10-10: Overview on systemic exposure of riociguat at steady state in the carcinogenicity studies and margins of exposure compared to human exposure

Daily dose	Sex	Total exposure				Unbound exposure			
		C _{max} [µg/L]	MoE	AUC(0-24) [µg-h/L]	MoE	C _{max, u} [µg/L]	MoE	AUC(0-24) _u [µg-h/L]	MoE
[ppm]									
Carcinogenicity study in mice (Module 4.2.3.4.1, PH-36818)									
50	M	58	0.3	917	0.2	11.7	1.2	184	0.9
100	M	157	0.8	2324	0.6	31.6	3.1	467	2.3
200	M	320	1.6	4308	1.0	64.3	6.4	866	4.2
50	F	69	0.3	1223	0.3	13.9	1.4	246	1.2
100	F	172	0.8	2925	0.7	34.6	3.4	588	2.8
200	F	416	2.0	6622	1.6	83.6	8.3	1331	6.4
[mg/kg]									
Carcinogenicity study in rats (Module 4.2.3.4.1, PH-36817)									
5	M	92	0.5	1526	0.4	14.5	1.4	240	1.2
10	M	155	0.8	2812	0.7	24.3	2.4	441	2.1
20	M	325	1.6	5923	1.4	51.0	5.1	930	4.5
5	F	104	0.5	1967	0.5	16.3	1.6	309	1.5
10	F	303	1.5	4999	1.2	47.6	4.7	785	3.8
20	F	515	2.5	9099	2.2	80.9	8.0	1429	6.9
Human exposure (Module 2.7.2, Section 2.6.2)									
7.5 mg ^a		203		4161		10.1		206.8	

MoE = margins of exposure (when compared to the human plasma levels at 7.5 mg/day)

a 7.5 mg riociguat/day corresponding to 0.125 mg/kg in a 60 kg patient

f_u mouse = 20.1 %, f_u rat = 15.7 %, f_u human = 4.97 % (see Module 2.6.4, Section 4.1)

Sponsor's proposed exposure ratios:

13 NONCLINICAL TOXICOLOGY

(b) (4)

CDER proposed exposure ratios:

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Fertility

Carcinogenesis: Carcinogenicity studies of riociguat were conducted in mice and rats. In mice, oral administration of riociguat (up to 25 mg/kg/day in males and 32 mg/kg/day in females) for up to two years did not reveal evidence of carcinogenic potential. Plasma riociguat exposure (AUC) of unbound drug at the highest dose was 6 times the human's exposure at the maximum recommended dose of 2.5 mg, t.i.d.

In rats, oral administration of riociguat (up to 20 mg/kg/day) for up to two years did not reveal evidence of carcinogenic potential. Plasma riociguat exposure (AUC) of unbound drug at the highest dose was 7 times the human exposure.

RECOMMENDATIONS: The difference in exposure ratios using the sponsor's numbers has been communicated to the sponsor for resolution.

No further action required at this time.

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/s/

ELIZABETH A HAUSNER
09/20/2013

THOMAS PAPOIAN
09/20/2013
Concur.

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: NDA204819
Supporting document/s: 31
Sponsor's letter date: July 12, 2013
CDER stamp date: July 12, 2013
Product: Adempas® (riociguat)
Indication: Chronic Thromboembolic Pulmonary Hypertension
and Pulmonary Arterial Hypertension
Sponsor: Bayer Healthcare
Review Division: Cardiovascular and Renal Products
Reviewer: Elizabeth Hausner, D.V.M.
Supervisor/Team Leader: Thomas Papoian, Ph.D.
Division Director: Norman Stockbridge, M.D., Ph.D.
Project Manager: Ed Fromm Pharm D., M.B.A.

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1 Executive Summary

The material in this submission was provided by the sponsor as part of the reply to the Information Request of May 10, 2013, in particular, the Division's request for an assessment of the bone findings from a veterinary pathologist with expertise in bone histopathology. The reviewing pathologist, Dr. Philip Long, assessed the bone findings from the repeat dose toxicology studies from rats and the dog 4 week, 13-week and 26 week studies.

Dr. Long noted that the alterations in the bones varied based on age, confirming this reviewer's assessment, made from the reviews of study reports. It was further noted that the changes indicated an increase in bone turnover with a bias towards osteoblast activity and a resulting net increase in bone. Due to the lack of apparent changes in the 2 year rat carcinogenicity study, it was suggested that the bone effects are reversible. Dr. Long also provided additional detail for two rats who demonstrated unscheduled mortality during the rat repeat dose mechanistic study. One rat was reported to have died due to septicemia and demonstrated bone changes consistent with that. This is in contrast to the original study report that stated only that the lesions were thought to be associated with a metabolic condition that remained undefined and undescribed. The second unscheduled mortality was a rat who was euthanized due to a gavage error that caused a perforated esophagus. Inflammation secondary to the traumatic injury was associated with changes in the sternum. This additional information and explanation about these two animals supports the sponsor's statements that these lesions were unlikely to be drug-related.

2 Background

Riociguat is a soluble guanylate cyclase (sGC) stimulator. Soluble guanylate cyclase catalyzes the generation of cyclic guanosine monophosphate (cGMP) from guanosine triphosphate (GTP). Riociguat is proposed to stabilize NO-sGC binding and directly stimulate sGC via a different binding site, independently of NO. One of the subsequent effects is smooth muscle relaxation.

The signaling molecule cGMP is also involved in cellular proliferation, fibrosis and inflammation. A sGC activator acts earlier in the biochemical pathway than does a phosphodiesterase inhibitor. Because PDE inhibitors increase cGMP levels by decreasing the hydrolysis of cGMP, the efficacy of the inhibitor may be limited by insufficient production of cGMP or compensation by other, non-inhibited, PDE isoforms. The sponsor proposes that the advantage of sGC activators for pulmonary hypertension is increased activity when NO production is limited.

2.1 Regulatory History

Adverse bone effects were reported in the original IND submission for riociguat and have been the subject of ongoing discussions with the sponsor. Both nonclinical and clinical aspects have been discussed and recorded in Minutes available in DARRTS. These communications are listed in the table below.

Reviewer's Summary of Official Communication Records During IND Phase

Date of communication	Filed in DARRTS
March 27, 2007. Teleconference with sponsor regarding questions from 30-day safety meeting, including bone	March 27, 2007
Internal meeting May 19, 2008. Preliminary responses, EOP2 meeting. Concerns for bone and endocrine effects noted.	May 22, 2008
EOP2 Meeting minutes May 29, 2008. Concerns for bone and endocrine effects noted.	June 14, 2008
Internal meeting October 2, 2009. Preliminary responses for Type C meeting: Clinical bone metabolism study discussed with recommendations for clinical monitoring.	October 8, 2009
Meeting with sponsor. October 9, 2009. Clinical bone study and nonclinical bone data discussed.	October 19, 2009
December 4, 2009. Email to sponsor. Clinical bone monitoring discussed.	December 4, 2009
June 14, 2011 telecon with sponsor regarding the nonclinical bone findings and implications for an osteoporotic population.	June 20, 2011
Advice/information email to sponsor. Necessity of juvenile animal studies prior to pursuing pediatric studies.	December 14, 2011

During the NDA review (April 18, 2013), photomicrographs were requested of the sponsor for purposes of showing the review team the age-related changes produced by the drug. The photomicrographs were shared with Luann Mckinney, DVM, DACVP, a Pharmacologist in CDER/DNP and a veterinary pathologist with expertise in bone. Her analysis of the images prompted several more questions that were provided to the sponsor in the April 24 information request.

To understand the reported bone effects, a 26 week mechanistic study in rats (Study A43289) was re-examined. This re-reading of the report prompted the questions that were sent to the sponsor on May 10 2013.

The nonclinical information requests sent to the sponsor during the NDA review pertaining to the bone issue are summarized in the reviewer's table below.

Reviewer's Summary of Information Requests Specifically Pertaining to Bone

Date of request	Date of response	Reviewer's request
April 18, 2013	April 19,2013	In preparation for our mid cycle meeting, please provide photomicrographs showing bone and/or articular cartilage effects from the following studies: 1. T2072968,4 week gavage study in rats 2. T0081434 Pilot study in neonatal rats 3. T4076731 13 week rat dietary administration study
April 24, 2013		A few more questions for the sponsor about the bone changes: 1. Please provide a magnification of the metaphyseal area of the bone section taken from the pilot juvenile animal study and a detailed description of the changes. Also provide a detailed description of the changes in the epiphyseal marrow of the same bone section. 2. Pertaining to the example of hyperostosis taken from the 26 week rat study: do you think this is endosteal or periosteal? 3. do you have any sections that were not decalcified and can be assessed for mineralization?
May 10, 2013	May 28, 2013	From Study A43289, 26 week mechanistic study in rats: Please provide detailed descriptions of the findings in the vertebrae, costae and humerus as well as an assessment of the findings from a veterinary pathologist with expertise in bone histopathology. Also provide photomicrographs of the findings to illustrate what you are describing and to show the spectrum of lesions from mild to severe. If Bayer feels that these findings are not relevant to the clinical situation, a detailed explanation of that position should be provided.

2.2 Current Submission

In the cover letter, the sponsor states that the current material is supplied in response to the May 10, 2013 information request. The current material contains:

- A review from Dr. Philip Long, DVM, DACVP and member of the INHAND working group on the skeletal system on the slides of bones from studies conducted with riocigugat.
- Photodocumentation on the variability of growth plate closure in dogs as well as birefringent collagen fiber distribution pattern in femurs of control and riocigugat treated rats.

3 Studies Submitted

Riociguat-related bone alterations

Documentation of morphological lesions

4 Study Reports

4.1 Anatomic Pathology Expert Review: Riociguat-related bone alterations

The author of the pathology expert review states that reports and bone slides from the following studies were examined (see sponsor's table below). In addition, bone and soft tissue from one moribund animal and one found dead in the Mechanistic Study on Bone Findings in Rats (report A43289) were also re-examined.

- 2-week Repeat-dose Juvenile Rat Study (Report PH-36257).
- 14-week Repeat-dose Juvenile Rat Study (Report PH-36659).
- 4-week Repeat-dose Rat Study (Report PH-34599).
- 13-week Repeat-dose Rat Study (Report PH-35987).
- 26-week Repeat-dose Rat Study (Report PH-35002).
- 2-year Rat Carcinogenicity Study (Report PH-36817).
- Chronic Mechanistic Study on Bone Findings in Rats (Report A43289).
- 2-week Mouse Study (Report PH-34519).
- 13-week Mouse Study (Report PH-34865)
- 2-year Mouse Carcinogenicity Study (Report PH-36818).
- 4-week Dog Study (Report PH-33454).
- 13-week Dog Study (Report PH-34778).
- 26-week Dog Study (Report PH-35050).

The report concluded that administration of riociguat to rats caused an accelerated bone turnover with a net increase in bone. The bone alterations varied in location as a function of age and the

severity of bone alterations was greater in males than females. These observations were noted as important for demonstrating that regional as well as sex-related differences in bone growth/cellular activity and biomechanical stress modulate riociguat bone effects. The age-related differences in lesions is summarized in the reviewer's table below.

Reviewer's Summary of Age-Related Bone Alterations

Study(ies)	Bone-region primarily affected	Pathologist's Comments
2-week juvenile	epiphysis	increased steoclasts, increased numbers of osteoclasts, and increased bone formation. Hematopoietic cells and fat cells in marrow displaced by stromal cells (osteoblast precursors) recruited to support accelerated bone turnover.
4-week	Metaphysis and physis	Metaphyseal bone spicules often lacked cartilage cores due to prior reorption/turnover and replacement, resulting in a net increase in bone. Intertrabecular regions contained prominent stromal cells (osteoblast precursors), which as in the epiphysis discussed above, were being recruited to support the accelerated bone turnover.
13- and 26-week	Cortical bone, posterior aspect more affected than anterior	increased remodeling within cortical bone seen as expansion of vascular channels within cortex and replacement of mature lamellar bone with woven bone.

Cortical perivascular cellularity and the amount of woven bone within the cortex were diminished in the 26 week study compared to the 13-week study, suggesting partial normalization. Animals in the 2-year study surviving to the end of the dosing phase had no detectable test article-related bone alterations, suggesting complete remodeling.

The reviewing pathologist makes a statement pertaining to osteoporosis. Referring to the 13-week rat study, Dr. Long notes that "Increased bone resorption was especially noticeable around vascular channels within cortical bone...Although bone resorption was increased, it is important that this not be mistaken for osteoporosis and to recognize that bone resorption was counterbalanced by a concurrent and disproportionate increase in new bone formation. The amount of new bone was so substantial that the cortical staining changed from predominantly eosinophilic to predominantly basophilic and the cortical thickness was noticeably increased. In contrast, in osteoporosis there is an uncoupling of bone formation and bone resorption in which resorption is increased and formation is decreased, resulting in net bone loss. "

A brief discussion was also given to two unscheduled mortalities from the mechanistic bone study (report A43289). Animal #221 was euthanized moribund due to a suspected dosing error. The patellar and bone lesions were consistent with secondary septicemia/infection and characterized by inflammation, marrow necrosis with intralesional bacteria. This is a somewhat different characterization than the original report which suggested a “metabolic disease” without further detail or explanation. Animal #225 who died on study due to a gavage accident was found to have esophageal perforation and subsequent inflammation of the sternum. The additional detail provided for these two animals appears to support the earlier statements that the bone lesions were not consistent with those associated with riociguat treatment.

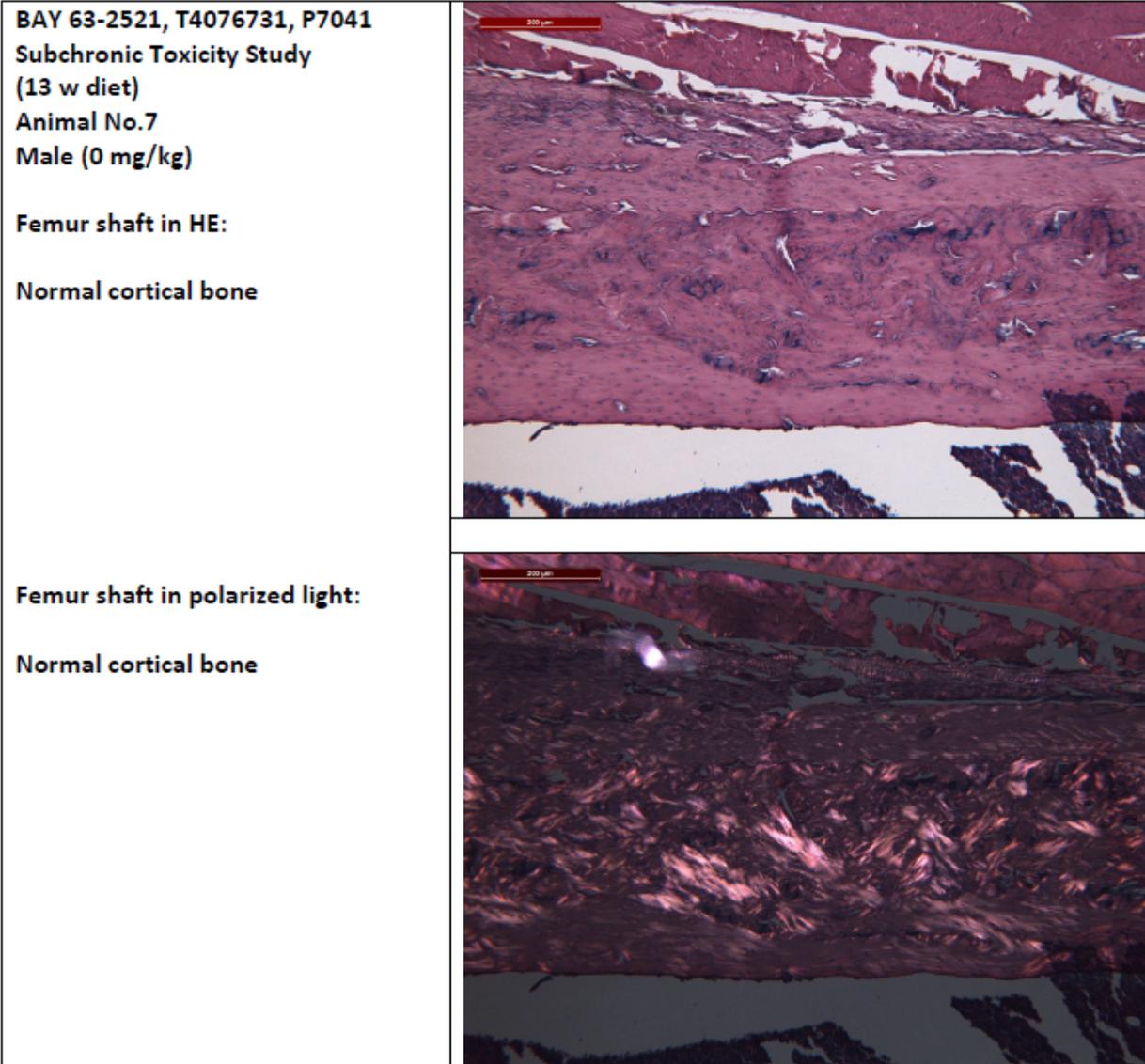
Overall pathology conclusion: Administration of riociguat to rats caused acceleration of bone turnover with a net increase in bone. The observations that the bone alterations varied in location as a function of age and that the severity of bone alterations was greater in males than in females are important and indicate that regional as well as sex-related differences in bone growth/cellular activity and biomechanical stress modulate riociguat bone effects. This is consistent with reports that NO is involved as a second messenger in mechanical and stress-induced bone formation and that it mediates the osteogenic effects of sex-steroid hormones.

Pathologist’s Listed Conclusions

- Riociguat-related bone alterations were restricted to rats and were characterized by increased bone turnover with a bias towards increased bone formation and a net increase in bone.
 - There was no evidence of bone fracture, deformity, inflammation, necrosis, hemorrhage, osteomalacia (defective mineralization), osteochondrosis, or arthritis. The presence of increased woven bone was not associated with fracture or deformity, indicating it was not adverse.
 - The bone alterations were reversible.
 - Findings are consistent with Riociguat acting on bone via the NO-sGC-cGMP pathway.
-
- The observation that there were no Riociguat-related bone alterations seen in dogs at systemic exposure levels that represented a multiple of the proposed human clinical exposure provides some assurance that the rat bone findings do not represent a safety concern for humans.
 - It is reasonable to assume that similar bone alterations in humans could be monitored for by using bone biomarkers and/or non-invasive techniques.

4.2 Documentation of Morphological Bone Lesions

In this report, the pathologist provided photomicrographs of control versus riociguat-treated animals. The photomicrographs compare the birefringent collagen fiber distribution patterns. The comparisons of the 13 week study are shown below as a representative example.



BAY 63-2521, T4076731, P7041

Subchronic Toxicity Study

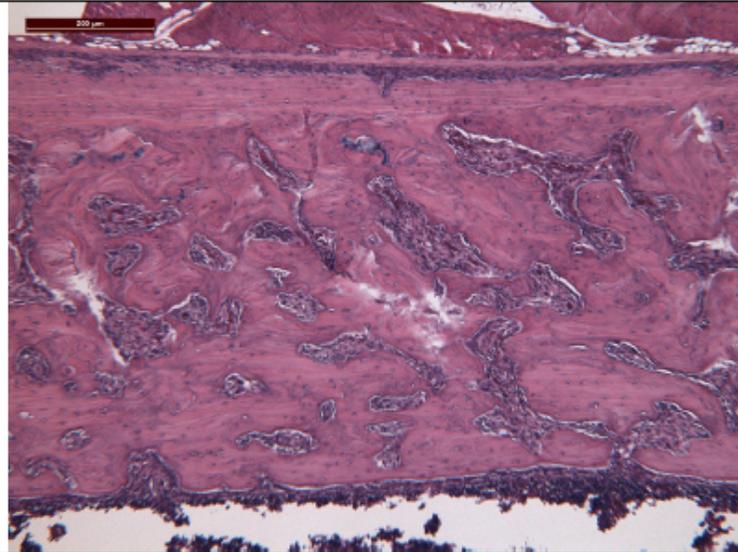
(13 w diet)

Animal No.38

Male (100 mg/kg)

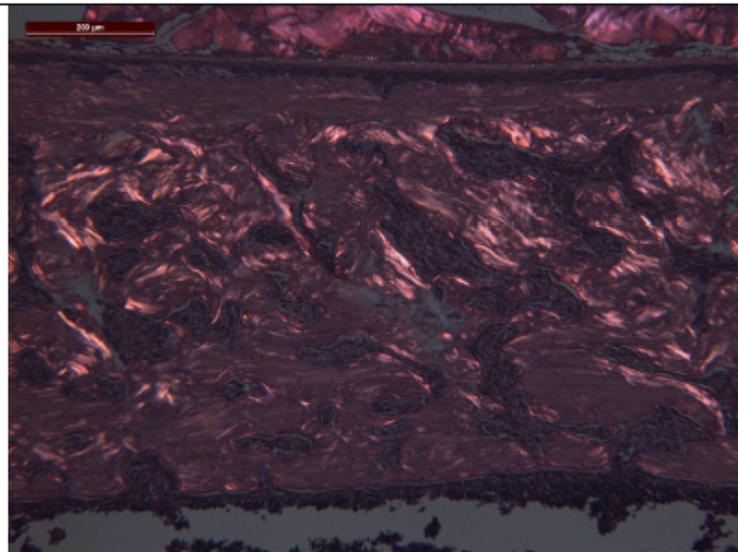
Femur shaft in HE:

**Increased remodeling/hyperostosis
grade 3**

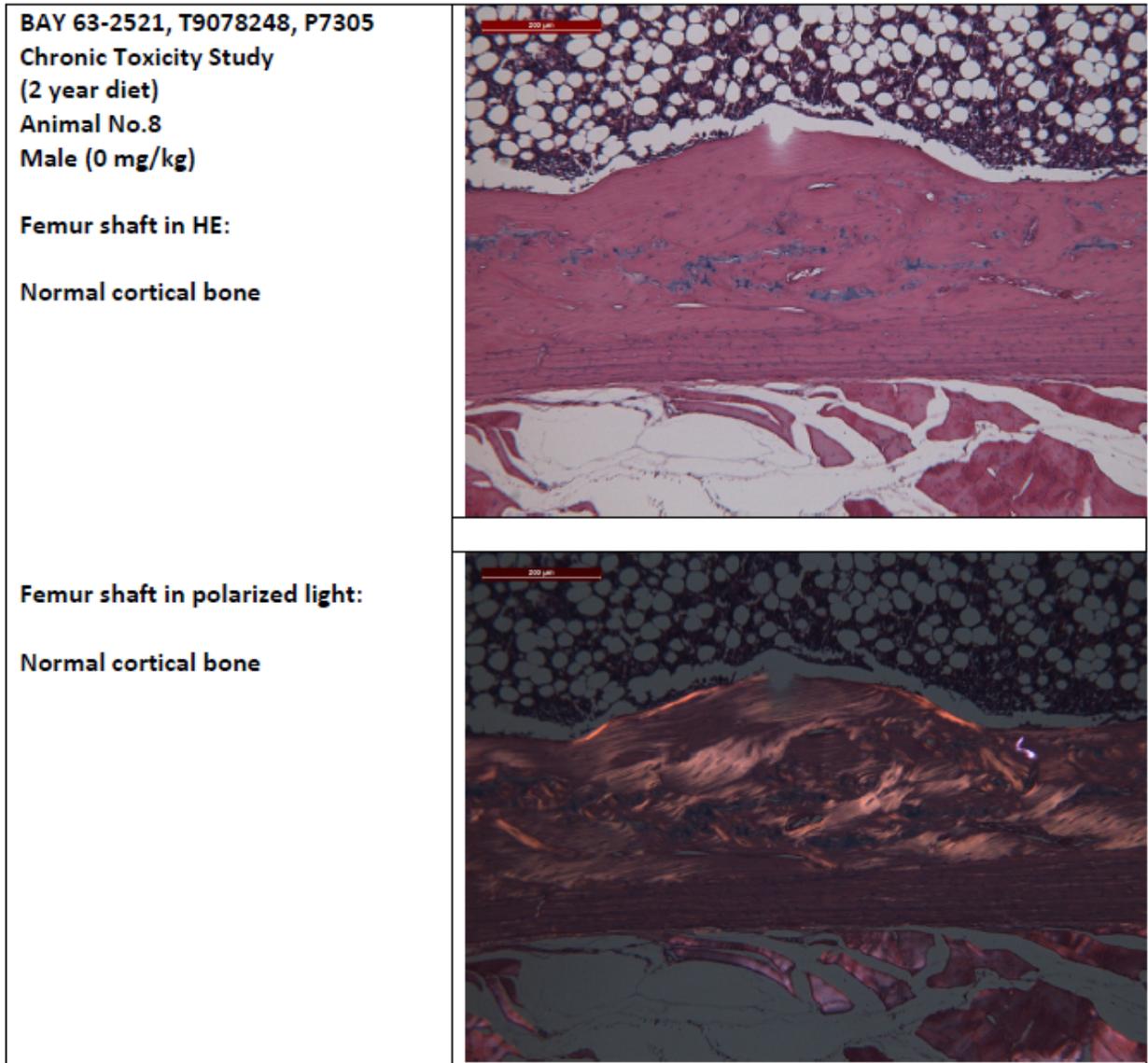


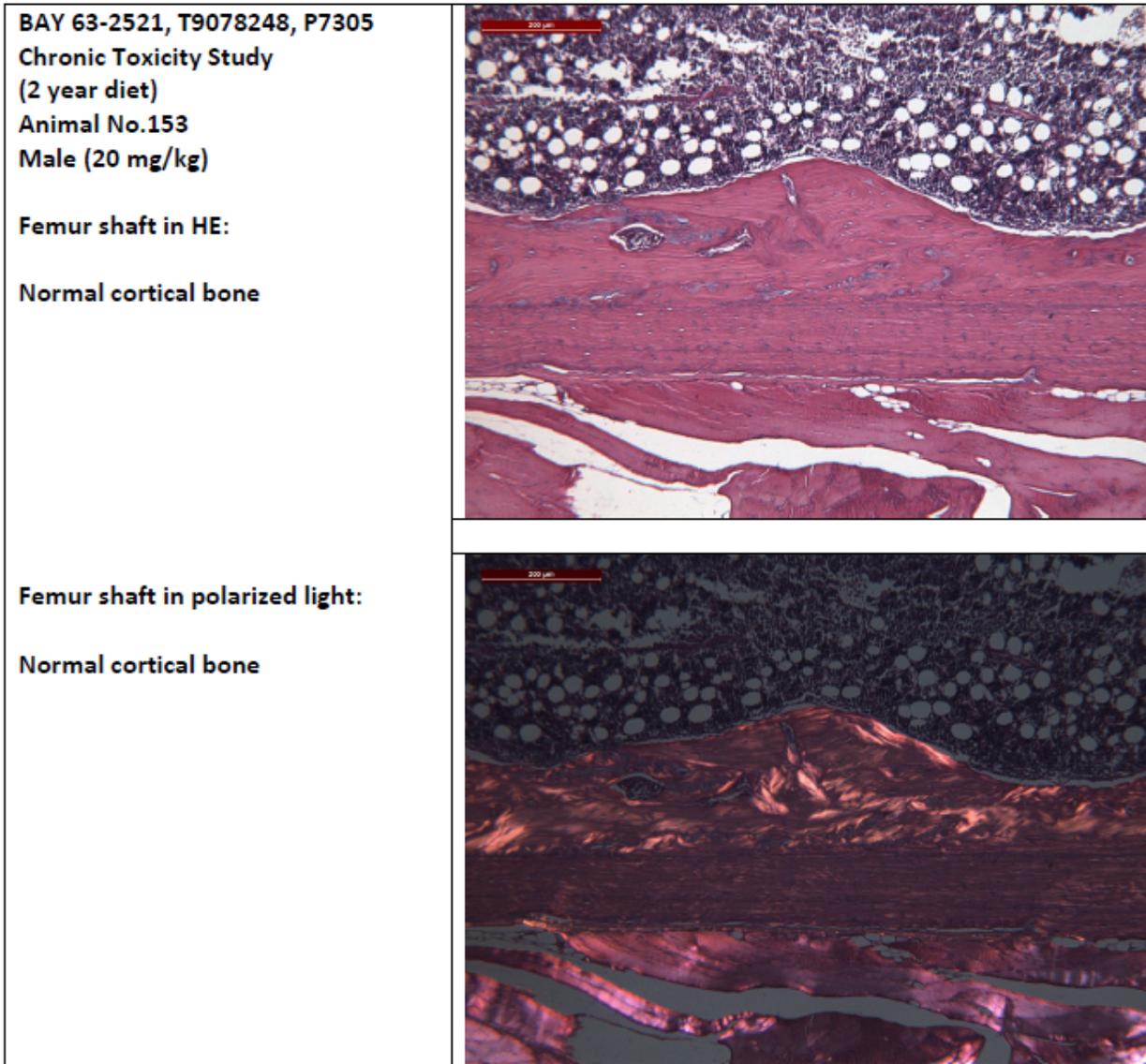
Femur shaft in polarized light:

**Increased amount of birefringent
collagen fibers, less regular fiber
orientation**



The photomicrographs for the 26 week study were similar to those shown above. The photomicrographs for the 2-year carcinogenicity study are shown below due to the variation in the birefringent pattern in the bone from the drug-treated rat.





It is a subjective observation of this untrained eye that there appears to be a difference in the birefringence pattern of the two examples from the 2-year study that might be described as a pattern of birefringence that appears to fall between normal controls and that seen in the drug-treated rat from the 13-week study.

The remainder of the expert report pertains to comparison of growth plate closure in the dog studies. This is primarily photomicrographs of head of the femur of control versus riociguat-treated dogs in the 13-week, 26-week and 52-week dog studies. There was very little text with the photos and I am unclear as to the overall direction and goal of this section.

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/s/

ELIZABETH A HAUSNER
07/19/2013

THOMAS PAPOIAN
07/19/2013
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**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

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Applicant's letter date: 05.30.2013

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Product: Adempas® (riociguat)

Indication: Chronic Thromboembolic Pulmonary Hypertension
and Pulmonary Arterial Hypertension

Applicant: Bayer Healthcare

Review Division: Cardiovascular and Renal Products

Reviewer: Elizabeth Hausner, D.V.M.

Supervisor/Team Leader: Thomas Papoian, Ph.D.

Division Director: Norman Stockbridge, M.D., Ph.D.

Project Manager: Ed Fromm R.Ph., RAC

Template Version: September 1, 2010 (Modified by DCRP: Feb. 6, 2013)

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The majority of pharmacology, safety pharmacology, and toxicology studies supporting this NDA were reviewed during the IND stage with the reviews available in DARRTS. This NDA also includes the rodent carcinogenicity studies and the definitive juvenile animal studies.

Summary of Reviews Filed During the IND Stage

Filing date	SDN	Topics
May 5, 2007	000, 001, 002, 003, 004, 005, 006	Riociguat: pharmacology, genetic toxicology, subacute toxicology, Fertility and early embryonic development, teratology in rats and rabbits, phototoxicity
June 29, 2007	008, 010, 011, 012	Riociguat Subacute oral toxicity in rats, dietary administration: 2 weeks and 13 weeks Subacute oral toxicity in mice, dietary administration: 2 weeks and 13 weeks Carcinogenicity protocols
May 5, 2008	015	Riociguat: 28 week rat study, 13 week dog study
August 18, 2008	027	M1 metabolite: BAY-60-4552 Safety pharmacology, phototoxicity, genetic toxicology, acute toxicity in mouse and rat after intravenous and oral administration, 4 week rat study, 2 week and 4week dog study
August 19, 2009	042, 068, 083	M1, BAY60-4552: rat 13-week gavage study Riociguat: 26 week and 52 week dog study, ADME studies, pharmacology
March 18, 2010	137	M1, BAY60-4552: pharmacology, ADME, 13 week dog study Riociguat: metabolism, bleeding time
May 25, 2011	130, 121, 098	Riociguat: pilot neonatal rat study, pre-and post-natal development, 26-week toxicity in rats. Bleeding time comparisons, ADME
July 5, 2011	132	ADME for riociguat. Renal mechanistic study for BAY60-4552
October 17, 2011	140	Preliminary bone effects in carcinogenicity studies, pharmacology

1 Executive Summary

1.1 Introduction (and Clinical Rationale)

Riociguat is a soluble guanylate cyclase (sGC) stimulator. Soluble guanylate cyclase catalyzes the generation of cyclic guanosine monophosphate (cGMP) from guanosine triphosphate (GTP). Riociguat is proposed to stabilize the NO-sGC binding and directly stimulate sGC via a different binding site, independently of NO. One of the subsequent effects is smooth muscle relaxation. The signaling molecule cGMP is also involved in cellular proliferation, fibrosis and inflammation. A sGC activator acts earlier in the biochemical pathway than do a phosphodiesterase inhibitors. Because PDE inhibitors increase cGMP levels by decreasing the hydrolysis of cGMP, the efficacy of the inhibitor may be limited by insufficient production of cGMP or compensation by other, non-inhibited, PDE isoforms. For treating pulmonary hypertension, PDE5 is important due to the selectivity of the approved drugs for this isoform. PDE3 is involved in control of cardiac contractility and PDE6 is found in the retina, which is involved in the phototransduction pathway. The sponsor proposes that the advantage to sGC activators is activity when NO production is limited.

1.2 Brief Discussion of Nonclinical Findings

Riociguat has been demonstrated to be negative in the standard battery of genotoxicity assays, the rodent two-year carcinogenicity studies and in special studies of phototoxicity and immunotoxicity. The toxicities for riociguat include teratogenicity, effects on bone metabolism, potential renal effects, and variable effects on serum glucose, thyroid hormones and the liver. The effects on serum glucose, liver and thyroid may be individual phenomena or collectively may be a manifestation of decreased gastrointestinal efficiency secondary to smooth muscle relaxation. Some inhibition of platelet aggregation has been demonstrated and may be considered either adverse or beneficial. With the available data, it is not possible to assess whether a parathyroid effect also exists.

Studies submitted for the active metabolite (M1 or BAY60-4552) indicate similar pharmacologic activity with approximately 10 fold lower potency. The sponsor attributes adverse renal effects to the metabolite. Neurologic effects are apparent with direct dosing of BAY60-4552 that are not apparent following administration of riociguat.

Interpretation of the nonclinical findings is complicated by the pharmacology of smooth muscle relaxation and decreased blood pressure. For effects on the renal, cardiovascular, and endocrine systems, it is unclear what, if anything, is due to a primary effect, and what may be due to decreased perfusion of a given organ.

The bone toxicity is incompletely described with minimal examination of bones other than the sternum, femur and tibia in the standard approaches used for animal safety assessment studies. There are a number of safety assessment studies with apparent changes in serum calcium and phosphorous levels. Whether this is spurious, within normal variability, secondary to bone effects or involves a parathyroid effect, cannot be determined from the data in hand.

There do not appear to be developmental effects as determined by the pre- and post-natal development (Segment III) study. A pilot juvenile animal study showed adverse effects of riociguat on developing bone. A definitive juvenile animal study using lower doses than the pilot study showed effects on serum electrolytes but without histologic correlates in bone or any other apparent effects.

1.3 *Recommendations*

1.3.1 *Approvability:*

Approvability depends upon clinical findings and whether the potential benefit is appropriate for the potential risk. From a pharmacology and toxicology perspective, the effects on bone suggest that caution is warranted in using this drug in children. Questions remain what effect, if any, this drug has on aging bone and the process of osteoporosis.

1.3.2 *Additional Non Clinical Recommendations*

A study conducted in dogs similar to studies used to address issues seen in the bisphosphonates may be appropriate and would include an in-life portion of 1 to 3 years (Chennekatu et al, 1996) and one year or more in rats (Bauss and Dempster, 2007). A well designed animal study that includes examination of bones such as mandible, nasal turbinates, calvarium, vertebrae, humerus, femur (including the neck) and tibia and clinical chemistry parameters such as levels of vitamin D and metabolites, parathyroid hormones, and urinary calcium excretion may help to address the issue of exacerbation of osteoporosis. Assessment of the mechanical properties of bone may also be prudent. A clinical trial with appropriate monitoring and imaging (e.g. high resolution MRI) may also be of value.

1.3.3 *Labeling*

Note: the sponsor's calculations of margins of exposure were based on plasma levels from healthy volunteers (AUC_{0-24} 1446 $\mu\text{g}\cdot\text{hr}/\text{l}$). The reviewer's exposure ratios are based on the plasma exposure in pulmonary hypertension patients reported in study 12166 (AUC_{0-24} 4161 $\mu\text{g}\cdot\text{hr}/\text{l}$).

The sponsor has proposed the following:

8.1 Pregnancy

Pregnancy Category X:  (b) (4)

 (b) (4)

This should be modified to read:

Use of <Tradename> is contraindicated in females who are or may become pregnant. While there are no adequate and well-controlled studies in pregnant females, animal studies show that riociguat may cause fetal harm when administered during pregnancy. In rats administered riociguat orally (1, 5, 25 mg/kg/day) throughout organogenesis, an increased rate of cardiac ventricular-septal defect was observed at the highest dose tested, which represents an exposure approximately 2.5 times that in humans at the MRHD of 2.5 mg three times a day based on AUC comparison. This dose also produced evidence of maternal toxicity (reduced body weight). Incomplete ossification of the 4th sacral vertebrae was apparent from the lowest dose of 1 mg/kg/day, or an exposure approximately 0.15 that in humans, without evidence of maternal toxicity. Post-implantation loss was statistically significantly increased from the mid-dose of 5mg/kg/day. In rabbits given doses of 0.5, 1.5 and 5 mg/kg/day, an increase in spontaneous abortions was seen starting at the middle dose of 1.5 mg/kg and an increase in resorptions was seen at 5 mg/kg/day, a dose which represents an exposure approximately 15 times that in humans at the MRHD. The highest dose tested also produced evidence of maternal toxicity (reduced body weight).

Oral administration of riociguat (1.5, 5 and 15 mg/kg/day) to rats throughout organogenesis and lactation did not cause any significant effects. The lowest dose tested was four times the exposure for humans taking riociguat at the MRHD based on body surface area.

The sponsor proposes the following:

12.1 Mechanism of Action

Riociguat is a stimulator of soluble guanylate cyclase (sGC), an enzyme in the cardiopulmonary system and the receptor for nitric oxide (NO).

When NO binds to sGC, the enzyme catalyzes synthesis of the signaling molecule cyclic guanosine monophosphate (cGMP). Intracellular cGMP plays an important role in regulating processes that influence vascular tone, proliferation, fibrosis and inflammation.

Pulmonary hypertension is associated with endothelial dysfunction, impaired synthesis of nitric oxide and insufficient stimulation of the NO-sGC-cGMP pathway.

Riociguat has a dual mode of action. It sensitizes sGC to endogenous NO by stabilizing the NO-sGC binding. Riociguat also directly stimulates sGC via a different binding site, independently of NO.

(b) (4)

This is acceptable with removal of the last sentence and should thus read:

12.1 Mechanism of Action

Riociguat is a stimulator of soluble guanylate cyclase (sGC), an enzyme in the cardiopulmonary system and the receptor for nitric oxide (NO).

When NO binds to sGC, the enzyme catalyzes synthesis of the signaling molecule cyclic guanosine monophosphate (cGMP). Intracellular cGMP plays an important role in regulating processes that influence vascular tone, proliferation, fibrosis and inflammation.

Pulmonary hypertension is associated with endothelial dysfunction, impaired synthesis of nitric oxide and insufficient stimulation of the NO-sGC-cGMP pathway.

Riociguat has a dual mode of action. It sensitizes sGC to endogenous NO by stabilizing the NO-sGC binding. Riociguat also directly stimulates sGC via a different binding site, independently of NO, leading to increased generation of cGMP.

[Redacted] (b) (4)

The sponsor proposes the following:

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

[Redacted] (b) (4)

[Redacted] (b) (4)

[Redacted] (b) (4)

Impairment of Fertility

In rats, no effects on male and female fertility were [Redacted] (b) (4)

This should be changed to read:

Carcinogenesis

Carcinogenicity studies of riociguat were conducted in mice and rats. In mice, oral administration of riociguat at 6, 12, 25 mg/kg/day in males and 8, 16 and 32 mg/kg/day in females for up to two years did not result in evidence of carcinogenic potential. Systemic exposure (AUC) at the highest dose was 1.6 times the human exposure at the MRHD.

In male and female rats, oral administration of riociguat at 5, 10, 20 mg/kg/day for up to two years did not result in evidence of carcinogenic potential. Systemic exposure (AUC) of riociguat at the highest dose was 2 times the human exposure at the MRHD.

Mutagenesis

Riociguat and its active metabolite did not show genotoxic potential in the *in vitro* bacterial reverse mutation (Ames) assay, the *in vitro* chromosomal aberration assay in human peripheral blood lymphocytes, or the *in vivo* micronucleus assay in the rat.

Impairment of Fertility

In male and female rats, oral administration of riociguat (3, 10, 30 mg/kg/day) prior to and throughout the mating period had no effect on fertility. The highest dose of 30 mg/kg/day is 117 times the MRHD based on a mg/m² comparison.

2 Drug Information*2.1 Drug*

CAS Registry Number
625115-55-1

Generic Name
Riociguat

Code Name
BAY63-2521, BAY63-2521 micronized

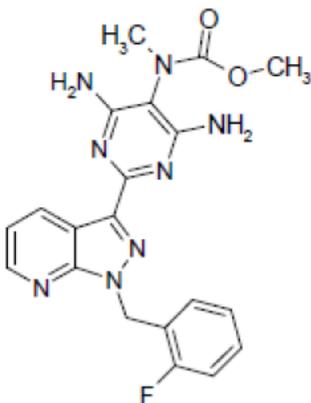
Chemical Name

Methyl 4,6-diamino-2-[1-(2-fluorobenzyl)-1H-pyrazolo[3,4-b]pyridin-3-yl]-5-pyrimidinyl (methyl)carbamate
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Molecular Formula/Molecular Weight

C₂₀H₁₉FN₈O₂/ 422.42

Structure or Biochemical Description



Pharmacologic Class

Soluble guanylate cyclase (sGC) activator

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND75629

Pre-IND (b) (4) is for

(b) (4)

This was submitted for

(b) (4)

2.3 Drug Formulation

The immediate release clinical formulation contains: riociguat and the excipients lactose, microcrystalline cellulose, crospovidone, magnesium stearate, hypromellose and sodium lauryl sulfate. The tablets are coated with hydroxypropyl cellulose, hypromellose, propylene glycol, titanium dioxide and/or iron oxide (red and/or yellow).

2.4 Comments on Novel Excipients

None of the excipients are novel.

2.5 Comments on Impurities/Degradants of Concern

Email communication with the review chemists February 28, 2013 indicates that there are no impurities of concern.

2.6 *Proposed Clinical Population and Dosing Regimen*

Persistent/recurrent chronic thromboembolic pulmonary hypertension (CTEPH) (WHO Group 4) after surgical treatment or inoperable CTEPH to improve exercise capacity and WHO functional class. Pulmonary arterial hypertension (PAH) (WHO Group 1) to improve exercise capacity, improve exercise capacity, improve WHO functional class and to delay clinical worsening. The sponsor recommends that treatment be initiated at 1 mg three times daily (t.i.d.), 6 to 8 hours apart, with or without food. The dosage is increased by 0.5 mg increments in approximately 2-week intervals.

2.7 *Regulatory Background*

IND75629 was presented to the Division February 20, 2007. At that time, the drug had already been tested in four clinical pharmacology studies in Europe. The nonclinical studies suggested adverse bone and cardiac effects from riociguat. The bone effects were reported for pre-natal rats (teratology) and young rats in standard toxicology studies. This is consistent with published information suggesting that sGC modulates osteoclast/osteoblast balance. Cardiac effects were also noted in teratology studies and in the adult animal toxicology studies. The adult effects included both QTc prolongation and apparent cardiac morphologic damage. The above-mentioned points were discussed with the sponsor in a telecon March 27, 2007 (minutes filed in DARRTS March 27, 2007).

The effects on bone were addressed further in submissions 98, 121 and 130 (review for these three submissions in DARRTS, entry date May 25, 2011). This material included a pilot study in neonatal rats, pre- and post-natal (Segment III) developmental study and a time course study in mature rats. A second neonatal rat study was submitted with the NDA. A teleconference was held between the sponsor, the medical officer, the pharm/tox reviewer and the respective team leaders to discuss the possible bone effects of BAY63-2521 noted in the neonatal pilot study (minutes available in DARRTS, filed June 20, 2011). In response to this telecom, the sponsor provided:

1. data on the interaction of riociguat with the natriuretic peptides and the natriuretic peptide receptors.
2. summary of bone-related findings from the 2-year carcinogenicity studies
3. update on enrollment in the Phase III development program and rationale behind the current entry criteria in clinical studies as it relates to the inclusion of patients with a history of osteoporosis or bone fractures
4. life-time risk of osteoporosis in the target populations
5. rationale behind the current approach for monitoring for potential bone effects and specifically for using carboxy-terminal cross-linking of telopeptide of type I collagen (CTX) measurements to assess for effects in humans.

An exploratory renal effects study was provided in sequence number 132 (review in DARRTS, filed July 5, 2011). This study examined several biomarkers of renal damage in conjunction with the main metabolite, M1 (BAY 60-4552) treatment.

The BAY60-4552, is also pharmacologically active and was in development as a separate entity for biventricular heart failure. Based upon a face to face meeting held October 9, 2009 (minutes filed in DARRTS October 19, 2009) and a request from the sponsor (correspondence dated

November 13, 2009) for clarification of several items discussed at the October 9th meeting, the Division requested that Bayer HealthCare "...submit all clinical information and additional preclinical information not previously submitted since April 30, 2008 that is available for BAY60-4552." This request is available in DARRTS (Advice/Information Request, filed December 4, 2009 in DARRTS). The sponsor has provided almost an entire development package, more information than is typically available for an active metabolite.

Photomicrographs of the bone lesions were requested of the sponsor for purposes of illustration to the review team. The reviewer requested photomicrographs from studies T2072968 (4 weeks dosing, 2 weeks recovery), T4076731 (13 weeks dosing), T0081434 (pilot juvenile animal study, 14 days of dosing). The sponsor provided this information in SDN008. In consultation with L. McKinney, DVM, DACVP, currently a reviewer in the Division of Neurologic Products, the photomicrographs of the lesions raised additional questions. These have been posed to the sponsor and answered in SDN 0016.

The communications with the sponsor pertaining to the bone issue or in which it was discussed during the IND stage are summarized in the reviewer's table below.

Reviewer's Summary of Official Communication Records

Date of communication	Filed in DARRTS
March 27, 2007. Teleconference with sponsor regarding questions from 30-day safety meeting, including bone	March 27, 2007
Internal meeting May 19, 2008. Preliminary responses, EOP2 meeting. Concerns for bone and endocrine effects noted.	May 22, 2008
EOP2 Meeting minutes May 29, 2008. Concerns for bone and endocrine effects noted.	June 14, 2008
Internal meeting October 2, 2009. Preliminary responses for Type C meeting: Clinical bone metabolism study discussed with recommendations for clinical monitoring.	October 8, 2009
Meeting with sponsor. October 9, 2009. Clinical bone study and nonclinical bone data discussed.	October 19, 2009
December 4, 2009. Email to sponsor. Clinical bone monitoring discussed.	December 4, 2009
June 14, 2011 telecon with sponsor regarding the nonclinical bone findings and implications for an osteoporotic population.	June 20, 2011
Advice/information email to sponsor. Necessity of juvenile animal studies prior to pursuing pediatric studies.	December 14, 2011

To understand the reported bone effects, Study A43289, a 26 week mechanistic study in rats was re-examined. This re-reading of the report prompted the questions that were sent to the sponsor May 10 2013.

The nonclinical information requests sent to the sponsor during the NDA pertaining to the bone issue are summarized in the reviewer's table below.

The communications to the sponsor pertaining to the bone issue during the NDA stage are summarized in the reviewer's table below.

Reviewer's Summary of Information Requests Specifically Pertaining to Bone

Date of request	Date of response	Reviewer's request
April 18, 2013	April 19,2013	In preparation for our mid cycle meeting, please provide photomicrographs showing bone and/or articular cartilage effects from the following studies: 1. T2072968,4 week gavage study in rats 2. T0081434 Pilot study in neonatal rats 3. T4076731 13 week rat dietary administration study
April 24, 2013		A few more questions for the sponsor about the bone changes: 1. Please provide a magnification of the metaphyseal area of the bone section taken from the pilot juvenile animal study and a detailed description of the changes. Also provide a detailed description of the changes in the epiphyseal marrow of the same bone section. 2. Pertaining to the example of hyperostosis taken from the 26 week rat study: do you think this is endosteal or periosteal? 3. do you have any sections that were not decalcified and can be assessed for mineralization?
May 10, 2013	May 28, 2013	From Study A43289, 26 week mechanistic study in rats: Please provide detailed descriptions of the findings in the vertebrae, costae and humerus as well as an assessment of the findings from a veterinary pathologist with expertise in bone histopathology. Also provide photomicrographs of the findings to illustrate what you are describing and to show the spectrum of lesions from mild to severe. IF Bayer feels that these findings are not relevant to the clinical situation, a detailed explanation of that position should be provided.

3 Studies Not Reviewed

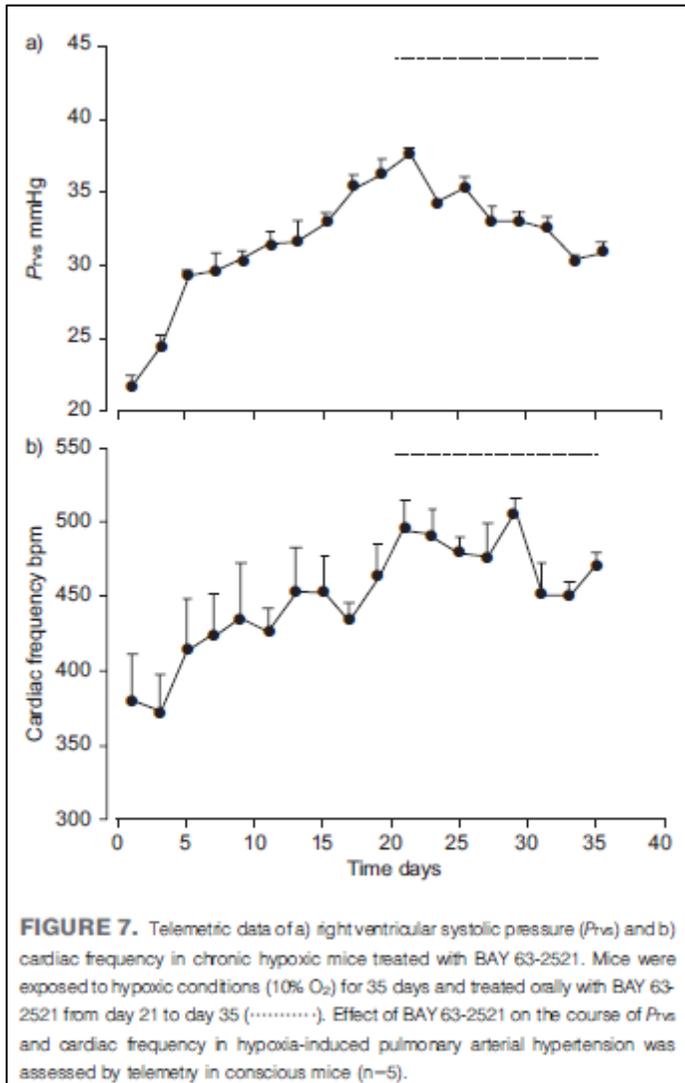
None

4 Pharmacology

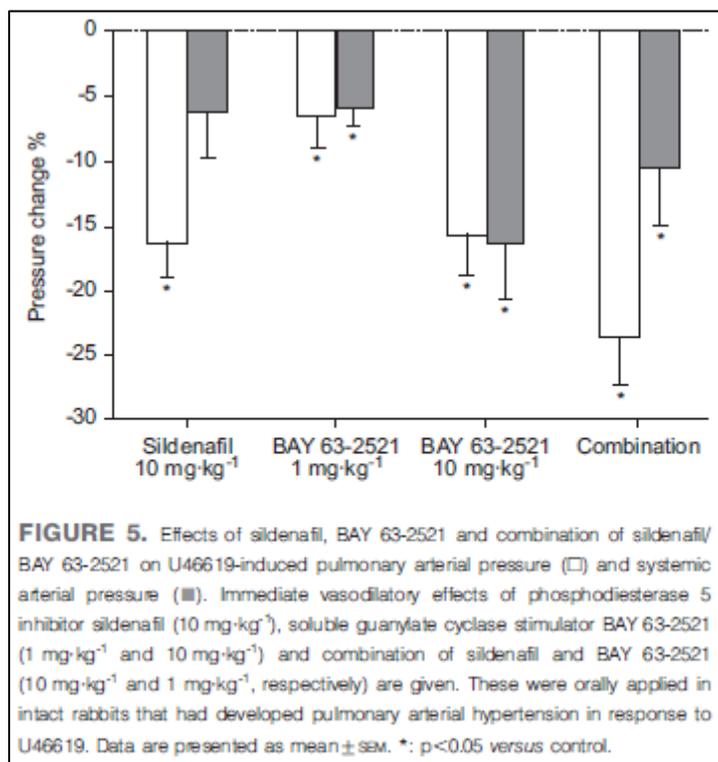
In vitro and cell culture preparations demonstrated riociguat-induced stimulation of sGC by increased cGMP concentration. Organ culture preparations of isolated vasculature demonstrated the ability of riociguat to relax phenylephrine-constricted vessels that were unresponsive to nitrates. Langendorff preparations were used to show reduced coronary perfusion pressure after riociguat administration. Normotensive rats, spontaneously hypertensive rats and normal dogs were used to demonstrate riociguat's hemodynamic effects of decreased mean arterial pressure, increased coronary blood flow and oxygen saturation. The sponsor demonstrated by immunohistochemistry the presence of increased sGC in pulmonary arterial vessels of idiopathic PAH lungs from human transplant patients compared to healthy human donors. (Schemuly et al 2008).

The sponsor also used three models of induced pulmonary hypertension to evaluate the pharmacology of riociguat. The effect of riociguat on the *development* of PAH was not investigated, rather, treatment was initiated 3 weeks after chronic hypoxia or chemical induction

of pathology when the elevated pulmonary arterial pressure, vascular remodeling and right heart hypertrophy were *established*. Mice subjected to chronic hypoxia showed changes in the heart suggestive of pulmonary hypertension. Administration of riociguat decreased right ventricular hypertrophy, and structural remodeling of the lung vasculature when compared to untreated controls. The sponsor's graph is shown below



Rabbits with PAH induced by U46619 (undefined) showed decreased pulmonary arterial pressure after treatment with riociguat, sildenafil or the combination of the two drugs. The sponsor's graph of this is shown below.



Monocrotaline-induced pulmonary hypertension in rats was treated with 10 mg/kg riociguat daily for 14 days. Right ventricular systolic pressure (RVSP) was significantly decreased compared to vehicle treated rats. Total pulmonary resistance was also decreased but systemic arterial pressure

did not change in response to treatment. (Schermuly et al 2008).

Combined exposure to the vascular endothelial growth factor receptor antagonist SU5416 and hypoxia (10% O₂) (SUHx) caused a severe PAH in rats. Administration of riociguat significantly decreased right ventricular hypertrophy, increased cardiac output and decreased total pulmonary resistance compared with vehicle treated animals. The studies using models of PAH were submitted as journal publications rather than study reports.

4.1 Primary Pharmacology

BAY63-2521

A CHO cell line over-expressing sGC was used to characterize different activators of sGC. BAY63-2521 stimulated the reporter cell line with a minimal effective concentration of 100 nM (0.1 μM). In a second study, BAY63-2521 dose-dependently stimulated the recombinant reporter CHO cell line with an EC₅₀ of 180 nM. Addition of the NO-donor SIN-1 to BAY63-2521-treated cells caused a potentiation of the EC₅₀ values. A standard receptor screen showed no significant levels of affinity, defined as ≥50% inhibition or displacement, of the test article for any of the receptors tested

Tab. 1: Displacement of various radioligands by BAY 63-2521.

CAT. #	TARGET	BATCH	SPP.	n=	CONC.	% INHIBITION					
						-100	-50	0	50	100	
						%	↓	↓	↓	↓	↓
118050	CYP450, 1A2	69607	hum	2	10 µM	3					
118070	CYP450, 2C19	69609	hum	2	10 µM	7					
118060	CYP450, 2C9	69608	hum	2	10 µM	15					
118080	CYP450, 2D6	69681	hum	2	10 µM	-1					
118090	CYP450, 3A4	69610	hum	2	10 µM	19					
200510	Adenosine A ₁	69082	hum	2	10 µM	7					
200610	Adenosine A _{2A}	69083	hum	2	10 µM	7					
200800	Adenosine A _{2B}	69084	hum	2	10 µM	10					
203100	Adrenergic α _{1A}	69126	rat	2	10 µM	-3					
203200	Adrenergic α _{1B}	69127	rat	2	10 µM	-4					
203400	Adrenergic α _{1C}	69128	hum	2	10 µM	9					
203620	Adrenergic α _{2A}	69130	hum	2	10 µM	-15					
203710	Adrenergic α _{2B}	69131	hum	2	10 µM	5					
204010	Adrenergic β ₁	69226	hum	2	10 µM	4					
204110	Adrenergic β ₂	69227	hum	2	10 µM	-4					
204410	Adrenergic, Norepinephrine Transporter	69208	hum	2	10 µM	-29					
212500	Bradykinin B ₁	69110	hum	2	10 µM	3					
212610	Bradykinin B ₂	69168	hum	2	10 µM	5					
214510	Calcium Channel Type L, Benzothiazepine	69218	rat	2	10 µM	-1					
214600	Calcium Channel Type L, Dihydropyridine	69134	rat	2	10 µM	-1					
216000	Calcium Channel Type N	69426	rat	2	10 µM	-6					
219500	Dopamine D ₁	69182	hum	2	10 µM	16					
219600	Dopamine D _{2A}	69183	hum	2	10 µM	-4					
219800	Dopamine D ₂	69184	hum	2	10 µM	-3					
219900	Dopamine D _{4L}	69468	hum	2	10 µM	6					
220320	Dopamine Transporter	69209	hum	2	10 µM	3					
224010	Endothelin ET _A	69230	hum	2	10 µM	-3					
224110	Endothelin ET _B	69231	hum	2	10 µM	-7					
225500	Epidermal Growth Factor (EGF)	69161	hum	2	10 µM	32					
226010	Estrogen ERα	69116	hum	2	10 µM	6					
226400	GABA Transporter	69342	rat	2	10 µM	-3					

CAT. #	TARGET	BATCH	SPP.	n=	CONC.	% INHIBITION					
						-100	-50	0	50	100	
226500	GABA _A , Agonist Site	69344	rat	2	10 µM	7					
226600	GABA _A , Benzodiazepine, Central	69429	rat	2	10 µM	2					
228510	GABA _A	69099	rat	2	10 µM	-4					
232010	Glucocorticoid	69167	hum	2	10 µM	4					
232700	Glutamate, Kainate	69104	rat	2	10 µM	-6					
232810	Glutamate, NMDA, Agonism	69105	rat	2	10 µM	11					
232910	Glutamate, NMDA, Glycine	69106	rat	2	10 µM	10					
233000	Glutamate, NMDA, Phencyclidine	69107	rat	2	10 µM	-1					
239510	Histamine H ₁ , Central	69164	gp	2	10 µM	9					
239700	Histamine H ₂	69162	gp	2	10 µM	16					
239800	Histamine H ₃	69163	rat	2	10 µM	0					
241000	Imidazoline I ₃ , Central	69165	rat	2	10 µM	-15					
243510	Interleukin IL-1 α	69111	mouse	2	10 µM	-1					
250510	Leukotriene B ₄	69166	hum	2	10 µM	11					
250600	Leukotriene D ₄	69169	gp	2	10 µM	6					
252600	Muscarinic M ₁	69186	hum	2	10 µM	7					
252700	Muscarinic M ₂	69187	hum	2	10 µM	-2					
252800	Muscarinic M ₃	69188	hum	2	10 µM	-5					
257000	Neuropeptide Y ₁	69094	hum	2	10 µM	1					
257110	Neuropeptide Y ₂	69118	hum	2	10 µM	2					
258600	Nicotinic Acetylcholine, Central	69101	rat	2	10 µM	9					
260110	Opiate δ	69170	hum	2	10 µM	-12					
260210	Opiate κ	69171	hum	2	10 µM	3					
260410	Opiate μ	69172	hum	2	10 µM	-4					
264500	Phorbol Ester	69112	mouse	2	10 µM	-3					
265010	Platelet Activating Factor (PAF)	69345	hum	2	10 µM	-1					
265600	Potassium Channel [K _{ATP}]	69343	syh	2	10 µM	-10					
268700	Purinergic P _{2U}	69194	rabbit	2	10 µM	-8					
268810	Purinergic P _{2V}	69109	rat	2	10 µM	15					
271110	Serotonin 5-HT _{1A}	69217	hum	2	10 µM	-8					
271650	Serotonin 5-HT _{1A}	69141	hum	2	10 µM	6					
271910	Serotonin 5-HT ₂	69136	hum	2	10 µM	-4					
274020	Serotonin Transporter	69210	hum	2	10 µM	14					
278110	Sigma σ_1	69137	hum	2	10 µM	-4					
278200	Sigma σ_2	69138	rat	2	10 µM	3					
279510	Sodium Channel, Site 2	69140	rat	2	10 µM	1					
255510	Tachykinin NK ₁	69329	hum	2	10 µM	-11					
285010	Testosterone	69214	rat	2	10 µM	3					

Vascular rings were used *in vitro* to demonstrate vaso-relaxant properties of riociguat. Glycerol trinitrate inhibited phenylephrine-induced contraction with IC₅₀ of 13 nM in control vessels and IC₅₀ of 65 nM in nitrate tolerant vessels. Riociguat inhibited the phenylephrine-induced

contraction in normal and nitrate-tolerant saphenous artery rings with IC₅₀ values of 4.8 nM and 5.6nM, respectively.

vessel	drug	IC ₅₀
Saphenous artery rings	Glycerol trinitrate	13nM
Nitrate-tolerant saphenous artery rings	Glycerol trinitrate	65nM
Saphenous artery rings	Riociguat	4.8nM
Nitrate-tolerant saphenous artery rings	Riociguat	5.6 nM
Rabbit aortic rings	Riociguat	95 nM
Rabbit saphenous artery rings	Riociguat	200 nM
Canine femoral vein rings	Riociguat	340 nM
Rabbit corpus cavernosum	Riociguat	110 nM

Effects on blood pressure were reported. The influence of BAY63-2521 on cardiovascular function and respiration was examined in anesthetized, artificially ventilated Beagles after single intraduodenal administration of 0, 0.01, 0.03, 0.1 and 0.3 mg/kg body weight. Beginning after administration of 0.1 mg/kg, vasodilatory effects occurred, with a reported decrease of total peripheral resistance by up to 44% at the HD and a decrease in left ventricular end diastolic pressure (LVEDp) in the same group

Cardiovascular effects of the main metabolite compared to the parent drug were examined in anesthetized dogs after 1 hour of IV infusions. There seemed to be equivalent efficacy between 30 µg/kg/hour BAY 63-2521 and 100 µg/kg/hour M1. However, there is no data to show that a lower dose of M1 is less effective, i.e. that a plateau had been reached.

Decrease in mean aortic pressure after infusion

	Time after infusion			
	15 minutes	30 minutes	45 minutes	60 minutes
BAY63-2521 (30 µg/kg/hr)	7.0	15.3	22.5	28.0
M1 (100 µg/kg/hr)	7.6	13.4	18.3	20.9%

Following infusion with either compound, the decrease in mAoP was accompanied by a decrease in LVP, LVEDP, -dP/dt, CVP, PAP and SVR. There were mild reflex increases in HR and +dP/dt. The hemodynamic effects lasted beyond the 3 hour observation period.

BAY60-4552

A comparison of BAY63-2521 and BAY60-4552 showed different IC₅₀ values for inhibition of phenylephrine-induced vasorelaxation. There was approximately a 10-fold difference in effective inhibitory concentrations.

Reviewer's summary of vasorelaxant effects

Species/vessel	IC ₅₀ BAY63-2521	IC ₅₀ BAY60-4552
Rabbit saphenous artery rings	554 nM	5470 nM
Rabbit aortic rings	640 nM	6862 nM
Porcine coronary artery rings	601 nM	5220 nM

A radioligand binding screen showed no significant interaction, defined as $\geq 50\%$ inhibition or displacement, of the test article with any of the tested receptors or enzymes (reviewed in 050.doc, available in DARRTS). The M1 metabolite was also tested for interaction with human phosphodiesterase enzymes 11A, 9A, 8A, 7B, 4B, 3B, 2A and 5 and against bovine PDE6 and 1. The IC_{50} values were $>10^{-4}$ M for the isoforms evaluated, suggesting little affinity.

BAY 1077251 (M4)

Metabolite M4 is the N-glucuronide of BAY60-4552. It has been demonstrated as lacking in biological activity against isolated sGC up to concentrations of 10 μ M and on phenylephrine-induced contractions of rabbit aortic rings.

Soluble and Particulate Guanylate Cyclases

The distinction between the soluble and particulate guanylyl cyclases was part of the characterization of riociguat's effects on bone. A comparison of the different forms of the guanylyl cyclases may be found in the review filed May 25, 2011. In response to a request from the Division, the sponsor investigated the specificity of BAY63-2521 for particulate GC receptors. Chinese hamster ovary cells stably expressing aequorin were co-transfected for cyclic nucleotide gated cation channel (CNGA2) followed by rat guanyl cyclase A (specific for atrial natriuretic peptide) or guanyl cyclase B (specific for C natriuretic peptide).

Under the conditions used, BAY63-2521 did not appreciably stimulate either the GC-A or the GC-B reporter cell line. As a positive control, ANP stimulated the GC-A cell line with an EC_{50} value of 1.9×10^{-10} M. The positive control for the GC-B reporter cell line, CNP, was reported to have an EC_{50} value of 2.8×10^{-10} M in the appropriate test system. Under the conditions of the experiments, riociguat has little affinity for the particulate guanylyl cyclases.

4.2 Secondary Pharmacology

PH34507 The sGC isoforms are found in platelets in relatively high concentration. By formation of cGMP as a second messenger, sGC plays a role in platelet aggregation and adhesion. Vasodilator-stimulated phosphoprotein (VASP) is a cGMP-dependent protein kinase (PKG) substrate and has been characterized as an important substrate of both cAMP-dependent protein kinase (PKA) and PKG in human platelets. In vitro, riociguat increased both VASP phosphorylation and cGMP levels as did sodium nitroprusside (SNP) and DEA/NO. Riociguat in combination with SNP or DEA/NO produced synergistic effects on both measured parameters. The levels of cAMP were not significantly altered.

The levels of cAMP did not change either with BAY60-4552 alone or in combination with sodium nitroprusside (SNP) or DEA. In combination with DEA/NO at $\geq 3 \mu$ M, BAY60-4552 (0.1 μ M) caused a slight increase in cAMP.

A direct assessment of drug effect on platelet aggregation, that is, use of *ex vivo* aggregometry methods, was presented for both riociguat and BAY60-4552. These are summarized in the reviewer's table below.

Summary of Platelet Aggregation Effects in BAY63-2521 and BAY60-4552

Aggregating stimulus	Human platelet rich plasma		Washed human platelets
	BAY63-2521	BAY60-4552	BAY60-4552
U46619	Not done	33.4 μ M	3.0 μ M
Collagen	59 μ M	27.5 μ M	4.8 μ M
TRAP 6	34 μ M	15.9 μ M	6.1
ADP	41 μ M	18.2 μ M	15.5
thrombin	Not done	Not done	2.2 μ M

4.3 Safety Pharmacology

Both BAY63-2521 and BAY60-4552 were evaluated for overt neurological effects (rats), cardiovascular effects (anesthetized dogs and conscious telemetered dogs), renal function and clinical chemistry (rats), and gastrointestinal transit (rats). This has been summarized in the reviewer's table below.

Summary of Single Dose Safety Pharmacology Study Results for Riociguat (BAY63-2521) and M1 Metabolite (BAY60-2552)

Test, species	BAY63-2521		BAY60-4552	
	doses	effects	doses	effects
Overt neurological rats	0, 0.3, 1, 3 mg/kg	Delayed response to painful stimulus (nocifensive response): 21% greater than control, p<0.05. No NOAEL	0,1,3,10 mg/kg	Piloerection, vocalization, ↓activity HD animals also showed tremor, irregular respiration, stereotypic chewing. No effect on nocifensive response ↑behavioral signs with ↑dose.
Cardiovascular, Anesthetized Beagles,	intraduodenal administration 0, 0.01, 0.03, 0.1,0.3 mg/kg	≥0.1 mg/kg: vasodilation, ↓TPR 44% ↓ systolic b.p. ↓ diastolic b.p. ↑ cardiac output (46%) 0.3 mg/kg : ↓LVEDp 45% ↓ blood pressure 22% Respiratory: no effect	Iv dosing 10, 30, 100, 1000 µg/kg	30 µg/kg (0.3 mg/kg): ↓tpr
Conscious, telemetered dog	Oral dosing, 0, 0.05, 0.1, 0.3 mg/kg	Dose related ↓ systolic blood pressure: up to 25% ↓ at highest dose	Oral dosing 0.15- 1.5 mg/kg	0.5 mg/kg: ↓systolic pressure
Conscious telemetered rat	Oral dosing, 0, 0.5, 1 and 3 mg/kg	Dose-related ↓ systolic blood pressure: up to 23% ↓ at highest dose	Oral dosing 0,1,3,10 mg/kg	Dose related increase in heart rate (38% at HD).Blood pressure not measured.
Respiratory Anesthetized, dogs	intraduodenal administration 0, 0.01, 0.03, 0.1, 0.3mg/kg	No discernible effects	Intraduodenal administration 0.3-1.0 mg/kg	No discernible effects
In vitro hERG, HEK 293 cells	0.1, 1.0, 10 µM	No discernible effect	1, 10, 100 µM	62% inhibition at 100µM IC ₅₀ 57µM
Renal Effects and clinical chemistry, rats	Oral dosing 0, 0.3,1,3 mg/kg	3 mg/kg:↓ urine volume 32% (p<0.05) Anemia (~12%) ↑ blood glucose	1-10 mg/kg	10 mg/kg:↓ urine volume 60 % (p< 0.05) ↓HCT (11%, p<0.05) ↑ blood glucose
Gastrointestinal transit, rats	0, 0.3, 1, 3 mg/kg	Dose dependent inhibition of transit: 29% at 3 mg/kg, p<0.05	1-10 mg/kg	Dose-dependent decrease in gastrointestinal motility
Bleeding time, rats	0, 0.3, 1, 3 mg/kg	No significant effect	0, 0.3,1,3 mg/kg	23% (ns)increase in bleeding time at HD

TPR= total peripheral resistance; b.p.= blood pressure

4.3.1 Riociguat Safety Pharmacology

Effects consistent with the pharmacology of riociguat were seen on blood pressure, cardiac output and gastrointestinal transit time, following single oral doses. In addition, effects were seen for renal function that may be extensions of the pharmacology.

Vasodilation in anesthetized Beagles occurred after single intraduodenal administration of 0.1 mg/kg riociguat. Total peripheral resistance was decreased by up to 44% at the high dose of 0.3 mg/kg with accompanying decrease in LVEDp. Dp/dt (mm Hg/s) was decreased in a dose-dependent manner from 0-0.1 mg/kg and increased at the highest dose. Cardiac output was increased at ≥ 0.1 mg/kg. The PQ interval was decreased at the 2 highest doses and QRS intervals were decreased at all doses. QTc was prolonged at the high dose only when calculated by Bazett's and in the two highest doses when calculated by Fridericia's. A transient increase in QTc was seen at 5 or 10 minutes in most animals including the vehicle controls. The high dose showed increases in QTc from ~20-45 minutes after dosing reaching a maximum of 22 msec (the individual animals showed maximum increases of 20, 22 and 9 msec).

Decreased gastrointestinal transit was determined in rats with no NOEL identified. The high dose of 3 mg/kg, caused approximately a 30% decrease ($p < 0.05$) in mean distance traveled relative to control.

A single oral dose study in rats identified effects of riociguat on renal function, serum glucose, thrombin time and circulating lipids. Urine volume was significantly decreased (~30%, $p < 0.05$) at the high dose of 3 mg/kg, but electrolyte excretion was not affected. Dose-related decreases in hematocrit (up to 12%), RBC (up to -11%) and hemoglobin were reported. It was stated that hemolysis was not present. The anemia reported here is repeated throughout the nonclinical studies for this drug. Decreased blood pressure, as demonstrated in other studies, may account for the change in urine volume and the apparent anemia. Serum glucose was increased in both fed and fasted rats. At the highest dose of 3 mg/kg, fasted rats showed increases of 14% of mean baseline ($p < 0.05$) and fed rats showed 2% increase ($p < 0.05$) over baseline. There were no significant effects found on either triglycerides or total cholesterol.

Effects upon hemostasis were varied. Thrombin time showed a statistically significant decrease but the magnitude of change was small and probably biologically insignificant. A separate study examined the effect of 0, 0.3, 1 and 3 mg/kg BAY63-2551 on transected tail bleeding time in male rats. At the HD, there was a 24% increase (n.s.) in bleeding time compared to the control group. Both riociguat and BAY60-4552 have been shown to inhibit platelet aggregation with IC_{50} values in the micromolar range.

The CNS safety pharmacology assessment of BAY63-2521 did not suggest overt neurological effects. A dose-related, statistically significant increase in time to respond to a painful stimulus (nocifensive) was reported with no No Observed Effect Level (NOEL) identified. At 0.3 mg/kg, a 25% increase (n.s.) compared to control was reported. At the highest dose of 3 mg/kg, the mean reaction time was essentially doubled ($p < 0.05$) relative to the control group.

4.3.2 Comparison of Riociguat and BAY60-4552(M1)

Conscious, telemetered Beagles were given single oral doses of BAY60-4552 at 0.15, 0.5 and 1.5 mg/kg or vehicle. Drug effects were compared against pre-drug values and against the vehicle control group. Drug related cardiovascular effects seemed to reach their maximum from 2-4.5 hours after dosing.

Summary of blood pressure effects

Parameter	Dose	Magnitude of maximum effect (compared to pre-drug value)
Systolic blood pressure	0.15mg/kg, 0.5 mg/kg 1.5 mg/kg	-8-11% -27%
Diastolic blood pressure	0.15mg/kg, 0.5 mg/kg 1.5 mg/kg	-10-14% -20%

Some ECG changes were noted in this study, in contrast to the study using intraduodenal dosing.

Summary of ECG observations

parameter	dose	Observation (change compared to pre-drug)
QRS	all	No effect
PQ	1.5 mg/kg	Shortened up to 31ms (26%)
QT	0.15 mg/kg	Increased up to 40 ms
QT		Shortened up to 37ms (16%)
QTc Bazette's	0.5 mg/kg	Increased 23 ms (9%)
	1.5 mg/kg	Increased 41 ms (16%)
QTc Fridericia's	1.5 mg/kg	Increased 12ms (5%)

Distinct from the parent drug, single oral doses of 0,1,3, or 10 mg/kg BAY60-4552, produced neurologic effects increasing in incidence and variety with increasing dose. The LD and MD animals showed a few instances of piloerection, vocalization when touched and decreased activity. The HD animals showed increased incidences of these signs and also ptosis (sedation), irregular respiration, tremor, stereotypic chewing, decreased muscle tone and decreased activity.

There was no apparent effect on seizure threshold or duration of hexobarbital sleeping time. Contrary to the parent drug, there was no effect on nocifensive response.

The sponsor examined riociguat and BAY 60-4552 in several different hERG assays. Different from the parent drug, a dose-related inhibition of the hERG channel was apparent. The IC₅₀ value was reported as 57µM, making this a low potency hERG inhibitor.

The assessment of renal function and lipid metabolism showed a dose-related increase in urine volume with no apparent effect on electrolyte excretion. There was no apparent effect on leucocytes, but a dose-related decrease in erythrocytes with a corresponding decrease in hematocrit and hemoglobin, similar to the changes noted with riociguat. There was a slight decrease in triglycerides (up to 8%) and a slight decrease in cholesterol (up to 10%). The changes in plasma triglycerides and cholesterol might be due to normal variability as it would be unusual to see changes in these parameters manifested within 2 hours of a single drug dose.

The decrease in urine volume may be due to a decrease in blood pressure although that also raises questions as to overcoming the autoregulation of the kidney, something possible with an acute drop in blood pressure. A direct renal toxic effect is also possible.

Similar to the parent drug, fasted rats given a single oral dose of 10 mg/kg BAY60-4552 showed increased serum glucose within half an hour after administration (5%, n.s.) that was still increasing (37% increase over control value, $p < 0.05$) at the final determination. There was a slight increase in blood glucose (up to 12%, n.s.) in all drug-treated groups compared to the vehicle group at 120 and 180 minutes. Non-fasted rats showed an increase in blood glucose compared to vehicle control at 30 minutes. The effect persisted until 180 minutes in the HD group but not in the LD or MD groups. Within the HD group, the effect was diminished at 120 minutes compared to 30 minutes.

Reviewer's Summary of Serum Glucose Effects

Dose BAY60-4552 (mg/kg)	Blood glucose in fed rats		Blood glucose in fasted rats	
	Blood glucose concentration (mmol/l \pm SD) Time after treatment		Blood glucose concentration (mmol/l \pm SD) Time after treatment	
	% Δ from vehicle 30 minutes	% Δ from vehicle 180 minutes	% Δ from vehicle 30 minutes	% Δ from vehicle 180 minutes
1	10%		-2%	+15%
3	10%		-2%	+12%
10	17%	8%	+5%	+37%
* $p < 0.05$ compared to vehicle controls				

Similar to the parent drug, BAY60-4552 caused a decrease in gastrointestinal transit.

Decreases in Gastrointestinal Transit Relative to Control

Dose BAY60-4552 mg/kg	# of animals	Length of intestine covered by BaSO ₄ (cm)
1	5	13% *
3	5	15% *
10	5	35% *
* $p < 0.05$ compared to control		

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

The validation of methodology for determination of BAY60-4552 and BAY63-2521 in rat, dog, rabbit and mouse plasma was presented. For the plasma of each species, the sponsor evaluated precision and accuracy of the methodology, drug stability and matrix effects. For each of the species evaluated, BAY60-4552 and BAY63-2521 in plasma stored in polypropylene were determined to be stable at 37°C (2 hours), -15°C (6 months) and -15°C (3 freeze-thaw cycles). Matrix effects minimally decreased the amount of BAY60-4552 detected in dog and rat plasma but increased the detected quantity in mouse and rabbit plasma. When characterizing the methodology for BAY63-2521, matrix effects seemed to be important for dog plasma only.

Absorption

The absolute bioavailability of riociguat is moderate to high in rats, dogs, and humans. This and the human oral bioavailability are summarized in the reviewer's table below.

species	bioavailability
Rat	35-65%
Dog	50-80%
Human	94%

Distribution

Single oral doses of [¹⁴C]-riociguat at 3 mg/kg were administered to both albino (Wistar) and pigmented (Long Evans) rats. The radioactivity was widely distributed with specific affinity for melanin-containing tissues in the pigmented rats. The greatest concentrations were found in the thyroid, liver, kidneys, and adrenal gland. Concentrations were not calculated for the brain, for the stated reason of too few concentrations above the lower limits of quantitation. The sponsor reported "significant enrichments and delayed elimination occurred in pigmented tissues, e.g. eye wall ($t_{1/2} = 114$ h) and pigmented skin ($t_{1/2} = 120$ h)."

Distribution was also studied in pregnant albino rats at gestation day 19. The radioactivity from a single oral dose of 3 mg/kg [¹⁴C]-riociguat was distributed to both maternal and fetal tissues. Mammary glands showed approximately 3-fold higher exposure than maternal blood. The average AUC₀₋₂₄ for fetuses was approximately 56% of what was reported for maternal blood. In contrast to the dams, a higher fetal blood/brain penetration occurred. Fetal brain exposure accounted for approximately 46% of total fetal exposure and was 4.6-fold higher than the exposure in the maternal brain, possibly reflecting the age-expected differences of fetal versus mature blood-brain barrier.

Metabolism

Neither BAY63-2521 nor BAY60-4552 appeared to induce CYP1A2 or CYP3A4 protein or activity. There were also no apparent inhibitory effects on CYP1A2, 2A6, 2B6, 2C8, 2C9, 2D6, 2E1, 2J2, and 3A4.

After single oral administration of riociguat, parent compound was a major component of the plasma of humans, dog, mouse and rat. The main metabolic pathway in rat, dog, human and mouse is microsomal N-demethylation leading to M1 or BAY60-4552. Metabolite M4, the N-glucuronide of BAY60-4552, was a major component of human plasma (6-26% of radioactivity AUC) but was not detected in the plasma of the other species. The M3 metabolite is formed by N-dealkylation. No human-specific primary metabolic pathways and no major species differences were identified in the *in vitro* metabolic studies, which seem to have results consistent with the *in vivo* mass balance studies. The sponsor's summary of the main metabolites for humans and the nonclinical species is shown below.

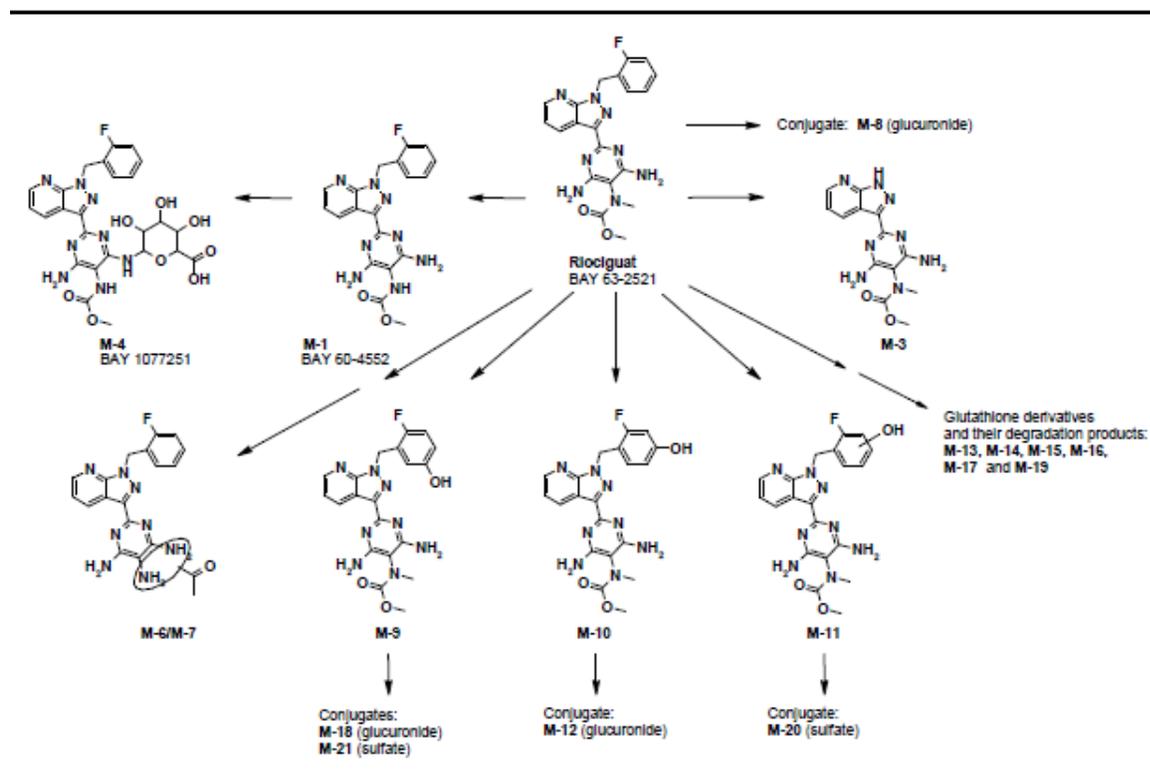
The presence of major human metabolites in the nonclinical species is important for accurate hazard characterization of the drug. The sponsor has noted that there is some variability in the level of M1 exposure in humans, possibly due to genetic variations in CYP1A1. The sponsor's comparison of parent drug, M1 and M4 (glucuronide of M1) is shown below.

Table 5-15: Biotransformation *in vivo*. Main components in plasma of male CD1-mouse, male Wistar rat, female Beagle dog, and man following single oral administration of [¹⁴C]riociguat

Species	Mouse	Rat	Dog	Man	
Dose	3 mg/kg	3 mg/kg	0.6 mg/kg	1.04 – 1.09 mg	
Analyzed interval	0 – 24 h	0 – 24 h	0 – 24 h	0 – 48 h	
Component	% of AUC (total radioactivity)				
				Subj. 1-3	Subj. 4
Riociguat	74.8	75.0	38.3	26.1-39.8	73.6
M-1	16.6	9.50	56.3	41.9-59.0	10.5
M-4				8.40-25.6	6.14

The sponsor's proposed overall metabolic schema is shown below:

Figure 5-1: Proposed metabolic pathways of riociguat *in vivo* (selected metabolites)



Protein Binding

The parent drug and active metabolite are highly protein bound in humans and only moderately protein bound in the nonclinical species. The sponsor's summary table is shown below.

Reviewer's Summary of Plasma Protein Binding of BAY63-2521

species	Fraction not bound to plasma proteins (F_u)	
	BAY63-2521	BAY60-4552
Human	5	4
Rat	16	11
Dog	17	17
Mouse	20	17

The sponsor examined the binding of both riociguat and M1 to α 1-acidic glycoprotein, human serum albumin, α -globulin, γ -globulin, and low density lipoprotein (LDL). The highest level of riociguat binding was to human serum albumin, followed by α 1-acidic glycoprotein, LDL, α globulin and the lowest level of binding (~16%) to γ -globulin. The main binding protein of M1 in human plasma is albumin with a free fraction of 16%.

Summary of Pharmacokinetics after Oral Administration

parameter	BAY63-2521		BAY60-4552 discreet administration		human
	rat	dog	rat	dog	
Clearance l/kg hr	1.3	0.3	0.8	0.159	0.04 nonsmokers
Vd ss l/kg	1.2	0.7			0.38 l/kg
T _{1/2} hours	1.2	3.0	1-4	5	6 (nonfasted humans)
Oral absorption	60%	78%	30-40%	88%	
Absolute bioavailability	35-65%	50-80%			94%

Excretion

Riociguat is eliminated by three routes: oxidative biotransformation, excreted as unchanged drug in feces or as unchanged drug in urine.

The rat and dog excreted >80% of orally administered [¹⁴C]-riociguat via the biliary/fecal route. Humans excrete 33-45% via the kidneys and 48-49% of a dose by the fecal route.

Reviewer's Summary of Excretion for Riociguat and BAY60-4552

parameter	BAY63-2521		BAY60-4552 discreet administration		BAY63-2521 human
	rat	dog	rat	dog	
Excretion (after oral administration)	Biliary >80% Extrabiliary: 24% of dose	Biliary >80% Renal 14%	Fecal 72% Renal 18%		Fecal 48-59% Renal 33-45%

6 General Toxicology

6.1 *Single-Dose Toxicity*

Single dose toxicity studies were conducted in mice, by oral and intravenous routes, and rats, by the oral route only. The highest non-lethal oral dose in both species was 300 mg/kg. The lowest lethal oral dose in both species was 2000 mg/kg. The lowest lethal intravenous dose in mice was 30 mg/kg. The signs were described as “nonspecific.”

6.2 *Repeat-Dose Toxicity*

Repeat dose toxicity studies included daily administration of parent drug (riociguat) or metabolite (BAY60-4552) to rats for up to 6 months or dogs for up to 12 months. The dog was sensitive to the pharmacologic effect of the parent drug with a steep dose-response curve for the limiting toxicities. This prevented the use of doses that might have produced toxicologically significant findings.

Gastrointestinal effects were reported across species and studies. These effects included decreased gastrointestinal transit (bloating, distension), secondary gastrointestinal irritation/inflammation, and diarrhea and may reasonably be attributed to the extended pharmacology of smooth muscle relaxation. Similarly, cardiovascular effects of decreased total peripheral resistance were also apparent across species and may similarly be considered extended pharmacology.

The target organs of toxicity appear to be the heart, bone, kidney, liver, endocrine and reproductive systems for both parent drug and metabolite. The sponsor did several ancillary studies to investigate bone (parent drug) and renal (metabolite) effects.

6.2.1 PH-35002 Chronic oral toxicity study in rats (26-weeks administration by gavage)

Report number: PH-35002

Study number: T5076778

Study initiated: May 26, 2006

GLP: statement included

QA: yes

Study location: Wupperthal, Germany

Test article: BX028BL, 97.3% pure

BAY63-2521 was administered by oral gavage to 20 male and 20 female Wistar (HsdCpb:WU) rats per dose group at daily doses of 0, 2.5, 10 or 40 mg/kg body weight for a period of 187-188 days. The drug was administered as a suspension in 0.5% aqueous tylose®.

Observations

Morbidity/mortality: 2x/day, 1x on weekends, holidays

Open field observation: weekly

Determination of :

Body weights weekly

Food consumption: weekly

Water consumption: weekly

Ophthalmoscopic exam: pre-tx(all groups), end of study(control and high dose)

Clinical laboratory:

Hematology: week 13 and 26 (first 10 living animals)

Clinical chemistry: week 13 and 26 (first 10 living animals)

Urinalysis: week 12 and 25 first 10 living animals

Necropsy: week 27/28

Determination of liver enzymes and substrate content: liver sampling during necropsies (first 10 animals)

Toxicokinetics: days 1, 28 and 182

0.5, 1,2,4 and 7 hours after dosing

3 animals per time point

Results

Signs: Reddening of ears and extremities were seen in all treated animals (≥ 2.5 mg/kg).

Males with penis erections were dose-dependent ≥ 10 mg/kg (6/20 MD and 17/20 HD) from day 11 to day 60.

Increased girth in females (4/20) at 40 mg/kg: the cecum was found to be dilated but histological correlates were not seen. The reason for the observation was hypothesized to be a functional effect.

Ophthalmoscopy: no treatment related effects reported.

Body weights

The male LD and MD groups gained more weight than the controls while the HD group gained at a lower rate. All of the female drug-treated groups gained weight at a greater rate than the control group.

Figure 6-1: Body Weights - Males

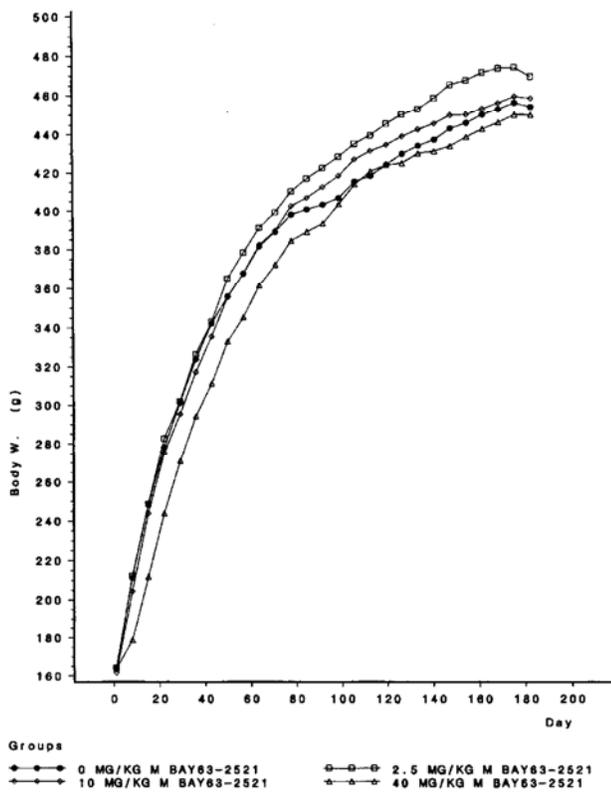
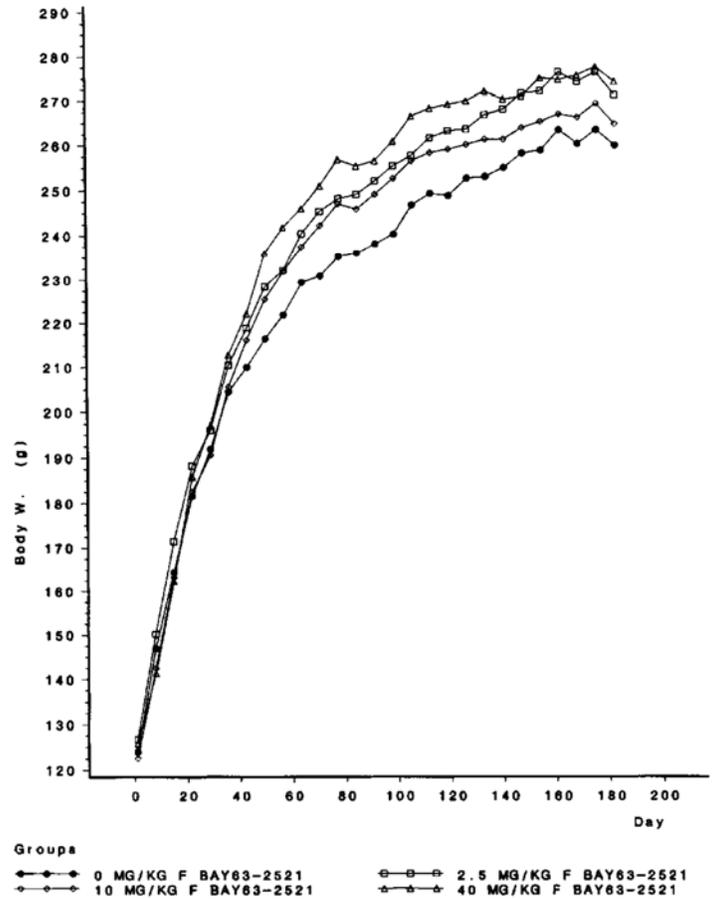


Figure 6-2: Body Weights - Females



When normalized for body weight, food intake was increased in the drug-treated groups.

Table 6-4: Cumulative and Mean Daily Food Intake

Dose mg/kg	Days	Mean Food Intake g/animal		g/kg body weight	
		total	per day	total	per day
males					
0	1 - 183	4288	23.6	11509	63.3
2.5	1 - 183	4543	25.0	11835	65.0
10	1 - 183	4531	24.9	12124	66.6
40	1 - 183	4446	24.4	12399	68.2
females					
0	1 - 183	3091	17.0	13659	75.1
2.5	1 - 183	3269	18.0	13816	76.0
10	1 - 183	3300	18.2	14225	78.2
40	1 - 183	3386	18.6	14081	77.4

Water consumption was also increased in both sexes.

Table 6-5: Cumulative and Mean Daily Water Intake

Dose mg/kg	Days	Mean Water Intake g/animal		g/kg body weight	
		total	per day	total	per day
males					
0	1 - 183	4374	24.1	11835	65.0
2.5	1 - 183	4617	25.4	12150	66.8
10	1 - 183	4759	26.2	12855	70.6
40	1 - 183	5309	29.2	15230	83.7
females					
0	1 - 183	3534	19.5	15673	86.2
2.5	1 - 183	3847	21.1	16328	89.7
10	1 - 183	3944	21.7	17056	93.7
40	1 - 183	4816	26.5	20390	112.1

Hematology

There were some minor changes in the hematology that could either be normal variability or very minimal effects consistent with a mild regenerative anemia. There were no findings of toxicological significance in the WBC material.

Clinical chemistry

Serum triglycerides were decreased in the MD/HD groups of both sexes. Other than that, there were no consistent findings of significance.

Summary of serum triglycerides

Dose mg/kg/day	Day 89/90 (mmol/l)		Day 180/181 (mmol/l)	
	m	f	m	f
0	0.76	0.80	0.89	1.01
2.5	0.65	0.61+	0.79	1.11
10	0.62	0.48++	0.76	0.69
40	0.42++(-45%)	0.47++ (-33)	0.48+ (-46)	0.74

+p<0.05, ++p<0.01

Summary of serum potassium

Dose mg/kg/day	Day 89/90 (mmol/l)		Day 180/181 (mmol/l)	
	m	f	m	f
0	4.7	4.5	5.4	4.6
2.5	4.8	4.5	4.9+	4.7
10	4.6	4.4	4.9+	4.8
40	4.9	4.5	4.9+	4.7

+p<0.05, ++p<0.01

Thyroid hormones

T3 was slightly but significantly decreased in females at 10 and 40 mg/kg on day 180. Two out of 10 values of both dose groups were outside the range of historical data according to the sponsor. The difference from control in the female T4 values was apparent at day 89 but not at day 181.

Table 6-11: Clinical Chemistry: Hormones

Dose mg/kg	T3	T4	TSH
	nmol/l	nmol/l	mcg/l
m Day 90			
0	1.42	79	7.44
2.5	1.33	70	7.78
10	1.33	70	7.62
40	1.31	67	7.23
m Day 181			
0	1.18	58	6.98
2.5	1.18	59	7.27
10	1.21	58	6.83
40	1.05	52	6.99
f Day 89			
0	1.53	47	6.13
2.5	1.40	49	6.06
10	1.48	57	5.95
40	1.56	71 +	6.08
f Day 180			
0	1.60	35	4.47
2.5	1.55	40	5.53
10	1.36 +	39	5.18
40	1.36 +	41	5.35

Urinalysis

A dose related increase in urine volume was reported. Density seemed to adjust appropriately.

Summary of HD volume changes

	Day 84		Day 174/5	
	Vol (ml)	%diff from control	Vol (ml)	%diff from control
Males: control	9.3		8.5	
Males: HD	14.4	56	12.4	46
Females: control	7.2		5.4	
Females: HD	11.7 ++	63	7.6	41

++p<0.01

Determinations in liver tissue

O-demethylase activity showed an increase in activity while N-demethylase showed a decreased activity.

Table 6-13: Determinations in Liver Tissue

Dose mg/kg	O-DEM	N-DEM	P 450	TRIGL
	mU/g	mU/g	nmol/g	mcmol/g
m	Day 190- 194			
0	9.8	125.4	39.5	6.99
2.5	11.5	96.8 ++	39.2	6.98
10	11.1	91.1 ++	39.2	6.59
40	13.1 ++	90.2 ++	36.0	5.88
f	Day 190- 194			
0	9.3	50.2	34.5	6.28
2.5	10.5 +	37.4	35.4	6.60
10	10.1	47.1	36.6	6.48
40	12.0 ++	51.3	35.9	6.44

Toxicokinetics

The sponsor reports that there were no sex-related differences in results and that therefore the data presented are the means after pooling the results of the sexes.

Day 182		BAY 63-2521	BAY 60-4552	BAY 63-2521	BAY 60-4552	BAY 63-2521	BAY 60-4552
Dose	[mg/kg]	2.5		10		40	
AUC(0-7)	[µg·h/L]	441	46.6	n.c.	193	n.c.	1008
AUC(0-7) _{norm}	[kg·h/L]	0.176	0.0193	n.c.	0.0200	n.c.	0.0261
AUC(0-24)	[µg·h/L]	594 ^b	68.2 ^b	2283	300 ^b	13927	1784
AUC(0-24) _{norm}	[kg·h/L]	0.238 ^b	0.0282 ^b	0.228	0.0310 ^b	0.348	0.0461
C _{max}	[µg/L]	116	10.9	524	53.5	2107	211
C _{max, norm}	[kg/L]	0.0462	0.00450	0.0524	0.00553	0.0527	0.00547
C(24) / C _{max}	[%]	n.c.	n.c.	0.393	n.c.	2.98	5.13
t _{max}	[h]	1.00	1.00	0.500	1.00	0.500	1.00
R _{A1}	[%]	101	130	186	230	302	255
R _{A3}	[%]	97.6	99.8	116	139	172	252
MR	[%]	n.c.	11.9	n.c.	13.1	n.c.	13.2

BAY 63-2521_T5076778.xls \ report Summary \ Kch \ 05.07.07

n.c. = not calculated

R_{A1} = ratio of accumulation regarding C_{max} Day 182 / C_{max} Day 1 in [%]R_{A3} = ratio of accumulation of BAY 63-2521 regarding AUC(0-24) Day 182 / AUC(0-24) Day 1 in [%],
ratio of accumulation of BAY 60-4552 regarding AUC(0-7) Day 182 / AUC(0-7) Day 1 in [%]MR = metabolic ratio AUC_{norm} BAY 60-4552_{D182} / AUC_{norm} BAY 63-2521_{D182} in [%]

b = extrapolated from 7 to 24 h

Organ Weights

Normalized adrenal weight was increased at the MD and HD of both sexes. The normalized brain weight was decreased in both sexes. Liver, spleen and kidney weight was also increased in both sexes.

Table 6-17: Necropsy - Relative Organ Weights

Dose	Body W.	Brain	Adrenals	Thymus	Heart	Liver	Spleen	Kidneys
mg/kg	G	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g
m	Terminal Sacrifice							
0	460	469	11	63	303	3358	145	604
2.5	477	443	11	58	293	3383	148	624
10	467	448	13 ++	67	307	3564 +	150	650 +
40	461	450	14 ++	72	319	3684 ++	155	627
f	Terminal Sacrifice							
0	264	731	25	99	377	3387	178	664
2.5	276	710	24	99	346 +	3484	172	682
10	271	707	27	100	354	3697 ++	188	695
40	279	692	28	103	366	4277 ++	201 ++	703

The normalized weight of epididymides (-11%, p<0.05), seminal vesicles (-16%, p<0.05) and prostate (-15%, p<0.05) were decreased in the drug treated groups compared to the control group.

Dose mg/kg	Body W. G	Testes mg/100g	Epididym. mg/100g	Prostate mg/100g	Seminal vesicle mg/100g	Ovaries mg/100g	Uterus mg/100g
m	Terminal Sacrifice						
0	460	745	362	289	391		
2.5	477	714	333	258	366		
10	467	723	337	264	354		
40	461	735	325 +	245 +	330 +		
f	Terminal Sacrifice						
0	264					60	403
2.5	276					58	349
10	271					62	436
40	279					61	392

Gross Observations

Elongated intestines were increasingly observed in both sexes ≥ 10 mg/kg. Dilated cecums were found in the 40 mg/kg females. Both elongated intestines and dilated cecums were without histological correlates. Enlarged livers occurred in males ≥ 2.5 mg/kg and in females at 40 mg/kg.

Summary of histopath findings (n=20 per group examined)

	Males (dose group: mg/kg)				females(dose group: mg/kg)			
	0	2.5	10	40	0	2.5	10	40
Liver								
• Hypertrophy/cytoplasmic					0	0	0	5
• Periportal fat decreased	4	2	2	0	4	4	0	0
Kidneys								
• Pigment deposits in proximal tubules	0	0	0	3	0	1	6	17
• Bone remodeling/hyperostosis femur	0	0	1	14	0	0	0	7
Hypertrophy adrenal zona glomerulosa	3	4	18	17	1	1	17	18
Mesenteric veins: plexiform change	4	6	5	13	1	2	3	7
Prominent/vacuolated Paneth cells					0	0	0	5

Summary

The observations of possible effects on the reproductive organs had been discussed with the sponsor at the time of the IND submission. The changes in male reproductive tract weights reported here therefore did not raise new issues. The potential for cardiac toxicity was discussed with the sponsor at the time of the original submission.

There may be some mild effect on thyroid hormones at the HD. This may be connected to the effects on liver enzyme activity or due to alterations in the efficiency of the gastrointestinal tract. The gross necropsy observations of distended intestines are consistent with in-life observations of distention and bloating.

The bone histopathology is also consistent with findings reported in other rat toxicology studies. This appears at ≥ 10 mg/kg riociguat in males and at 40 mg/kg in females. The highest dose that was proposed in the US clinical trials was 2.5 mg, given three times a day. Doses of 5 mg were used in Europeans. Reported clinical toxicity included cardiac palpitations (1/10 at 5 mg) and vertigo (1/132 at 2.5 mg). At the time that this study report was submitted to the Division, the following pharmacokinetic data was available:

AUC 348 $\mu\text{g}\cdot\text{h/l}$ for a human taking a 2.5 mg tablet and that value was used in the reviewer's table below.

Rat dose mg/kg	AUC0-24 for rat dose	Multiple of human AUC
Male rats		
2.5	594	1.7x
10	2283	6.6X
40	13927	40X

The low margins of safety were also noted in the review of the original submission.

6.2.2 26 Week Chronic Oral Toxicity Study in Beagles

Report Number: PH35050

Study Number: T007024

Study Location: Bayer HealthCare, Wuppertal, Germany

Experimental Start Date: September 4, 2006

GLP: statement included

QA: yes

Test Article: micronized BAY63-2521, batch BX028BL, purity 97.3%

vehicle: 0.5% tylose

Beagles, (4/sex/group) approximately 5 months of age at the start of the study were orally administered BAY63-2521 in doses of 0, 0.3, 1 and 3 mg/kg/day.

Observations

Appearance and behavior: daily

Feed intake: daily

Body weight: weekly

Reflexes (papillary, corneal, patellar, extensor, postural, flexor): pre-study, weeks 5, 13 and 26.

Ophthalmoscopy: pre-study, week 5, 13 and 26

ECG: pre-study, prior to and 2 hours after dosing in weeks 13 and 26 of the study.

Blood pressure measurements: direct measurement from the femoral artery
 Hematology and clinical chemistry samples: collected prestudy and weeks 6, 13, and 26 of the study

Sponsor's List of Hematology Parameters Measured

LEUCO	Leucocytes
ERY	Erythrocytes
HB	Hemoglobin
HCT	Hematocrit
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
THRO	Platelets/Thrombocytes
NEUTRO	Neutrophils
LYMPH	Lymphocytes
MONO	Monocytes
EOS	Eosinophils
BASO	Basophils
ATYP	Atypical Leucocytes
DIFF Com.	DIFF Comment: 0 (no abnormalities have been found) or 1 (comments see additional text)
RETI	Reticulocytes

Also measured: ESR-1/2: Erythrocyte sedimentation rate
 PTT: Partial Thromboplastin Time – kaolin-activated test
 PT; Thromboplastin Time

Clinical Chemistry Parameters Measured: ALT, Albumin, alkaline phosphatase, AST, total bilirubin, cholesterol, creatine kinase, GGT, glutamate dehydrogenase, glucose, lactate dehydrogenase, magnesium, inorganic phosphate, total protein, triglycerides, urea, calcium, potassium, sodium, chloride, triiodothyronine (T3), thyroxine (T4).

Urinalysis: pre-study, weeks 6, 13 and 26. Animals were placed individually in metabolism cages for a 6 hour period. Parameters measured: specific gravity, volume, creatinine, MultiStix evaluations of bilirubin, blood, glucose, ketone bodies, pH, protein, and urobilinogen. Urinary sediment was examined by microscopy.

Toxicokinetics: Day and week 25, plasma was collected before and 0.5, 1, 2, 4, 7 and 24 hours after administration.

Liver determinations: N-demethylase (NDEM), O-demethylase (ODEM), CYP450, and triglycerides.

Tissues collected at necropsy The report does not contain a simple declarative statement as to which animals were subject to histological examination. From the histologic summary tables, it appears that all main study animals were examined.

Adrenal glands	Oviducts
Aorta	Pancreas
Bone marrow cylinder	Pharynx #
Brain (cerebrum, cerebellum, brain stem, medulla oblongata)	Pituitary gland
Epididymides	Parotis
Esophagus	Prostate
Eyes*	Sciatic nerve
Femur with Bone marrow	Skeletal muscle (thigh)
Gallbladder	Skin (mammary region)
Heart +	Spinal cord (cervical, thoracic, lumbar)
Intestine/Peyer's Patches	Spleen
- Duodenum	Sternum
- Jejunum	Stomach
- Ileum	Testes*
- Caecum	Thymus
- Colon	Thyroid glands (with parathyroid glands)
- Rectum	Tonsils
Kidneys*§	Tongue
Larynx	Trachea
Liver	Ureters
Lungs	Urinary bladder
Lymph nodes, mandibular	Uterus with uterine cervix
Lymph nodes, mesenteric	Vagina
Lymph nodes, popliteal	
Mandibular and sublingual gland	Organs and tissues with macroscopic findings
Mesentery (vessels)	
Nasal cavity/Teeth #	
Optic nerves *	
Ovaries	Physical identifier #

- * fixation in Davidson's solution except animal 68
 § additional specimen fixed in 10 % neutral buffered formalin
 # no histopathology performed
 + from the heart, specimens of the following localizations were investigated: left and right atrium, left chamber (2 papillary muscles), right chamber, septum.

Results

Clinical signs of soft feces were seen in all dose groups but at much higher frequency in the HD group. Salivation was also reported at high frequency in the HD group.

Unscheduled mortality was reported. One HD female was found dead in week 6. There was a generalized lymphoid condition in that the thymus and other lymphoid organs were described as atrophied. The sponsor attributed this to an overall deterioration of condition unrelated to treatment. No other cause for deteriorating condition was offered.

There were no consistent body weight findings.

MALES		MEAN BODY WEIGHTS (KG)				
		0 MG/KG	0.3 MG/KG	1 MG/KG	3 MG/KG	
WEEK	1	MEAN	6.88	6.60	6.53	6.28
		S.D.	1.034	0.816	0.629	0.818
		N	4	4	4	4
WEEK	13	MEAN	8.88	8.93	8.05	7.98
		S.D.	1.031	0.750	1.500	1.245
		N	4	4	4	4
WEEK	26	MEAN	9.45	9.63	8.73	9.00
		S.D.	0.988	1.050	1.592	1.203
		N	4	4	4	4
Absolute difference from Week1			2.57	3.03	2.20	2.72

Body Weight continued

Females also showed no apparent drug-related changes in body weight.

FEMALES		MEAN BODY WEIGHTS (KG)				
		0 MG/KG	0.3 MG/KG	1 MG/KG	3 MG/KG	
WEEK	1	MEAN	4.85	5.18	5.18	5.00
		S.D.	0.858	0.650	0.574	0.829
		N	4	4	4	4
WEEK	13	MEAN	6.55	7.00	7.22	6.97
		S.D.	1.103	0.864	0.359	1.050
		N	4	4	4	3
WEEK	26	MEAN	7.30	7.45	8.15	7.47
		S.D.	1.163	0.947	0.646	1.206
		N	4	4	4	3
Absolute difference from Week1			2.45	2.27	2.97	2.47

Food consumption : was not reported in a way that it could be examined for effects.

Reflex testing, body temperature, ophthalmoscopy: No effects were noted.

Hematology: There was a possible signal in the erythrocyte sedimentation rate, an indicator of systemic inflammation.

Week	ESR-1 mm/h	ESR-2 mm/2h	Week	ESR-1 mm/h	ESR-2 mm/2h	Week	ESR-1 mm/h	ESR-2 mm/2h	Week	ESR-1 mm/h	ESR-2 mm/2h
0 mg/kg Male PO control			0.3 mg/kg Male PO group I			1 mg/kg Male PO group II			3 mg/kg Male PO group III		
-2	Mean 1	2	-2	Mean 1	2	-2	Mean 1	2	-2	Mean 1	2
	Med. 1	2		Med. 1	2		Med. 1	2		Med. 1	2
	S.D. 0.0	0.5		S.D. 0.0	0.5		S.D. 0.0	0.0		S.D. 0.0	0.6
	Min. 1	1		Min. 1	1		Min. 1	2		Min. 1	1
	Max. 1	2		Max. 1	2		Max. 1	2		Max. 1	2
	N 4	4		N 4	4		N 4	4		N 4	4
6	Mean 1	1	6	Mean 1	2	6	Mean 3	8	6	Mean 1	2
	Med. 1	1		Med. 1	2		Med. 3	6		Med. 1	2
	S.D. 0.0	0.5		S.D. 0.0	0.0		S.D. 2.9	8.1		S.D. 0.0	1.0
	Min. 1	1		Min. 1	2		Min. 1	2		Min. 1	1
	Max. 1	2		Max. 1	2		Max. 7	19		Max. 1	3
	N 4	4		N 4	4		N 4	4		N 4	4
13	Mean 1	2	13	Mean 2	5	13	Mean 2	4	13	Mean 1	2
	Med. 1	2		Med. 2	4		Med. 2	4		Med. 1	2
	S.D. 0.0	0.5		S.D. 1.4	3.6		S.D. 0.5	1.0		S.D. 0.0	0.0
	Min. 1	1		Min. 1	2		Min. 1	2		Min. 1	2
	Max. 1	2		Max. 4	10		Max. 2	4		Max. 1	2
	N 4	4		N 4	4		N 4	4		N 4	4
26	Mean 1	2	26	Mean 2	4	26	Mean 2	5	26	Mean 1	2
	Med. 1	2		Med. 2	5		Med. 2	5		Med. 1	2
	S.D. 0.6	0.5		S.D. 0.6	1.0		S.D. 1.5	3.3		S.D. 1.0	1.9
	Min. 0	1		Min. 1	3		Min. 1	2		Min. 0	1
	Max. 1	2		Max. 2	5		Max. 4	9		Max. 2	5

Week	ESR-1 mm/h	ESR-2 mm/2h	Week	ESR-1 mm/h	ESR-2 mm/2h	Week	ESR-1 mm/h	ESR-2 mm/2h	Week	ESR-1 mm/h	ESR-2 mm/2h
0 mg/kg Female PO control			0.3 mg/kg Female PO group I			1 mg/kg Female PO group II			3 mg/kg Female PO group III		
-2	Mean 1	1	-2	Mean 1	1	-2	Mean 1	2	-2	Mean 1	2
	Med. 1	1		Med. 1	1		Med. 1	2		Med. 1	2
	S.D. 1	0.5		S.D. 1	0.5		S.D. 1	0.6		S.D. 1	0.8
	Min. 1	1		Min. 1	1		Min. 1	1		Min. 1	1
	Max. 1	2		Max. 1	2		Max. 1	2		Max. 1	3
	N 1	4		N 1	4		N 1	4		N 1	4
6	Mean 1	2	6	Mean 1	2	6	Mean 1	2	6	Mean 1	2
	Med. 1	2		Med. 1	2		Med. 1	1		Med. 1	2
	S.D. 1	0.6		S.D. 1	0.5		S.D. 1	1.0		S.D. 1	0.5
	Min. 1	1		Min. 1	1		Min. 1	1		Min. 1	1
	Max. 1	2		Max. 1	2		Max. 1	3		Max. 1	2
	N 1	4		N 1	4		N 1	4		N 1	4
13	Mean 1	2	13	Mean 1	1	13	Mean 1	1	13	Mean 1	2
	Med. 1	2		Med. 1	1		Med. 1	1		Med. 1	2
	S.D. 1	0.6		S.D. 1	0.0		S.D. 1	0.0		S.D. 1	0.6
	Min. 1	1		Min. 1	1		Min. 1	1		Min. 1	1
	Max. 1	2		Max. 1	1		Max. 1	1		Max. 1	2
	N 1	4		N 1	4		N 1	4		N 1	3
26	Mean 1	5	26	Mean 1	2	26	Mean 1	3	26	Mean 1	2
	Med. 1	3		Med. 1	2		Med. 1	2		Med. 1	2
	S.D. 1	5.0		S.D. 1	1.2		S.D. 1	1.0		S.D. 1	0.6
	Min. 1	1		Min. 1	0		Min. 1	2		Min. 1	2
	Max. 1	12		Max. 1	3		Max. 1	4		Max. 1	3
	N 1	4		N 1	4		N 1	4		N 1	3

Clinical Chemistry

There were no findings of apparent toxicological significance in the data as presented.

A summary table in an appendix presented the liver enzyme data in a different format, making it apparent that there was a small, statistically insignificant, decrease in enzymatic activity at the highest dose.

Sponsor's Table

Dose [mg/kg]	ECOD	EROD	ALD	EH	GS-T	GLU-T
0	19.4	0.93	15.2	2086	78	3566
0.3	16.7	0.65	15.5	2127	74	3559
1	17.9	0.89	13.0	1995	79	3232
3	16.5	0.58	12.5	1819	88	2899

Sponsor's Table

Table 2-4 Enzyme activities in the liver of dogs (f) after administration of BAY 63-2521 for 26 weeks (mean values)

Dose [mg/kg]	ECOD	EROD	ALD	EH	GS-T	GLU-T
0	22.0	0.76	11.7	1789	74	2988
0.3	21.6	0.62	12.7	1720	74	3369
1	18.8	0.62	10.5	1710	100	2515
3	15.5	0.39	9.1	1358	88	2223

Urinalysis

There were no apparent findings of toxicological significance.

Blood Pressure and ECG

The effects on blood pressure apparent at week 13, 2 hours after dosing, not indicated as significant, were also apparent at week 26, 2 hours after dosing.

Sponsor's Table

SUMMARY OF BLOOD PRESSURES (mmHg)

MALES		0 MG/KG	0.3 MG/KG	1 MG/KG	3 MG/KG	
WEEK -2	SYSTOLIC	MEAN	170	139	164	164
		S.D.	13.1	15.0	6.7	10.2
		N	4	4	4	4
DIASTOLIC	MEAN	92	80	89	91	
	S.D.	8.9	6.6	7.5	17.5	
	N	4	4	4	4	
WEEK 13 HOUR 0	SYSTOLIC	MEAN	182	143	146	152
		S.D.	24.8	9.3	23.3	13.6
		N	4	4	4	4
DIASTOLIC	MEAN	88	72	61	95	
	S.D.	12.0	15.2	15.6	8.2	
	N	4	4	4	4	
WEEK 13 HOUR 2	SYSTOLIC	MEAN	164	126	99	115
		S.D.	7.5	37.1	21.4	19.2
		N	4	4	4	4
DIASTOLIC	MEAN	90	64	50	69	
	S.D.	12.0	20.3	11.7	8.6	
	N	4	4	4	4	
WEEK 26 HOUR 0	SYSTOLIC	MEAN	163	154	156	155
		S.D.	6.5	28.7	36.7	10.9
		N	4	4	4	4
DIASTOLIC	MEAN	86	84	78	82	
	S.D.	19.0	15.1	22.6	10.1	
	N	4	4	4	4	
WEEK 26 HOUR 2	SYSTOLIC	MEAN	140	129	130	138
		S.D.	22.0	25.4	29.7	25.6
		N	4	4	4	4
DIASTOLIC	MEAN	68	72	71	77	
	S.D.	19.3	28.0	9.8	15.3	
	N	4	4	4	4	

FEMALES		SUMMARY OF BLOOD PRESSURES (mmHg)			
		0 MG/KG	0.3 MG/KG	1 MG/KG	3 MG/KG
WEEK -2 SYSTOLIC	MEAN	130	156	175	135
	S.D.	33.6	21.1	26.1	34.3
	N	4	4	4	4
DIASTOLIC	MEAN	67	81	89	58
	S.D.	18.6	6.6	15.1	13.6
	N	4	4	4	4
WEEK 13 HOUR 0 SYSTOLIC	MEAN	142	174	195	147
	S.D.	36.3	26.6	37.2	21.3
	N	4	4	4	3
DIASTOLIC	MEAN	74	83	103	90
	S.D.	22.6	25.5	23.5	24.8
	N	4	4	4	3
WEEK 13 HOUR 2 SYSTOLIC	MEAN	167	159	144	127
	S.D.	15.9	43.1	22.9	22.3
	N	4	4	4	3
DIASTOLIC	MEAN	88	85	68	68
	S.D.	19.6	34.2	25.6	10.4
	N	4	4	4	3
WEEK 26 HOUR 0 SYSTOLIC	MEAN	153	170	184	188
	S.D.	18.4	23.9	22.9	8.3
	N	4	4	4	3
DIASTOLIC	MEAN	84	85	110	118
	S.D.	26.2	10.2	14.7	7.1
	N	4	4	4	3
WEEK 26 HOUR 2 SYSTOLIC	MEAN	133	150	147	142
	S.D.	49.7	38.6	8.6	37.1
	N	4	4	4	3
DIASTOLIC	MEAN	73	72	80	78
	S.D.	23.8	32.3	6.1	11.5
	N	4	4	4	3

Inconsistent increases in corrected QT were seen in both sexes of drug-treated animals, primarily at the highest dose.

Males: week 13

Group	HF		QT _{cB}		QT _{cF}	
	0h	2h	0h	2h	0h	2h
Control	86	78	237	239	224	229
0.3 mg/kg	95	104	260	273	241	249
1 mg/kg	89	126	269	260	251	230
3 mg/kg	121	149	282	289	251	249

Males: week 26

Group	HF		QT _{cB}		QT _{cF}	
	0h	2h	0h	2h	0h	2h
Control	93	81	244	240	227	229
0.3 mg/kg	91	98	243	257	227	237
1 mg/kg	90	142	257	298	241	259
3 mg/kg	116	138	296	302	265	263

Females: week 13

Group	HF		QT _{cB}		QT _{cF}	
	0h	2h	0h	2h	0h	2h
Control	96	97	247	241	228	222
0.3 mg/kg	98	110	274	255	252	230
1 mg/kg	116	142	257	272	232	237
3 mg/kg	117	151	262	310	235	266

Females: week 26

Group	HF		QT _{cB}		QT _{cF}	
	0h	2h	0h	2h	0h	2h
Control	97	92	249	251	230	234
0.3 mg/kg	94	123	249	268	232	238
1 mg/kg	106	128	275	283	251	250
3 mg/kg	131	145	275	299	242	259

Organ Weights

In males, the absolute and normalized weights of the thyroid and adrenal glands were greater than control (up to 32% and 31%, respectively, n.s.).

SUMMARY OF ABSOLUTE ORGAN WEIGHT DATA

MALES

		0 MG/KG	0.3 MG/KG	1 MG/KG	3 MG/KG
THYROID WITH PARATHYROID g	MEAN	0.725	0.822	0.840	0.918
	S.D.	0.0686	0.1040	0.1122	0.1338
	N	4	4	4	4
ADRENALS g	MEAN	1.222	1.313	1.457	1.533
	S.D.	0.3278	0.1413	0.2333	0.2414

SUMMARY OF ORGAN-TO-BODY WEIGHT RATIOS

MALES

		0 MG/KG	0.3 MG/KG	1 MG/KG	3 MG/KG
THYROID WITH PARATHYROID Ratio	MEAN	0.0078	0.0087	0.0097	0.0103
	S.D.	.00127	.00186	.00106	.00177
	N	4	4	4	4
ADRENALS Ratio	MEAN	0.0132	0.0138	0.0170	0.0173
	S.D.	.00414	.00133	.00317	.00366
	N	4	4	4	4

In HD females, there was a striking decrease from concurrent control in the weight of the reproductive tract. The uterus/cervix/oviduct and ovarian weights, normalized to body weight, were decreased by 71% (n.s.) and 61% (n.s.) respectively compared to control.

FEMALES		SUMMARY OF ABSOLUTE ORGAN WEIGHT DATA			
		0 MG/KG	0.3 MG/KG	1 MG/KG	3 MG/KG
PANCREAS g	MEAN	18.3	18.3	17.0	16.7
	S.D.	4.11	5.74	4.16	2.89
	N	4	4	4	3
UTERUS/CERVIX/OVIDUCT g	MEAN	10.8	11.0	8.0	3.3
	S.D.	7.63	7.02	8.68	0.58
	N	4	4	4	3
OVARIES g	MEAN	1.283	1.425	0.955	0.503
	S.D.	0.5321	1.1246	0.6026	0.0611
	N	4	4	4	3

FEMALES		SUMMARY OF ORGAN-TO-BODY WEIGHT RATIOS			
		0 MG/KG	0.3 MG/KG	1 MG/KG	3 MG/KG
PANCREAS Ratio	MEAN	0.25	0.25	0.21	0.23
	S.D.	0.036	0.089	0.046	0.077
	N	4	4	4	3
UTERUS/CERVIX/OVIDUCT Ratio	MEAN	0.14	0.15	0.09	0.04
	S.D.	0.086	0.088	0.092	0.005
	N	4	4	4	3
OVARIES Ratio	MEAN	0.0175	0.0188	0.0113	0.0068
	S.D.	.00520	.01462	.00615	.00134
	N	4	4	4	3

Histopathology

Hyperplasia of the adrenal zona glomerulosa was identified in both sexes.

SEX :					MALE
DOSE GROUP:	01	02	03	04	
NO. ANIMALS:	4	4	4	4	

ADRENAL GLANDS :	4	4	4	4
- Hyperpl.Zona Glomer.:	-	3	4	4
Grade 1:	-	2	-	1
Grade 2:	-	1	4	3

SEX :					FEMALE
DOSE GROUP:	01	02	03	04	
NO. ANIMALS:	4	4	4	4	

ADRENAL GLANDS :	4	4	4	4
- Hemorrhage/s :	-	-	-	1
Grade 3:	-	-	-	1
- Hyperpl.Zona Glomer.:	-	-	1	4
Grade 1:	-	-	1	-
Grade 2:	-	-	-	4

Toxicokinetics

The sponsor found no sex-related differences in the toxicokinetic data. At steady state, the trough plasma values were 2-7% of the Cmax values.

Sponsor's Table

Dose	[mg/kg]	0.3		1		3*	
		BAY 63-2521	BAY 60-4552	BAY 63-2521	BAY 60-4552	BAY 63-2521	BAY 60-4552
AUC(0-t _n)	[μg·h/L]	1059	808	3089	1573	4654	4969
AUC(0-t _n) _{norm}	[kg·h/L]	3.53	2.78	3.09	1.63	1.55	1.71
AUC(0-24)	[μg·h/L]	1103	808	3089	1573	4654	4969
AUC(0-24) _{norm}	[kg·h/L]	3.68	2.78	3.09	1.63	1.55	1.71
C _{max}	[μg/L]	186	61.2	367	111	852	398
C _{max, norm}	[kg/L]	0.621	0.211	0.367	0.115	0.284	0.137
C(24) / C _{max}	[%]	3.35	19.0	6.60	24.7	1.99	16.0
t _{max}	[h]	1.30	4.00	2.00	4.29	1.22	4.33
R _{A1}	[%]	129	183	129	169	222	246
R _{A3}	[%]	153	202	146	164	231	244

T0077024_BAY 63-2521_Tables.xls \ report summary \ Nie \ 02.04.07

* n = 7
R_{A1} = ratio of accumulation regarding C_{max} Week 25 / C_{max} Day 1 in [%]
R_{A3} = ratio of accumulation regarding AUC(0-24) Week 25 / AUC(0-24) Day 1 in [%]
Equivalent doses were used for calculation of dose-normalized parameters of M-1 using a factor of 0.967

The sensitivity of the dog to riociguat's pharmacology limits the doses that can be used in the toxicology studies. There were few findings of obvious toxicological significance. The slight changes in normalized organ weights may be primary or secondary to alterations in blood flow and perfusion.

6.2.3 Systemic Toxicity Study in Male and Female Dogs with Daily Intra-gastric Administration for 52 Weeks

Report: A45725

Study Number: TXST20070080 (T1077502)

Study Location:

Study Initiation: June 4, 2007

GLP: statement included

QA: yes

Test Article: BAY63-2521, batch BX028BL, purity 97.1%

Formulation: microcrystalline suspension in 0.5% tylose

Beagles, 10 months of age, 4/sex/group, were administered BAY63-2521 at doses of 0, 0.3, 1 and 2 mg/kg/day for 52 weeks. The HD animals initially received 3 mg/kg for 9 days. Because the general condition of the animals was significantly impaired, treatment was stopped for 14 days (day 10 to 23). Dosing was restarted at 1 mg/kg for 7 days and subsequently increased to 2 mg/kg/day from day 31 until the end of the study.

Observations

- Mortality and clinical signs : 2X per day
- Food consumption : recorded daily
- Body weight: weekly
- Body temperature: pre-study and days 39, 86, 178, 268 and 362
- Ophthalmoscopy: pre-study, 38, 85, 176, 267 and 361
- Blood pressure: direct measurement pre study and during the study, prior to dosing and 2 hours after dosing (Tmax) on days 36, 87, 176, 269 and 358.
- ECG: pre study and during the study, prior to dosing and 2 hours after dosing (Tmax) on days 36, 87, 176, 269 and 358. Lead II was the only lead evaluated. QT correction by Bazett's, Fridericia and van de Water's formula.
- Nervous system function: observational studies on days 39, 86, 178, 268 and 362.
- Hematology and Clinical Chemistry: serum samples were collected pre-study and days 9, 40, 89, 180, 271 and 362
 - Hematology: rbc, wbc, Hb, PCV, MCH, MCHC, MCV, platelet count, differential, reticulocytes
 - Clinical chemistry: AST, ALT, ALP, GGT, GLDH, LDH, CK, total cholesterol, glucose, urea nitrogen, creatinine, sodium, potassium, calcium, chloride, total protein and protein electrophoresis, total bilirubin, triglycerides, magnesium, inorganic phosphate, iron and albumin, cardiac troponin T (cTnT) and cardiac troponin I (cTnI).
- Coagulation parameters: determined from citrated plasma pre-study and days 9, 40, 89, 271 and 362 thrombin time, thromboplastin time, activated partial thromboplastin time and fibrinogen
- Thyroid hormones: pre study and days 40, 89, 180, 271 and 362. T3, T4 and TSH
- Urinalysis: collected over 18-20 hours from all animals per group pre study, and days 37, 86, 178, 268 and 362.
- Toxicokinetics: The concentrations of BAY63-2521 and BAY60-4552 (metabolite M1) were determined in serum samples taken before and 0.5, 1, 2, 4, 7 and 24 hours after intragastric administration on study days 1/2, 9/10 and in weeks 26 and 50. For animals of the HD group only, serum concentration profiles were determined on day 24/25 and 31/32. LC/MS/MS methodology was used.
- Necropsy: the sponsor's summary table is shown below. There was no statement as to the animals examined. From the summary tables, it appears that all animals in all groups were examined histologically.

Sponsor's Summary of Organs Weighed and Tissues Collected for Histopathology

Organ	W	Histol. examination	Organ	W	Histol. examination
Liver	yes	P	Ovaries	yes	P
Gallbladder		P	Oviducts		P
Kidneys	yes	P	Uterus	yes	
Urinary bladder		P	- horn		P
Ureter		P	- corpus		P
Heart	yes	P	- cervix		P
Atrium		P	Vagina		P
Lung *	yes	P	Mammary glands (<i>with skin</i>)		P
Aorta (<i>thoracic</i>)		P	Skin (<i>back-lumbar region</i>)		Fo
Vein (<i>vena cava caudalis</i>)		Fo	Thymus	yes	P
Trachea		P	Spleen	yes	P
Esophagus		P	Mandibular lymph node		P
Larynx		P	Iliac lymph node	yes	P
Tongue		P	Mesenteric lymph node		P
Salivary glands			Tonsils		P
- mandibular gland	yes	P	Peyers Patches		P
- sublingual gland		P	Sternum (<i>bone marrow</i>)		P
- parotid gland		P	Femur (<i>incl. joint/bone marrow</i>)		P
Stomach		P	Brain	yes	
Duodenum		P	- cerebrum		P
Jejunum		P	- cerebellum		P
Ileum		P	- medulla oblongata		P
Cecum		P	Spinal cord		
Colon		P	- cervical		P
Rectum		P	- thoracic		P
Pancreas	yes	P	- lumbar		P
Pituitary gland	yes	P	Lacrimal gland		Fo
Thyroid glands with parathyroid glands	yes	P	Eyes (<i>with optical nerve</i>)		P
Adrenal glands	yes	P	Periph. nerve (<i>Sciatic nerve</i>)		P
Testes	yes	P	Skeletal muscle		P
Epididymides		P	Macroscopic findings (<i>if necessary for diagnostic evaluation</i>)		P
Prostate	yes	P			

Organs/Tissues in **bold print** = paired examination

W = weight determination

P = processed and examined histologically

Fo = fixation of organ/tissue samples, not processed for histological examination

* fixation by instillation after weight determination

Results

Mortality

One HD male died on day 279 after showing signs of decreased food consumption and accompanying weight loss, frequent diarrhea, vomiting, apathy, and general debilitation from the start of the study. The necropsy showed myocardial degeneration.

Signs

No signs were reported for 0.3 mg/kg BAY63-2521.

At doses ≥ 1 mg/kg, the following drug related findings were noted with dose-dependent incidence: sialorrhea, vomiting and diarrhea. Emaciation was noted in one male at 1 mg/kg/day.

At 2 mg/kg/day, the following compound related effects were reported: apathy, sedation, decreased activity, emaciation, transient exsiccosis, foul smelling breath.

At 3 mg/kg, muscle tremor was reported sporadically between day 4 and 9. This is not consistent with the 26 week dog study where no neurologic signs were reported.

Food consumption

In the first 9 days of the study, food consumption in the HD group was depressed to approximately $\frac{1}{4}$ that of the control group ($p < 0.01$). Consumption increased to control levels as soon as the HD group was given a drug-free period starting day 10. When dosing was resumed at 2 mg/kg, the food consumption gradually decreased until Day 39.

The time allowed for this group to consume food was extended from 2 to 6 hours and consumption generally increased although there were some sporadic episodes of decrease. Some individual animals were offered additional “wet” (canned?) food.

TT 3: Food consumption relative to control

Group	Food consumption relative to control animals	
	Males in week 52 (day 364)	Females in week 52 (day 364)
0.3 mg/kg BAY 63-2521	110 % (93 %)	105 % (95 %)
1.0 mg/kg BAY 63-2521	93 % (78 %)	107 % (108 %)
2.0 mg/kg BAY 63-2521	103 % (42 %)*	95 % (28 %)

*: n = 3 animals

Body Weight

The sponsor did not provide graphs of body weight. The reviewer's summary of body weight changes is shown below.

Reviewer's Summary of Male Body Weights: Mean \pm SD

group	Day 1	Day 365	Δ from baseline	% difference from control Δ from baseline
Control	9.2 \pm 1.0	10.7 \pm 1.0	1.5	--
0.3 mg/kg BAY63-2521	8.9 \pm 0.5	10.8 \pm 0.8	1.9	27%
1.0mg/kg BAY63-2521	8.9 \pm 0.6	9.9 \pm 0.5	1.0	50%
2.0/kg BAY63-2521	9.3 \pm 0.3	10.6 \pm 0.4	1.3	13%

Reviewer's Summary of Female Body Weights: Mean \pm SD

group	Day 1	Day 365	Δ from baseline	% difference from control Δ from baseline
Control	6.6 \pm 0.8	8.7 \pm 1.0	2.1	--
0.3 mg/kg BAY63-2521	6.8 \pm 0.6	7.8 \pm 0.7	1.0	52%
1.0mg/kg BAY63-2521	6.7 \pm 0.9	7.8 \pm 0.7	1.1	48%
2.0/kg BAY63-2521	6.7 \pm 0.6	8.4 \pm 0.3	1.7	19%

Body Temperature

There were no changes of toxicological significance apparent in the data.

Blood pressure

From day 36, systolic pressure was decreased relative to concurrent control in drug-treated animals of both sexes. Persistent reduction of blood pressure 24 hours after dosing suggested lingering drug effects. There was no change in the magnitude of effect from day 87 to day 176 in the males. In females, the magnitude of effect continued to increase to day 177. Blood pressure lowering was maintained in both sexes until the final determination on day 358.

Reviewer's summary of blood pressure changes 2 hours after dosing

	Maximum systolic pressure decrease (mm Hg)	Maximum diastolic pressure decrease (mm Hg)
Males day 87	75mm Hg, p<0.01	25 mm Hg, p<0.01.
Females day 88	42mm Hg, p<0.01	7 mm Hg, n.s.
Females day 177	75 mm Hg, p<0.01	18 mm Hg, p<0.01

Sponsor's tables: blood pressure for males and females combined

Day: 36 relative to Start Date Time Slot: Routine			
		Syst. pressure mmHg	Diast. pressure mmHg
Group	Sex	Identity	Identity
1m	Mean	195.0	81.8
	S.D.	5.9	4.5
	N	4	4
2m	Mean	165.3*	77.3
	S.D.	19.0	8.7
	N	4	4
3m	Mean	179.8	85.0
	S.D.	18.9	9.9
	N	4	4
4m	Mean	155.5**	74.0
	S.D.	2.4	2.8
	N	4	4

Day: 36 relative to Start Date Time Slot: 2 h			
		Syst. pressure mmHg	Diast. pressure mmHg
Group	Sex	Identity	Identity
1m	Mean	190.3	78.8
	S.D.	7.1	4.6
	N	4	4
2m	Mean	148.3*	71.0
	S.D.	27.4	8.8
	N	4	4
3m	Mean	148.8*	66.8
	S.D.	17.1	10.8
	N	4	4
4m	Mean	126.0**	64.8
	S.D.	3.4	7.7
	N	4	4

Statistics Test: Dunnett Test: * - 5% significance level;
 ** - 1% significance level;
 n - Data not appropriate for statistical analysis;
 n1 - This group has only one value;

Arithmetic Mean Values Presented

Nominal Dose: Group 1 - 0mg/kg Group 2 - 0.3mg/kg
 Group 3 - 1.0mg/kg Group 4 - 3.0/1.0/2.0mg/kg

Heart Rate and ECG

The only consistent effect was reflex tachycardia, apparent in both sexes at 2 hours after dosing. There were occasional statistically significant findings that were without time or dose dependence and therefore of questionable, if any, biological significance.

Assessment of nervous system function

This was a very limited evaluation that included pupillary light reflexes, corneal reflex, extensor postural thrust reaction, and patellar reflex. No deviations from normal reflexes were noted.

Ophthalmoscopy

No deviations from normal were noted.

Hematology

By day 180, an anemia was apparent in both sexes of drug treated animals. While the reticulocyte count was not apparently affected in the males, a dose-related decrease was seen in the females. This was significant at the HD. However, this relative anemia did not persist.

Sponsor's Table

		Day: 180 relative to Start Date Time Slot: Routine						
		RBC 10*12/L	HGB mmoL/L	PCV L/L	MCV fL	MCH fmoL	MCHC mmoL/L	RETC 10*12/L
Group	Sex	Identity	Arc Sine SQRT1.6	Arc Sine SQRT	Identity	Identity	Identity	Identity
1m	Mean	6.235	9.18	0.4110	65.98	1.475	22.340	0.0293
	S.D.	0.527	0.73	0.0315	0.91	0.017	0.151	0.0142
	N	4	4	4	4	4	4	4
2m	Mean	5.990	8.68	0.3888	64.98	1.455	22.393	0.0330
	S.D.	0.540	0.69	0.0300	2.34	0.037	0.372	0.0262
	N	4	4	4	4	4	4	4
3m	Mean	5.583	8.23	0.3760	67.38	1.475	21.865	0.0235
	S.D.	0.279	0.46	0.0175	1.86	0.047	0.248	0.0013
	N	4	4	4	4	4	4	4
4m	Mean	5.363	7.75*	0.3528*	65.78	1.443	21.948	0.0268
	S.D.	0.504	0.82	0.0355	1.15	0.042	0.301	0.0103
	N	4	4	4	4	4	4	4

Sponsor's Table

		Day: 180 relative to Start Date Time Slot: Routine						
		RBC 10**12/L	HGB mmol/L	PCV L/L	MCV fL	MCH fmoL	MCHC mmol/L	RETC 10**12/L
Group Sex	Identity	Arc Sine SQRT1.6	Arc Sine SQRT	Identity	Identity	Identity	Identity	Identity
1f	Mean	6.300	9.50	0.4228	67.18	1.513	22.500	0.0338
	S.D.	0.485	0.57	0.0240	2.03	0.042	0.201	0.0119
	N	4	4	4	4	4	4	4
2f	Mean	5.870	8.75	0.3933	67.13	1.495	22.303	0.0205
	S.D.	1.217	1.62	0.0769	1.00	0.042	0.367	0.0071
	N	4	4	4	4	4	4	4
3f	Mean	5.920	8.40	0.3845	64.95	1.425*	21.920	0.0193
	S.D.	0.981	1.30	0.0636	2.14	0.031	0.522	0.0087
	N	4	4	4	4	4	4	4
4f	Mean	5.668	8.20	0.3773	66.60	1.450	21.745*	0.0173*
	S.D.	0.115	0.14	0.0054	1.68	0.035	0.077	0.0022
	N	4	4	4	4	4	4	4

Clinical Chemistry

Day 9, serum potassium levels and chloride levels were decreased in the HD animals of both sexes, consistent with emesis. Day 40, the MD and HD males showed a non-significant increase in BUN (17% compared to control) and a decrease in serum potassium (7% compared to control), confirmatory of the decreased appetite and emesis noted by the sponsor. At the same time point, MD and HD females showed slight, non-significant increases in BUN (-11%). Only the HD females showed a decrease in mean potassium levels compared to the control group (-12%, $p < 0.05$). For the remainder of the sampling periods, there were sporadic changes in several different parameters. These changes were lacking in consistency, dose- or time-dependence. It is unlikely that these apparently random changes are of toxicological significance.

APTT was increased in the HD groups of both sexes on Day 40 (the first determination after dosing began). In the HD males, this relative increase persisted to day 270.

In HD females, there was a slight, statistically significant, increase on day 40. A non-significant increase persisted in the high dose group until the end of the study.

Summary of APTT Values in Males

Dose group Mg/kg/day	Day 40	Day 89	Day 180	Day271	Day 362
0	11.33	11.35	11.58	11.43	11.45
0.3	12.40	12.13	12.95	11.98	12.20
1	11.65	11.73	12.48	11.83	12.05
3/2	13.03**	12.88**	13.65*	12.55*	12.10

* $p < 0.05$, ** $p < 0.01$ by Dunnett's test

Summary of APTT Values in Females

Dose group Mg/kg/day	Day 40	Day 89	Day180	Day 271	Day 362
0	11.90	11.75	12.28	11.90	12.08
0.3	11.90	11.60	12.23	11.53	11.85
1	12.35	12.33	12.83	12.45	12.05
3/2	13.70*	12.93	12.93	12.63	12.28

*p<0.05, **p<0.01 by Dunnett's test

Histopathology Findings

There were no striking or consistent findings in the histopathology. There was also a lack of consistency with the histopathology findings from the 26 week dog study.

The decreases in the weight of the female reproductive tract seen in the 26 week study were not apparent here, nor were the changes in adrenal weight apparent in this study.

	males				Females			
	0	0.3	1	3/2	0	0.3	1	3/2
Dose: mg/kg								
Myocardial degeneration	0/4	0/4	0/4	1/3	0/4	0/4	0/4	0/4
Lung: osseus metaplasia	0/4	0/4	0/4	1/3	0/4	0/4	0/4	0/4
Cardiac vascular hypertrophy	0/4	0/4	1/4	0/3	0/4	1/4	1/4	1/4
Diestrus					4/4	3/4	2/4	1/4
Proestrus					0/4	0/4	2/4	2/4
Estrus					0/4	0/4	0/4	1/4
Metestrus					0/4	1/4	0/4	0/4

Summary of Organ Weight as Mean % of Body Weight: Males

	0 mg/kg	0.3 mg/kg	1 mg/kg	3/2mg/kg
gallbladder	0.12	0.15	0.17	0.17
thyroid gland	0.0069	0.00779	0.00931*	0.00613

Summary of Organ Weight as Mean % of Body Weight: Females

	0 mg/kg	0.3 mg/kg	1 mg/kg	3/2mg/kg
gallbladder	0.17	0.16	0.18	0.17
thyroid gland	0.0078	0.0080	0.0078	0.0081
Adrenal glands	0.015	0.014	0.016	0.018
ovaries	0.0093	0.0108	0.0183*	0.0122
uterus	0.034	0.057	0.158*	0.078
thymus	0.065	0.053	0.050	0.049

The sponsor reported that there were no sex-related differences in toxicokinetic parameters for either the parent drug or the M-1 metabolite (BAY60-4552). The sponsor's table (next page) presents the combined data for males and females.

Reviewer's Summary of PK parameters on Day 1

		BAY 65-2321	BAY 60-4552	BAY 65-2321	BAY 60-4552	BAY 65-2321	BAY 60-4552
dose	mg/kg	0.3		1		2	
AUC ₀₋₂₄	ug.hr/l	1251	582	1040	870	5139	3220
C _{max}	ug/l	172	44.1	199	77.1	534	211
T _{max}	h	1.41	4	1.09	3.7	1.65	9.01

Reviewer's Summary of PK parameters in Week 26

		BAY 65-2321	BAY 60-4552	BAY 65-2321	BAY 60-4552	BAY 65-2321	BAY 60-4552
dose	mg/kg	0.3		1		2	
AUC ₀₋₂₄	ug.hr/l	1123	580	1683	1586	3954	2839
C _{max}	ug/l	165	50	337	138	622	225
T _{max}	h	1.41	4	1.19	3.4	1.09	4

Sponsor's Table

The exposure in Week 50 (geometric means, n = 8; for dose 2 mg/kg n = 7) was as follows:							
		BAY 63-2521	BAY 60-4552	BAY 63-2521	BAY 60-4552	BAY 63-2521	BAY 60-4552
		Mean geom.	Mean geom.	Mean geom.	Mean geom.	Mean geom.	Mean geom.
Dose	[mg/kg]	0.3 (n=8)		1 (n=8)		2 (n=7)	
AUC(0-24)	[ug·h/L]	828	556	1205	1324	2980	2709
AUC(0-24) _{norm}	[kg·h/L]	2.76	1.92	1.21	1.37	1.49	1.40
C _{max}	[ug/L]	125	46.2	238	127	489	234
C _{max, norm}	[kg/L]	0.416	0.159	0.238	0.131	0.244	0.121
C(24)/C _{max}	[%]	1.61	12.1	0.795	7.21	1.64	10.9
t _{max}	[h]	1.00	4.29	1.30	3.67	0.906	4.00

Thyroid hormone values were presented only for the combined sexes. T3 and T4 values were slightly depressed for the HD group. There was no histological correlate for this and it's possible that the values are simply "sick euthyroid" or a basically healthy thyroid in a stressed animal. Changes in thyroid hormones can also occur secondary to gastrointestinal changes. The sponsor's values are shown below.

Sponsor's Table

Thyroid Hormones male/female combined group means and statistical evaluation			
Dose	T3	T4	TSH
	nmol/l	nmol/l	mcg/l
Week -3			
Control	1.27	27	0.14
(0.3mg/kg)	1.43 nc	27 nc	0.20 nc
(1.0mg/kg)	1.41 nc	25 nc	0.14 nc
(3.0mg/kg)	1.37 nc	27 nc	0.24 nc
Week 6			
Control	1.31	30	0.13
0.3mg/kg	1.40 -	30 -	0.13 -
1.0mg/kg	1.34 -	29 -	0.11 -
3.0mg/kg	1.28 -	19 -	0.09 -
Week 13			
Control	1.57	30	0.17
0.3mg/kg	1.52 -	29 -	0.12 -
1.0mg/kg	1.59 -	28 -	0.13 -
3.0mg/kg	1.10 +	24 -	0.20 -
Week 26			
Control	1.39	31	0.19
0.3mg/kg	1.40 -	31 -	0.20 -
1.0mg/kg	1.25 -	24 -	0.24 -
3.0mg/kg	1.05 -	25 -	0.20 -
Week 39			
Control	1.42	28	0.17
0.3mg/kg	1.38 -	28 -	0.14 -
1.0mg/kg	1.34 -	24 -	0.22 -
3.0mg/kg	1.13 -	22 -	0.23 -
Week 52			
Control	1.32	29	0.13
0.3mg/kg	1.34 -	31 -	0.21 -
1.0mg/kg	1.30 -	30 -	0.24 -
3.0mg/kg	1.12 -	29 -	0.41 -
053855/08.001 A5012499			
Legends:			
+	significantly different at $p \leq 0.05$		
++	significantly different at $p \leq 0.01$		
-	no significance		
nc	no statistical test performed		

The sponsor provided this succinct summary of the study:

At the low dose of 0.3 mg/kg and higher:
decrease in systolic and diastolic blood pressure, increase in heart rate, increase in weight of the adrenal glands, vascular hypertrophy in the heart (predominantly arteries).

At the mid dose of 1.0 mg/kg and higher:
sialorrhea, vomiting, diarrhea, emaciation.

Additionally, at the high dose of 2.0 mg/kg:
apathy, decreased locomotive activity, eyelid closure, emaciation and drawn in flanks, exsiccosis (transient), foul-smelling breath, ruffled and/or dull fur, temporarily decreased food consumption and body weight, slight decrease in triiodothyronine (T3) levels from week 13 onwards.

At the high dose of 2.0 mg/kg one male animal died on day 279 of the study after showing signs of impaired general condition, especially frequent occurrence of diarrhea, almost continuously over the entire treatment period. Furthermore, temporarily reduced food consumption and body weight were noted. The postmortem examination revealed moderate to marked congested vessels and slight acute myocardial degeneration. The cause of death is considered a final circulatory decompensation, which may have been enhanced by the blood pressure lowering effect of the compound.

In addition, at the dose of 3.0 mg/kg which was administered from day 1 to day 9, significantly reduced food consumption and body weight loss, and muscle tremor were reported. Thyroxine (T4) was diminished transiently in one single male and female animals each when examined in week 6.

7 Genetic Toxicology

There was no evidence of genetic toxicology for riociguat in the reverse mutation bacterial (Ames) assay, cytogenetic assay (Chinese hamster V79 cells), *in vitro* chromosome aberration (Chinese hamster V79 cells), the *in vivo* mouse micronucleus assay or a cytogenetic assay in mice. The metabolite, BAY60-4552, was tested in the bacterial reverse mutation assay, the *in vitro* chromosome aberration assay (Chinese hamster V79 cells), and the *in vivo* mouse micronucleus study. There was no apparent genetic toxicology for the active metabolite.

7.1 *In Vitro*

7.1.1 Ames-Test Screening

Key findings: Increase in revertants not seen under the conditions of the assay.

Study no.: T2071789

Volume #, and page #: vol15,p 1.

Conducting laboratory and location: Mol and Gen Tox, Bayer HealthCare, Wuppertal, Germany

Date of study initiation: August 30, 2002

GLP compliance: no

QA reports: yes () no (x)

Drug, lot #, and % purity: BAY63-2521 in DMSO There were no batch numbers listed and no purity listed with the comment “not indicated by sponsor”.

Methods

Plate incorporation method used.

S9: from Sprague-Dawley rats

Strains: Salmonella typhimurium TA1535,TA100,TA1537,TA98,TA102

Concentrations : 0, 16, 50, 160, 500, 1600, 5000 µg per plate.

Positive controls: sodium azide, nitrofurantoin, 4-nitro1,2-phenylene diaminemitomycin C, cumene hydroperoxide, 2-aminoanthracene

Criteria for assay acceptance and positive results cited.

Precipitation : concentrations \geq 1600 µg per plate

Bacteriotoxic $>$ 500 µg per plate

Results: no increase in revertants under the conditions of the assay.

7.1.2 Cytogenetic screening with Chinese hamster V79 cells

Key findings: No increase in clastogenicity in this limited exploratory study.

Study no.: T 4071790

Volume #, and page #: vol 15, p.51

Conducting laboratory and location: Mol Gen Tox, Bayer, Wuppertal, Germany

Date of study initiation: September 22, 2002

GLP compliance: no

Drug, lot #, and % purity: batch WSF1026-12

There is a statement in the report under the chemical analysis (4.1 Test Substances) that the content “not indicated by sponsor”, suggesting that the study was performed at a contract facility, other than at Bayer.

4. Material and Methods

4.1. Test Substances

Name of test substance	: BAY 63-2521
Manufacturer	: BAYER HealthCare
Batch number	: WSF 1026-12
Content	: not indicated by the sponsor
Approved	: not indicated by the sponsor

Methods

Chinese hamster V79 cells were exposed \pm S9 for 4 hours to concentrations up to 810 µg/ml BAY63-2521. Cultures of all concentrations were harvested 18 hours after the beginning of the treatment. Concentrations for analysis of metaphases were selected based on cytotoxicity.

Colcemid was added to each flask 2 hours prior to the end of the incubation period. At least two slides were generated per culture. Mitotic indices and numbers of surviving cells (survival index) were used to indicate cytotoxicity.

Concentrations tested \pm S9: 0, 450, 540, 630, 720, 810.

Cytotoxicity seen at concentrations \geq 450 μ g/ml –S9
 \geq 100 μ g/ml +S9.

Precipitation of test article in the medium \geq 450 μ g/ml.

Chromosomes of approximately 100 metaphases per concentration, 50 metaphases from each of two parallel cultures were examined. The sponsor states that in most cases at least 100 assessable metaphases were present on one slide prepared from an individual culture. However, the sponsor goes on to state on page 69 that when 50 assessable metaphases could not be found on the first slide of a culture, the back up slides were evaluated until a total of 50 metaphases was reached. This is below the guidance recommendations for number of metaphases to be analyzed.

The sponsor analyzed slides for only 1 concentration. It was also the lowest concentration tested. The background for the vehicle control was very low.

The sponsor states the criteria for a positive response as a relevant and statistically significant increase in the aberration ratio. If one only examines one concentration, can such a finding be identified?

Based on the limited data available there seems to be insufficient support for lack of clastogenicity. The data that is available suggests the opposite. Reviewer's note: while reviewing study T0072920 I found the statement that the current report was for the exploratory study, something not noted within this report.

7.1.3 Salmonella/Microsome test: plate incorporation and preincubation method

Key findings: No increase in revertants under the conditions of the assay.

Study no.: T1072921

Volume #, and page #: vol 15, p.88

Conducting laboratory and location: Mol and Gen Tox of Bayer, Wuppertal, Germany

Date of study initiation: July 20, 2003

GLP compliance: statement included

QA reports: yes (x) no ()

Drug, lot #, and % purity: batch # BX01AD8

Methods

Strains: S. typhimurium TA1535, TA100, TA1537, TA98, TA102 \pm S9

Positive controls : sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine, mitomycin C, cumene hydroperoxide, 2-aminoanthracene.

Concentrations tested : 0, 16, 50, 158, 500, 1581, 5000 µg/plate

Results

Sponsor reported that there was no inhibition of growth and that only a weak, strain-specific bacteriotoxic effect was observed at higher concentrations.

There was no apparent change in revertants with different concentrations. Under the conditions of the assay there was no increase in mutagenic activity.

7.1.4 In vitro chromosome aberration test with Chinese hamster V79 cells

Key findings: Slight increase in aberrations that is within the historical ranges.

Study no.: T0072920/PH33094

Conducting laboratory and location: Mol. And Gen.Tox Unit of Health Care Toxicology, Bayer, Wuppertal, Germany

Date of study initiation: August 8, 2003

GLP compliance: statement included

QA reports: yes (x) no ()

Drug, lot #, and % purity: BX01AD8, 93% dissolved in DMSO

Positive controls: Mitomycin C and cyclophosphamide

Methods Chinese hamster V79 cells were exposed ±S9 for 4 hours to concentrations of 125, 250, 450, 500 and 550 µg/ml of BAY63-2521. Cultures of all concentrations were harvested 18 hours after the beginning of treatment. In addition, cells treated with 450, 500 and 550 µg/ml were harvested 30 hours after the beginning of treatment. Without S9, an additional experiment was performed using continuous treatment for 18 hours, harvest at the end of the treatment period and BAY63-2521 concentrations of 15, 30, 60, 90 and 120 µg/ml. Based on cytotoxicity determined at 8 hours after the beginning of treatment, concentrations were selected for reading metaphases. Colcemid was added 2 hours prior to the end of incubation to arrest cells in metaphase.

Concentrations used for 4 hours of treatment

Test groups	S9 mix	µg/ml in culture medium	TX time (hours)	Harvest time (Hours)
Solvent control	±	0	4	18
Bay63-2521	±	125	4	18
Bay63-2521	±	250	4	18
Bay63-2521	±	450	4	18
Bay63-2521	±	500	4	18
Bay63-2521	±	550	4	18
Positive controls				
Mitomycin c	-	0.1	4	18
cyclophosphamide	+	2.0	4	18
Solvent control	±	0	4	30
Bay63-2521	±	450	4	30
Bay63-2521	±	500	4	30
Bay63-2521	±	550	4	30

Concentrations used for 18 hours of treatment

Test groups	S9 mix	µg/ml in culture medium	Tx time hours	Harvest time hours
Solvent control	-	0	18	18
Bay63-2521	-	15	18	18
Bay63-2521	-	30	18	18
Bay63-2521	-	60	18	18
Bay63-2521	-	90	18	18
Bay63-2521	-	120	18	18
Mitomycin C	-	0.03	18	18

Mitotic index was determined by counting 1000 cells per culture. Duplicate cultures were processed and examined. Chromosomes of approximately 200 metaphases per concentration, 100 metaphases from each of two parallel cultures were examined. However, the sponsor then goes on to state that if they reached a total of 100 metaphases on a single slide, the second slide was typically not evaluated.

Results

- Precipitation was reported both \pm S9 \geq 450 μ g/ml.
- Altered cell morphology \geq 450 μ g/ml
- Non-attached cells \geq 500 μ g/ml

Concentrations the sponsor selected for reading

Test groups	S9 mix	μ g/ml	Harvest time (hrs)
Bay 63-2521	\pm	125	18
Bay 63-2521	\pm	250	18
Bay 63-2521	\pm	450	18
Bay 63-2521	\pm	450	30
Bay 63-2521	-	15	18
Bay 63-2521	-	30	18
Bay 63-2521	-	60	18

Mitotic index showed dose-related decreases with increasing concentration of test article. The sponsor dismissed statistically significant results as non-relevant in that they were within the historical ranges. However, the positive results repeated. They were not within all the historical ranges and some were at the upper limits of the historical ranges. There was however, no clear signal for a definite effect.

Chromosomal aberrations: 18 hr harvest time

-S9, 4 hours treatment +S9, 4hrs treatment

Experimental Group and Concentration in μ g/ml	Metaphases with Aberrations (%)			Metaphases with Aberrations (%)		
	incl. gaps	excl.gaps	exchanges	incl. gaps	excl.gaps	exchanges
DMSO 0	2.0	2.0	0.0	4.0	3.0	0.0
	1.0	1.0	0.0	1.0	1.0	0.0
	1.5	1.5	0.0	2.5	2.0	0.0
BAY 63-2521 125	3.0	3.0	2.0	5.0	5.0	1.0
	1.0	1.0	1.0	3.0	3.0	1.0
	2.0	2.0	1.5	4.0	4.0	1.0
BAY 63-2521 250	5.0	4.0	2.0	2.0	2.0	0.0
	1.0	1.0	1.0	1.0	1.0	0.0
	3.0	2.5	1.5	1.5	1.5	0.0
BAY 63-2521 450	2.0	2.0	1.0	5.0	5.0	1.0
	7.0	7.0	3.0	7.0	7.0	0.0
	4.5	4.5	2.0	6.0	6.0*	0.5
Mitomycin C 0.1	46.0	44.0	22.0	58.0	57.0	29.0
	60.0	58.0	33.0	59.0	58.0	24.0
	53.0**	51.0**	27.5**	58.5**	57.5**	26.5**

Chromosomal aberrations: 30 hr harvest time

**Chromosomal Aberrations without and with metabolic Activation
4 Hours Treatment**

Experimental Group and Concentration in µg/ml	Harvest Time in Hours	Culture Number	Cells scored	Gaps		Chromatid Type			Classes of Aberrations Chromosome Type				other				Metaphases with Aberrations (%)			
				g	ig	b	f	d	ib	if	id	ex	maE	ma	cd	incl. gaps	excl. gaps	exchanges		
without metabolic activation																				
DMSO 0	30	12	100	1	0	0	0	0	0	1	0	0	0	0	0	0	0	2.0	1.0	0.0
		18	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0	0.0
		200	200	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1.0	0.5	0.0
BAY 63-2521 450	30	20	100	0	0	2	0	0	2	0	0	3	1	0	0	0	0	4.0	4.0	3.0
		27	100	0	0	0	0	0	1	0	0	3	0	0	0	0	0	3.0	3.0	2.0
		200	200	0	0	2	0	0	3	0	0	6	1	0	0	0	0	3.5	3.5*	2.5*
with metabolic activation																				
DMSO 0	30	1	100	0	0	1	0	0	0	2	0	0	0	0	0	0	0	2.0	2.0	0.0
		14	100	0	0	0	0	0	0	0	2	0	0	0	0	0	0	2.0	2.0	0.0
		200	200	0	0	1	0	0	0	2	2	0	0	0	0	0	0	2.0	2.0	0.0
BAY 63-2521 450	30	3	100	0	0	2	0	0	0	0	2	4	0	0	0	0	0	5.0	5.0	2.0
		25	100	0	0	1	0	0	0	1	2	0	0	0	0	0	0	3.0	3.0	2.0
		200	200	0	0	3	0	0	0	0	3	6	0	0	0	0	0	4.0	4.0	2.0

* p < 0.05
** p < 0.01

7.2 In Vivo

7.2.1 Micronucleus test on the male mouse

Key findings: 1) Intraperitoneal dosing was used instead of oral dosing, possibly to ensure exposure; 2) Only males were used. This is also the second genotox study were there is a borderline effect based upon historical control ranges.

Study no.: T8072919 and T1073218

Conducting laboratory and location: [REDACTED] (b) (4)

Date of study initiation: December 8, 2003

GLP compliance: statement included

QA reports: yes (x) no ()

Drug, lot #, and % purity: BX01AD8, 93.3%

Positive control: cyclophosphamide

Animals: male mice, strain Hsd/Win:NMRI 5 mice/dose

Methods

T8072919: Male mice received 2 intraperitoneal injections of 200, 400 and 800 mg/kg 24 hours apart. The femoral bone marrow was prepared 24 hours after the last administration. The sponsor reported signs of systemic toxicity at all doses. All males survived to the scheduled euthanasia.

The text states that there was a pilot study conducted where 3 males and 3 females were used. Only 1 dose (1000 mg/kg) was used and that was to establish the HD for the current study.

T1073218: Male mice received 2 intraperitoneal doses of 100, 200 and 400 mg/kg. Males of the positive control group received single intraperitoneal injections of cyclophosphamide. The femoral bone marrow was prepared 24 hours after the last administration. The sponsor reported signs of systemic toxicity at all doses. 1 HD male died prematurely due to toxicity.

Signs included: apathy, roughened fur, weight loss, spasm, dyspnea, ptosis, diarrhea.

The number of normochromatic erythrocytes (NCE) per 2000 polychromatic ones was recorded. The sponsor defined a ratio of >6000 NCE per 2000 PCE as pathological and unrelated to treatment if other animals in the group do not show similar effects. A variation in micronuclei and NCE was considered statistically significant if its error probability was below 5% and the treatment group figure was higher than that of the negative control. A test was considered positive if there was relevant and significant increase in the number of PCE showing micronuclei in comparison to the negative control.

Summary of results: mean \pm SD (T8072919)

group	MNNCE per 2000 NCE	MNPCE per 2000 PCE
Negative control	2.2 \pm 1.8 (0-4.2)	2.2 \pm 1.3(1-4)
2x200 mg/kg ip	0.7 \pm 0.5(0-1.3)	6.0 \pm 1.2 (5-8)**
2x400 mg/kg ip	1.3 \pm 1.1(0-3.1)	5.6 \pm 2.5(2-9)
2x800 mg/kg ip	1.9 \pm 1.1(0.6-3.2)	5.4 \pm 1.5(3-7)
cyclophosphamide	1.8 \pm 2.4(0-5.8)	28.8 \pm 10.5(17-44)**

**p<0.01 in Wilcoxon test

Summary of results: mean \pm SD (T1073218)

group	MNNCE per 2000 NCE	MNPCE per 2000 PCE
Negative control	4.4 \pm 3.1(0-8.2)	2.8 \pm 1.3(2-5)
2x100 mg/kg ip	4.7 \pm 2.6(0-7)	4.6 \pm 1.8(3-7)
2x200 mg/kg ip	2.1 \pm 2.5(0-5.2)	3.2 \pm 2.2(0-6)
2x400 mg/kg ip	5.7 \pm 2.3(2.6-7.6)	6.0 \pm 3.7(2-10)
cyclophosphamide	3.2 \pm 1.9(0-4.8)	32.6 \pm 6.7(25-41)

While the sponsor notes that the results are within historical ranges, the most recent historical controls to the study are below the levels reached in the drug-treated mice.

Table 9-9: Negative Controls 2003

Sacrifice 24 hours, intraperitoneal treatment,
Results of Males based on 10,000 PCE per Study

Study Number	Vehicle	number of NCE per 2000 PCE	MNNCE per 2000 NCE	MNPCE per 2000 PCE
T 0072065	0.5% C	2222	1.8	3.8
T 5063303	0.5% C	1612	2.2	3.4
T 1063318	0.5% C	1106	2.4	5.3
T 5072907	0.5% C	1650	4.0	4.4
T 4063348	0.5% C	1440	1.8	3.8
T 2063346	0.5% C	1457	4.7	4.6
T 3072905	0.5% C	1185	3.1	2.6
T 3072923	0.5% C	2356	4.2	2.8
T 3072950	0.5% C	1446	0.9	2.4
T 3072905	0.5% C	1185	3.1	2.6
T 8073206	0.5% C	1349	2.2	2.6
T 8072919	0.5% C	1483	2.2	2.2
T 8072937	0.5% C	1266	2.3	3.6
T 1073218	0.5% C	812	4.4	2.8

7.2.2 In vivo bone marrow cytogenetic study in the male mouse

Key findings: Under the conditions of the assay there was no apparent increase in chromosome aberrations.

Study no.: T9073225

Conducting laboratory and location: Bayer, Molecular and Genetic Toxicology, Wuppertal, Germany

Date of study initiation: February 5, 2004

GLP compliance: statement included

QA reports: yes (x) no ()

Drug, lot #, and % purity: Batch # BX01AD8, 92%, suspended in a vehicle of 0.5% aqueous Cremophor.

Methods: Male NMRI mice received a single intraperitoneal injection of either BAY63-2521 or cyclophosphamide. Doses of BAY63-2521 were 200, 400 and 800 mg/kg. Femoral marrow was prepared 24 hours after administration. For the 800 mg/kg dose a sample was also prepared 48 hours after administration according to the abstract. The summary does not list a 48 hour time point. Positive control animals were euthanized 24 hours after dosing. Negative control animals were euthanized 24 and 48 hours after dosing.

Mitotic index was determined by counting 1000 cells per animal. Chromosomes of approximately 100 metaphases per animal were examined. The sponsor states that only metaphases containing the modal chromosome number were analyzed unless exchanges were detected. In this case, metaphases were evaluated even if the chromosome number was not equal to 40.

Animals treated with BAY63-2521 showed signs of toxicity at doses ≥ 200 mg/kg. One of fifteen animals died before the end of the study due to acute toxicity. Signs reported included apathy, roughened fur, weight loss, sternal recumbency, spasm, shivering, dyspnea, ptosis, diarrhea.

Group	Euthanasia time (hrs)	Metaphases with aberrations %		
		Incl gaps	Excl gaps	Only with exchanges
Neg control	24	1.6 \pm 1.9	0.8 \pm 1.3	0 \pm 0
200 mg/kg	24	1.8 \pm 1.1	1.0 \pm 1.2	0 \pm 0
400 mg/kg	24	2.8 \pm 2.6	1.4 \pm 1.3	0 \pm 0
800 mg/kg	24	2.0 \pm 1.2	1.4 \pm 0.5	0 \pm 0
Positive control	24	37.6 \pm 5.5	33.6** \pm 7.4	4.0** \pm 2.3
Neg control	48	1.2 \pm 0.8	0.8 \pm 0.8	0 \pm 0
800 mg/kg	48	3.4 \pm 3.0	2.0 \pm 2.0	0 \pm 0

**p<0.01 Chi squared test.

7.2.3 PH-33557/T6073899 Special study for toxicokinetic investigation in mice (single dose administration by intra-peritoneal injection)

This GLP study was done to support 2 of the genetic toxicology studies in which intraperitoneal dosing was used.

Groups of 21 male NMRI mice were given BAY63-2521 at doses of 100, 200, 400 and 800 mg/kg body weight. TK examinations were performed at 0.25, 0.5, 1, 2, 4, 7 and 24 hours post-dose. No unscheduled mortality was seen.

Plasma analysis by LC/MS/MS showed rapid absorption in all dose groups. 24 hours after dosing, plasma concentrations were $\leq 21\%$ of C_{max} for the 100 and 200 mg/kg dose groups. The highest dose group exhibited a plateau up to 24 hours after administration. The sponsor's summary of the TK is shown below.

Dose	[mg/kg]	100	200	400	800
AUC(0-24)	[$\mu\text{g}\cdot\text{h/L}$]	14113	28439	37294	102875
AUC(0-24) _{norm}	[kg $\cdot\text{h/L}$]	0.141	0.142	0.0932	0.129
C _{max}	[$\mu\text{g/L}$]	1329	2762	3820	4604
C _{max, norm}	[kg/L]	0.0133	0.0138	0.00955	0.00575
t _{max}	[h]	2.00	2.00	1.00	24.00

There was exposure of the mice to the test article at each dose in the in vivo gene tox studies.

8 Carcinogenicity

8.1 Carcinogenicity Study in Wistar Rats

Conducting laboratory and location: Bayer Pharma AG, Wuppertal, Germany

Study number(s): T9078248

Date of study initiation: October 31, 2007

Drug lot/batch number: a) BX028BL, purity 96.9% b) BX02RKK, 98.4%

Analyses done prior to study:

The test item concentration for all doses and homogeneity of the high and low doses were analyzed before the start of the study.

Analyses done during in-life phase:

The test item concentration for all doses and the homogeneity of the low and high dose preparations were checked at beginning and termination of the study and every three months in between.

Validation studies for these analyses were referenced. The homogeneity, stability and concentration were reported to be within the prespecified limits of acceptance.

GLP compliance: Yes

QA statement: Yes

Methods

Harlan Wistar rats (Hsd Cpb:WU) were administered BAY63-2551 as an admixture in the diet. The ppm concentrations of the diet were adjusted weekly up to week 26 to reach the scheduled effective dose. Thereafter the concentrations were adjusted only when there was a 10% difference in weight between current and most recent past measurement.

Sponsor's summary of study design

Group No.	Dose (mg/kg)	Sex	Number of Animals	Animal number	Start of Treatment
Main Groups					
1	0	male	50	1 - 50	5-NOV-2007
2	5	male	50	51 - 100	5-NOV-2007
3	10	male	50	101 - 150	5-NOV-2007
4	20	male	50	151 - 200	5-NOV-2007
5	0	female	50	201 - 250	6-NOV-2007
6	5	female	50	251 - 300	6-NOV-2007
7	10	female	50	301 - 350	6-NOV-2007
8	20	female	50	351 - 400	6-NOV-2007
Satellite Groups					
9	0	male	15	401 - 415	7-NOV-2007
10	5	male	15	416 - 430	7-NOV-2007
11	10	male	15	431 - 445	7-NOV-2007
12	20	male	15	446 - 460	7-NOV-2007
13	0	female	15	461 - 475	7-NOV-2007
14	5	female	15	476 - 490	7-NOV-2007
15	10	female	15	491 - 505	7-NOV-2007
16	20	female	15	506 - 520	7-NOV-2007

The observations made are summarized in the sponsor's table below.

Inspection of Animals: for Morbidity and Mortality	Twice daily, once daily on weekends and public holidays
Detailed Clinical Examinations	Main Groups Weekly
Determination of:	
Body Weight(s) (main groups)	Weekly
Food Intake (main groups)	Weekly
Water Intake (main groups)	Weekly for the first 13 Weeks Every 4 weeks thereafter up to termination
Ophthalmological Investigation:	Before start of treatment (all main group animals); month 12 (control and high dose main groups) and 18 and 24 (control, mid and high dose main groups)
Clinical Laboratory Investigation:	
Hematology/Clinical chemistry	First 10 alive animals per satellite group Month 6 (Day 182/183) Month 12 (Day 364/367) Month 18 (Day 546/547) Month 24 (Day 728/729)
Preparation of Blood Smears	All moribund rats (main groups) all alive main group rats after 12, 18 and 24 months
Urinalysis	First 10 alive animals per satellite group Month 6 (Day 182/183) Month 12 (Day 364/367) Month 18 (Day 546/547) Month 24 (Day 728/729)
Necropsy:	Week 105-107
Toxicokinetics:	days 2/3, 373/374, and 732 (satellite groups)

Hematological parameters analyzed in peripheral blood: erythrocyte count, hemoglobin concentration, hematocrit, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, reticulocyte count, erythrocyte morphology, thrombocyte count, thromboplastin time, leucocyte count, differential count. Differential determination was performed only on the blood smears of the following:

- All animals euthanized moribund
- All main group control and high dose animals alive near the end of the study

Clinical chemistry parameters analyzed: alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, glutamate dehydrogenase, gamma glutamyl transferase, glucose, cholesterol, triglyceride, creatinine, potassium, urea, total bilirubin, total protein, albumin, chloride, calcium, inorganic phosphate, sodium.

Urinalysis parameters determined: density, volume, protein concentration, creatinine concentration, protein excretion, creatinine excretion, protein/creatinine ratio, pH, blood, bilirubin, glucose, ketone bodies, urobilinogen, microscopy of sediment

Necropsy: Included gross observations, organ weights, histopathology. In case that histopathological examination was not possible in a main group animal, it was replaced by a corresponding satellite group member. Organs weighed and sampled are summarized in the sponsor's table shown below.

Sponsor's Summary of Organs Weighed and Collected

Organs fixed Group No.	Organ weights 1-8	Histopathological evaluation ³			
		1	2	3	4
Abnormalities		X	X	X	X
Adrenals	X	X	X	X	X
Aorta		X	X	X	X
Brain (3 regions)	X	X	X	X	X
Cecum		X	X	X	X
Colon		X	X	X	X
Duodenum		X	X	X	X
Epididymides	X	X	X	X	X
Esophagus		X	X	X	X
Exorbital lacrimal glands		X	X	X	X
Eyes		X	X	X	X
Eyelids		X	X	X	X
Femur (with bone marrow/joint)		X	X	X	X
Harderian glands		X	X	X	X
Head with skull cap					
Heart	X	X	X	X	X
Ileum		X	X	X	X
Jejunum		X	X	X	X
Kidneys	X	X	X	X	X
Larynx		X	X	X	X
Liver	X	X	X	X	X
Lungs		X	X	X	X
Lymph node, mandibular		X	X	X	X
Lymph node, mesenteric		X	X	X	X
Lymph nodes, popliteal					
Nasal Cavity/Nasopharynx		X	X	X	X
Ovaries / Oviducts	X	X	X	X	X
Optic nerves		X	X	X	X
Pancreas		X	X	X	X
Peyer's patches					
Pharynx					
Pituitary gland		X	X	X	X
Preputial gland		X	X	X	X
Prostate		X	X	X	X
Rectum		X	X	X	X
Salivary glands		X	X	X	X
Sublingual gland					
Submandibular gland					
Parotid gland					
Sciatic nerve		X	X	X	X
Seminal vesicles with Coagulation glands		X	X	X	X
Skeletal muscle		X	X	X	X

Organs fixed Group No.	Organ weights 1-8	Histopathological evaluation ⁵			
		1	2	3	4
Skin/mammary region		X	X	X	X
Spinal cord 3x		X	X	X	X
Spleen	X	X	X	X	X
Sternum (with bone marrow)		X	X	X	X
Stomach		X	X	X	X
Testes	X	X	X	X	X
Thymus		X	X	X	X
Thyroid / Parathyroids		X	X	X	X
Tongue		X	X	X	X
Trachea		X	X	X	X
Uterus with Cervix	X	X	X	X	X
Ureters		X	X	X	X
Urethra					
Urinary bladder		X	X	X	X
Vagina		X	X	X	X
Zymbal glands		X	X	X	X
Physical identifier					

Toxicokinetic sampling: The days and times of blood sampling are summarized in the sponsor's table below.

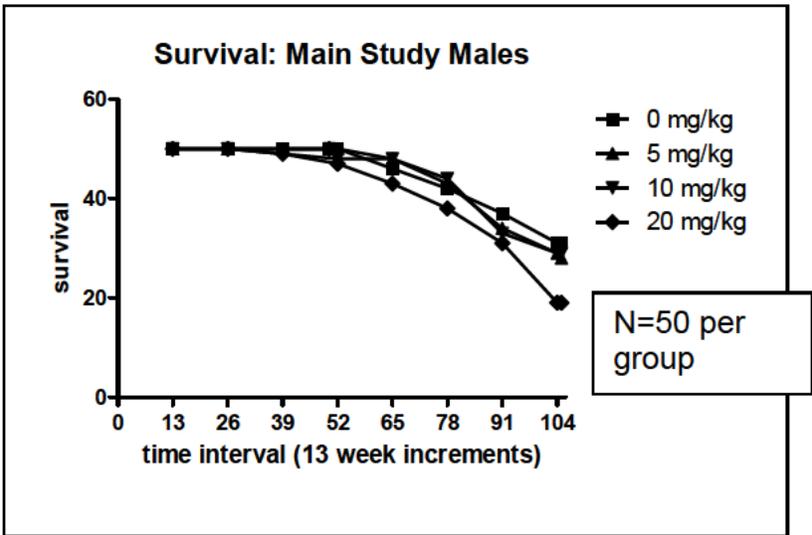
Toxicokinetics	
Days of Blood Sampling:	days 2/3, 373/374, and 732
Time Points at Days 2/3 and 373/374:	
- treated animals	6 (8, 13, 18, 23, 4 and 8 CET)
- control animals	3 (8, 23, and 4 CET)
Time Points at Day 732:	1 (23 CET)
Number of Animals per Time Point:	3 (scheduled per group)

Results

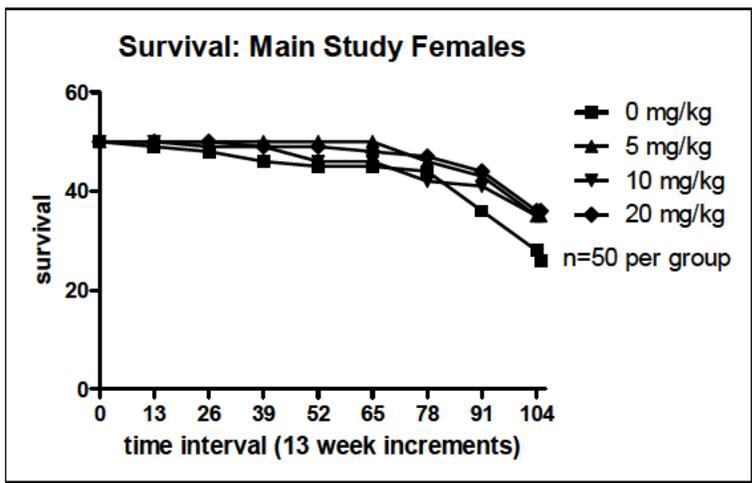
[Note: the CDER statistical review for the mouse and rat carcinogenicity studies is on file in DARRTs, filing date April 22, 2013]

Mortality

Males did not show early mortality in association with the test article. There was slightly greater mortality in the male 20 mg/kg group, but this did not become apparent until between weeks 52 and 60. According to the CDER statistical reviewer, the pairwise comparisons showed statistically significant increased mortality in the male high dose group compared to the control.



Increased early mortality was not apparent in the females. According to the CDER statistical reviewer, the pairwise comparisons showed statistically significant decreased mortality in the female rat high dose group compared to the control.



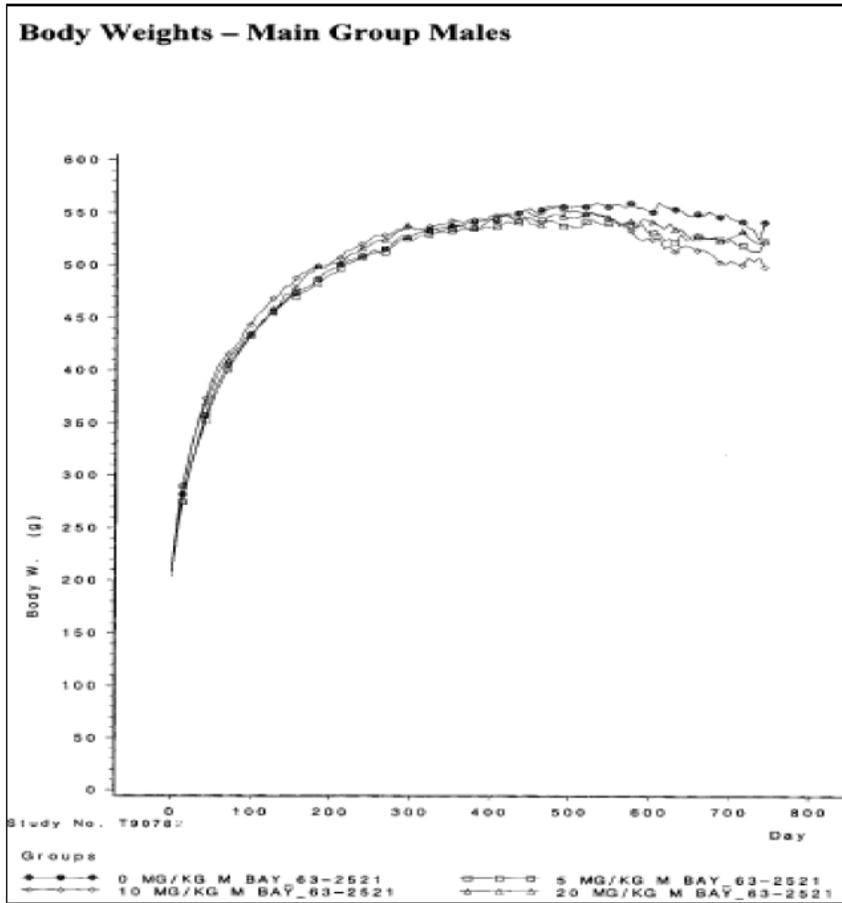
Clinical signs in the 10 mg/kg and 20 mg/kg group males included emaciation, accelerated and labored breathing. The incidences are summarized in the sponsor's table below.

Table 5-3: Remarkable Clinical Symptoms - Cumulative Incidences (Main Groups)

Sex Dose mg/kg No of rats at study start	Males				Females			
	0	5	10	20	0	5	10	20
	50	50	50	50	50	50	50	50
Emaciation	28	23	35	38	22	18	13	22
Accelerated breathing	1		3	5	2			
Labored breathing	1		3	4				1
Increased urine excretion (observed per cage)	15	27	27	34	2	2	4	

Body weights: Males

The drug-treated males gained on average less than the control group. No dose-response was apparent. The differences in body weight relative to the control group are summarized in the reviewer’s table below.

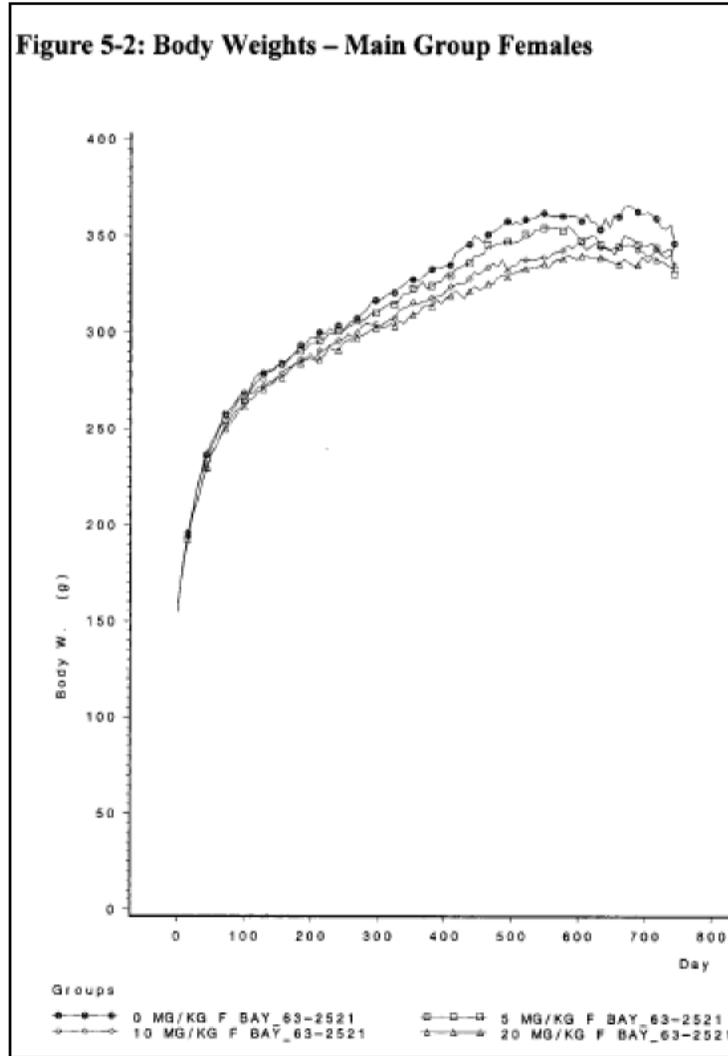


Summary of Body Weight Changes

Males: Main Dose Groups

Dose mg/kg/day	0	5	10	20
Day 1	208	203	210	211
Day 729	533	513	503	522
Δ day729-Day 1	325	310	293	311
% difference from control day 729	NA	-4	-6	-2

Body weights: females



The drug-treated females gained on average 3-5% less than the control group. The bodyweight changes relative to the control group are summarized in the reviewer’s table shown below.

Summary of Body Weight Changes	Females: Main Dose Groups			
Dose mg/kg/day	0	5	10	20
Day 1	158	156	155	156
205/206	297	295	286	285+
Day 205-day1	139	139 (0)	131 (-6)	129 (-7)
428	344	335	324++	317++
Day 428-day1	186	179 (-4)	169 (-9)	161 (-13)
Day 730	353	342	338	335
Δday730-Day 1	195	186	183	179
% difference from control day 730		-3	-4	-5

+ significantly different at p<0.05,++ significantly different at p<0.01

Exposure to Test Item

There were no significant differences in mean food intake between the groups. The sponsor's summary of mean effective doses are shown below. Both sexes consumed sufficient food to achieve within $\pm 10\%$ of the nominal dosages.

Table 5-7: Mean Effective Dosage - Main Groups

Sex Dose (mg/kg)	Males			Females		
	5	10	20	5	10	20
Average used concentration in the food (ppm):	97.54	177.88	354.75	73.58	141.09	273.27
Effective dose (mg/kg):	4.98	10.10	20.25	4.98	10.01	20.00
Difference (%):	-0.36	0.96	1.24	-0.19	0.10	0.00

Plasma level determination of drug and active metabolite demonstrated exposure in all dose groups.

BAY 63-2521							
Gender		male			female		
Dose	[mg/kg]	5	10	20	5	10	20
AUC(0-24)	[$\mu\text{g}\cdot\text{h/L}$]	1526	2812	5923	1967	4999	9099
AUC(0-24) _{norm}	[$\text{kg}\cdot\text{h/L}$]	0.305	0.281	0.296	0.393	0.500	0.455
C _{max}	[$\mu\text{g/L}$]	92.2	155	325	104	303	515
C _{max, norm}	[kg/L]	0.0184	0.0155	0.0163	0.0208	0.0303	0.0258
C _{min} /C _{max}	[%]	43.5	42.1	56.0	60.2	40.6	54.7
t _{max}	[h]	15.0	10.0	15.0	15.0	15.0	15.0
R _A C _{max}	[%]	168	139	147	195	252	245
R _A AUC	[%]	169	143	148	210	260	215
BAY 60-4552*							
Gender		male			female		
Dose	[mg/kg]	5	10	20	5	10	20
AUC(0-24)	[$\mu\text{g}\cdot\text{h/L}$]	233	470	940	141	408	850
AUC(0-24) _{norm}	[$\text{kg}\cdot\text{h/L}$]	0.0482	0.0486	0.0486	0.0292	0.0422	0.0440
C _{max}	[$\mu\text{g/L}$]	13.5	24.3	50.2	7.83	25.5	49.6
C _{max, norm}	[kg/L]	0.00280	0.00251	0.00260	0.00162	0.00264	0.00256
C _{min} /C _{max}	[%]	54.0	40.2	53.6	49.0	43.4	55.2
t _{max}	[h]	15.0	20.0	20.0	20.0	15.0	15.0
R _A C _{max}	[%]	221	166	171	231	256	255
R _A AUC	[%]	228	188	184	206	263	236
MR _{AUC}	[%]	15.8	17.3	16.4	7.4	8.4	9.7

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*Equivalent dose was used for the calculation of dose-normalized parameters of M-1 using a factor of 0.967.

The multiples of human exposure achieved are summarized in the reviewer's tables below.

Reviewer's Summary of Plasma Exposure to BAY63-2521 in Rats

	Male mg/kg/day			Female mg/kg/day		
Dose mg/kg	5	10	20	5	10	20
AUC ₀₋₂₄ µg.hr/l	1526	2812	5923	1967	4999	9099
Multiple of human exposure	0.4X	0.7X	1.4X	0.5X	1.2X	2.2X
Human: 2.5 mg t.i.d. based on multiple dose study #12166 in pulmonary hypertension patients : AUC ₀₋₂₄ 4161 µg hr/l						

Reviewer's Summary of Plasma Exposure to BAY60-4552 in Rats

	Male mg/kg/day			Female mg/kg/day		
Dose mg/kg	5	10	20	5	10	20
AUC ₀₋₂₄ µg.hr/l	233	470	940	141	408	850
Multiple of human exposure	0.1X	0.2X	0.4X	0.05X	0.2X	0.3X
Human: 2.5 mg t.i.d. sponsor estimated based on multiple dose study #12166 in pulmonary hypertension patients AUC ₀₋₂₄ 2604 µg.hr/l						

Hematology: Males

Hemoglobin and hematocrit were increased in drug-treated males for the first year. By the end of the study, hemoglobin was decreased 7% (n.s.) and hematocrit was decreased 5% (n.s.) in high dose males. At the same time point, the reticulocyte count in this group was increased over the control values. This suggests a regenerative anemia. However, the changes are small and of uncertain biological significance.

Summary of Hematology for the Males

	0 mg/kg	5 mg/kg	10 mg/kg	20 mg/kg
Hemoglobin g/l				
Day 182	145	152+	152+	155++
364	152	154	159	157
546	149	156	151	143
728	144	164+ (+8%)	151	144 (-7%)
HCT				
Day 182	0.466	0.493++	0.485	0.486
364	0.494	0.493	0.503	0.494
546	0.479	0.499	0.483	0.451
728	0.462	0.520 (+5)	0.477 (-2)	0.462 (-5)
MCV fl				
Day 182	56.2	57.1	56.3	56.0
364	55.2	55.3	54.5	54.5
546	55.5	56.0	54.7	54.3
728	54.3 (-3)	54.7 (-4)	53.6 (-5)	54.8 (-2)
MCHC g/l eryth				
Day 182	310	309	312	318++
364	308	312	316++	317++
546	310	313	312	317+
728	311	315 (+2)	317 (+2)	311 (-2)
Retic o/oo				
Day 182	15	16	17	15
364	14	14	15	13
546	14	15	17	14
728	14	15	15	20 (+30)
Thro 10e9/l				
Day 182	1289	1374	1267	1346
364	1404	1329	1293	1364
546	1047	969	1124	1212
728	1346	1178	1220	1215
ERY 10e12/l				
Day 182	8.29	8.63+	8.62	8.70+
364	8.95	8.90	9.23	9.06
546	8.64	8.93	8.83	8.30
728	8.51	9.53	8.90	8.43

Numbers in parentheses () are percent change from the group day 182 value.

Hematology: females

The erythrocyte count was increased in the first three determinations as was hemoglobin and hematocrit. While some of the changes are marked as statistically significant, the biological significance is uncertain. There were no reported bone marrow changes at the end of the study and no bone marrow samples taken earlier in the study for comparison with the peripheral changes.

Summary of Hematology for Females

	0 mg/kg	5 mg/kg	10 mg/kg	20 mg/kg
ERY10E122/1				
Day 182	7.58	8.18++	8.38++	8.29++
364	8.10	8.49	8.47	8.27
546	7.83	8.35+	8.63++	8.37+
728	8.44	8.38	8.63	8.33
Hemoglobin g/l				
Day 182	142	154++	157++	155++
364	152	161++	163++	159+
546	141	155++	158++	155++
728	149	154	157	153
HCT				
Day 182	0.449	0.485++	0.495++	0.489++
364	0.468	0.493++	0.491+	0.478
546	0.450	0.494++	0.506++	0.489++
728	0.484	0.488	0.494	0.481
MCV fl				
Day 182	59.3	59.2	59.1	59.0
364	57.9	58.2	58.0	57.9
546	57.5	59.2	58.7	58.5
728	57.3	58.3	57.3	57.8
MCHC g/l eryth				
Day 182	317	318	318	318
364	326	326	332	333+
546	314	313	313	317
728	309	316	317	319+
Retic o/oo				
Day 182	18	17	14	18
364	17	17	17	19
546	19	19	20	17
728	16	21	21	19
Thro 10e9/l				
Day 182	1091	1125	1149	1188
364	1128	1113	1133	1105
546	890	912	1038	961
728	899	857	901	960

There were no findings of toxicological significance in the differential data for either sex.
There were no findings of toxicological significance in the clinical chemistry for either sex

Urinalysis

Increased urine protein excretion was reported for both sexes of drug-treated animals, but more pronounced in the males. The effect in the males also appears earlier in the study and in all groups of the drug-treated animals compared to the females.

Dose mg/kg	VOL ml	Density g/l	pH	PROT g/l	CREA mmol/l	PROT* VOL mg	CREA* VOL mcmol	PROT /CREA mg/mmol
Males								
Day 182/ 183								
0	5.8	1037	7.4	4.63	19.65	21.2	95	228
5	7.1	1034	7.1	13.36	15.05	92.9	+	104
10	6.2	1041	7.2	14.27	19.25	85.3	++	110
20	7.8	1032	7.5	5.75	14.18	43.3	+	107
Males								
Day 364/ 367								
0	4.0	1043	7.6	10.69	23.76	42.1	93	473
5	6.7	1040	7.1	23.23	19.09	158.5	++	111
10	5.5	1041	7.2	20.57	17.72	114.3	++	97
20	5.1	1044	7.3	18.90	18.59	95.3	+	89
Males								
Day 546/ 547								
0	4.5	1045	7.1	14.22	17.77	64.1	74	851
5	8.5	1035	6.8	18.62	11.79	146.4	+	86
10	11.6	1026	6.8	16.79	8.74	175.7	+	84
20	9.9	1033	6.9	16.99	10.85	144.6	+	74
Males								
Day 728/ 729								
0	9.1	1029	6.7	12.32	9.44	111.3	78	1466
5	13.2	1026	6.6	12.84	7.66	179.3	93	1920
10	9.9	1033	6.9	14.76	11.37	141.7	96	1778
20	11.5	1027	6.5	14.71	8.02	170.3	83	2029
Females								
Day 182/ 183								
0	3.9	1037	6.7	1.40	17.26	5.1	63	74
5	8.9	1021	7.1	1.65	9.15	8.9	74	148
10	6.1	1026	7.2	1.14	11.31	6.9	67	108
20	9.3	1023	7.0	1.25	9.25	11.2	76	140
Females								
Day 364/ 367								
0	6.2	1030	6.9	4.11	11.62	21.7	68	328
5	6.0	1027	6.8	4.64	11.09	23.0	64	447
10	6.6	1025	7.2	3.15	10.21	19.7	65	309
20	8.0	1024	7.0	4.06	9.66	30.2	73	390
Females								
Day 546/ 547								
0	7.5	1028	7.0	5.86	8.60	47.7	61	799
5	8.6	1028	6.8	7.15	7.84	65.1	62	1055
10	7.6	1027	7.3	7.11	7.10	58.8	54	1060
20	10.9	1023	6.6	6.04	6.54	70.5	65	1078
Females								
Day 728/ 729								
0	7.3	1028	6.4	9.19	10.37	61.3	74	917
5	8.2	1027	6.8	9.04	9.18	60.0	70	981
10	10.5	1027	6.5	17.08	8.02	186.6	79	2324
20	11.3	1025	6.7	16.14	7.05	175.7	76	2526

+ significantly different at $p \leq 0.05$

++ significantly different at $p \leq 0.01$

Organ Weights

Liver weight normalized to body weight showed dose-related increases in both sexes of drug-treated animals (+17% high dose males, $p \leq 0.05$; +12% high dose females, n.s.) when compared to the control group values. Kidney weights were also increased in both sexes without obvious dose-response. High dose males were also reported to have increased spleen (+13%, $p < 0.01$) and testes (+19%, $p < 0.01$) relative to the control group. Ovarian weight was increased (+28%, $p < 0.05$)

Table 5-18: Relative Organ Weights - Main Groups

Dose (mg/kg)	Body W. g	Brain mg/100g	Adrenals mg/100g	Heart mg/100g	Liver mg/100g	Spleen mg/100g	Kidneys mg/100g	Testes mg/100g	Epididym. mg/100g
Males									
0	524	431	15	425	3755	225	792	649	292
5	509	444	18	448	3979	246	909 +	741 ++	295
10	500	459	16	460	4117 +	226	885	751 ++	294
20	519	439	19	465	4387 ++	255	967 ++	775 ++	290
Females									
0	355	583	20	500	3741	223	805	50	402
5	340	598	20	479	3948	207	860	55	396
10	339	597	21	494	3873	226	852	53	400
20	334 +	610	21	512	4185 +	214	870	64 +	445

+ significantly different at $p \leq 0.05$
 ++ significantly different at $p \leq 0.01$

Non-Neoplastic Findings

Gross observations from main group showed increased incidences of enlarged heart, small intestinal consistency changes and discolored mesenteric lymph nodes. None of the incidences reached statistical significance. (28% in HD males, 12% in HD females relative to control incidence).

Incidence of gross observation

Gross observation	males		females	
	control	high dose	control	high dose
Enlarged heart	8/50	14/50	2/50	6/50
Small intestine consistency change	0/50	7/50	0/50	9/50
Enlarged kidneys	4/50	10/50	1/50	0/50
Ovarian cysts			5/50	11/50
Mesenteric lymph node discoloration	1/50	3/50	0/50	3/50

The pathologist stated that there was no histopathological correlate to the cardiac enlargement.

The pathologist summarized the most common causes of premature mortality as follows:

Among the most common causes of death were		MALES			
DOSE [MG/KG]	n:	0	5	10	20
- chronic progressive nephropathy:		6/10	11/9		
- tumors (benign and malignant):		8/7	9/3		
- inflammation:		1/3	7/4		
- thrombosis (heart, kidneys, other):		0/2	4/5		
		FEMALES			
DOSE [MG/KG]	n:	0	5	10	20
- tumors (benign and malignant) :		17/11	10/9		
- inflammation:		2/0	2/2		
- chronic progressive nephropathy:		1/3	1/2		
- thrombosis (heart):		1/0	0/0		

And added these comments as well:

In the heart of decedents

- a higher incidence of gross enlargement was observed at 20 mg/kg in males (incidence 2/6/6/10). A histopathological correlate could not be found.
- vasculopathy/media hypertrophy of arteries was slightly more frequent in the myocardial vasculature at 10 and 20 mg/kg in both sexes. However, a dose-relationship was not present and the finding was rated mostly minimal or slight.
- atrial thrombosis was significantly increased in males at 20 mg/kg (0/3/3/5) in the pairwise comparison (p < 0.05). In one male dosed at 10 mg/kg, thrombosis was seen in the pulmonary artery. In female sex, atrial thrombosis was seen in one control only (1/0/0/0).

Table 5-19: Noteworthy Non-Neoplastic Changes

Sex	Dose (mg/kg)	Male				Female			
		0	5	10	20	0	5	10	20
Heart	- No. exam.	49	49	47	42	49	49	49	50
- thrombus atrial		2	3	3	5	1	0	0	0
- dilation		7	8	9+1	11+1	2	1	3	2
- cardiomyopathy		t* 37+1	39+1	39+3	38+7	27+1	21	23	20
- vasculopathy		t* 17	21	24+1	22+4	9	5	14	12
Mesent. lymph nodes	- No. exam.	49	48	47	40	48	48	50	50
- plexiform change of mesenteric veins		t* 6	17**	14**+1	25**+5	t* 2	3	3	14**
- erythrophagocytosis		t* 5	8	10	12*	t* 4	4	5	9
Liver	- No. exam.	49	49	47	42	49	49	50	50
- biliary cysts		t* 5	7	8+2	12*	9	14	10	16
Thyroid gland	- No. exam.	48	49	47	42	49	49	50	50
- hyperplasia of C-cells, diffuse		t* 1	1	0	5	8	15	11	16

* = p < 0.05, ** = p < 0.01 (one-tailed pairwise group comparison)

t* = p < 0.05 (trend-test);

Remark: The table gives the number of organs examined from main group animals, the incidence of the finding in main groups and the results of the statistical calculation for these data. In addition, organs of satellite animals were examined to achieve an overall number of organs examined of 50. If the respective finding was also observed in these additionally investigated organs of satellite group animals, this is indicated by e.g. +1.

The ocular findings are summarized in the reviewer's table. A drug-effect is not suggested.

Ocular Non-neoplastic Changes

	Dose mg/kg			
	0	5	10	20
Males				
Retinal atrophy	14/48	11/49	11/46	10/42
Lenticular degeneration	18/48	15/49	11/46	15/42
Females				
Retinal atrophy	16/49	27*/49	23/50	23/50
Lenticular degeneration	15/49	12/49	13/50	16/50

Neoplastic Findings

Because of the gastrointestinal findings in the mice, the rat findings are shown below. There are no apparent trends.

ORGAN/DOSE [MG/KG]	MALES				FEMALES			
	0	5	10	20	0	5	10	20
Tongue Tumor, granul.c.benign	0/	0/	0/	0/	1 /	0 /	0/	0/
Forestomach Hyperplasia	1/	4/	4/	2	1 /	2 /	1/	1
Gland. stomach Hyperplasia	0/	0/	1/	0	0 /	0 /	0/	0
Duodenum - Avill. hyperplasia	0/	0/	0/	2	0 /	0 /	0/	0
- Adenocarcinoma mal.	0/	0/	0/	1	0 /	0 /	0/	0
- Hyperpl.,Brunn.gld.	0/	0/	0/	1	0 /	0 /	0/	0
- Leiomyoma ben.	0/	0/	0/	0	0 /	0 /	0/	1
Jejunum - Hyperplasia	1/	0/	0/	0	0 /	0 /	0/	0
Ileum - Adenoma ben.	1/	0/	0/	0	0 /	0 /	0/	0
- Leiomyoma ben.	0/	0/	0/	0	0 /	0 /	0/	1
Rectum - Hyperplasia	0/	0/	1/	0	0 /	0 /	0/	1
Salivary glands - Adenocarcinoma	1/	0/	0/	0	0 /	0 /	0/	0
Parotid gland - Hyperplasia	0/	0/	1/	0	0 /	1 /	0/	0
Pancreas - Hyperplasia, exocrine	3/	4/	0/	0	0 /	0 /	0/	0
- Adenoma, exocrine	1/	0/	0/	0	0 /	0 /	0/	0
- Hyperplasia islet c.	1/	0/	0/	1	0 /	0 /	1/	2
- Adenoma, islet cell	0/	0/	2/	0	1 /	0 /	0/	0
- Carcinoma, islet cell	0/	1/	1 /	0	0 /	0 /	1/	0
Liver - Basophil.focus NOS	8+1/	5/11+1/	6		9 /	13 /	11/	16
- Basoph.focus tigr.	10 /	10/10+1/	7		10+1/	12 /	14/	16
- Clear cell focus	39+1/	41/31+2/	27+8		14+1/	20 /	22/	20
- Eosinophilic focus	7+1/	10/ 4 /	6		1 /	4 /	1/	5
- Adenoma, hepatocell.	0 /	1/ 0 /	0		0 /	0 /	0/	1
- Carcinoma, hepatocell.	0+1/	0/ 0 /	0		0 /	0 /	0/	0
- Hyperplasia, bile d.	37+1/	42/42+3/	37+8		31+1/	33+1/	39/	37
- Cholangioma ben.	0/	0/ 0 /	1		0/	0/	1/	1
- Cholangiocarcinoma	1 /	0/ 0 /	0		0/	0/	0/	0

In the main groups, a negative trend was calculated in males for clear cell focus in the liver (p= -0.0170) and hyperplasia, exocrine of the pancreas (p= -0.0212).

As seen from the table, all proliferative lesions occurred spontaneously and their distribution and grade were unrelated to dosing with the test compound. A slight numerical increase of foci of cellular alteration was seen in high dose females. This was however not of significance in the trend test and in the pairwise comparison.

While Leydig cell hyperplasia was reported for all groups including the control, only drug-treated animals were reported to have Leydig cell tumors.

Neoplastic findings

	Males (mg/kg)			
	0	5	10	20
Testes: Leydig cell tumor: main study	0/49	2/49	4/47	3/42
Hyperplasia, Leydig cell focal	8	7	6+1	6+3

Because of the bone effects noted in several toxicology studies, special attention was given to any bone effects in this long term study. No specific toxicologic findings are suggested in the data as presented.

Reviewer's Summary of Bone Effects in Rat 2-Year Carcinogenicity Study

	Dose mg/kg			
	0	5	10	20
Males				
Femur: osteodystrophy	9/48	15/49	12/47	11/42
Sternum: cartilage degeneration	22/49	19/49	22/47	16/42
Bone:				
Odontoma	0/48	1/49	1/47	0/42
Osteosarcoma	0/48	0/49	0/47	1/42
osteoma	0/48	0/49	0/47	0/42
Females				
Femur:osteodystrophy	0/49	1/49	2/50	1/50
Sternum: cartilage degeneration	18/49	15/48	20/47	15/50
Ovaries				
Tubulostromal adenoma	0/26	0/35	1/35	1/36
Benign luteoma	0	0	0	1/36
Benign thecoma	0	0	0	1/36
Granulosa CB tumor	0	0	2/35	1/36
Sex cord stromal MB tumor	0	0	1/35	0

The CDER statistical reviewer concurred with the above opinion that there were no significant tumor findings.

From The CDER Statistical Review

Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or Pairwise Comparisons of Treated Groups and Control in Rats										
Sex	Organ Name	Tumor Name	Cont	Low	Med	High	Dose Resp	P_Value		
								C vs. L	C vs. M	C vs. H
Male	ADRENAL GLANDS	Tumor medullary benign,	15	22	17	22	0.0540	0.1116	0.4112	0.0387

Based on the criteria of adjustment for multiple testing discussed above, none of the observed tumors was considered to have statistically significant dose response relationship in either sex. The pairwise comparison also did not show statistically significant increased incidence in any observed tumor type in any treated group in either sex compared to their respective control.

The study was adequate as the Exec CAC gave prior concurrence to the doses used and there was neither increased mortality nor new toxicities evidenced in the study.

8.2 Carcinogenicity Study in Mice

Conducting laboratory and location: Bayer Pharma AG, Wuppertal, Germany

Study number(s): T0078366

Date of study initiation: October 17, 2007

Drug lot/batch number: BAY63-2521, batch number BX028BL (purity 96.9%), BX02RKK (purity 98.4%)

Before the start of treatment the suitability of the formulation was confirmed by the analysis of concentration, homogeneity and stability of dosage forms prepared in the same way as it was done in the study. Analyses were carried out before the start of the study. The sponsor references F5011508.

Homogeneity: Dosage forms including the highest and lowest concentration were prepared. Three samples were taken from different places of the mixture and analyzed for concentration of the test material. Homogeneity of formulations was also checked eleven times during the study for the low and high dose groups.

Stability: the dosage forms prepared for homogeneity analysis were analyzed shortly after preparation and 15 days thereafter. The analysis revealed that the test item was stable over this period within the pre-defined limits.

Content checks: concentrations of samples of control and each test item dosage form prepared were determined eleven times during the study.

The homogeneity, stability and concentration were reported to be within the prespecified limits of acceptance.

GLP compliance: Yes

QA statement: Yes

BAY63-2521 was administered in the diet to 50 male and 50 female Cr:CD-1(ICR)BR mice per dose group in concentrations of 0, 50, 100 or 200 ppm for a period of 2 years until one day before scheduled euthanasia, until spontaneous death, or moribund euthanasia.

Dose selection was based on a maximally tolerated dose from a 13-week dose-ranging study. An additional 20 mice per sex per dose group received the same doses for the same period of time.

The satellite animals were used for toxicokinetics and hematological investigation. At the end of the treatment period, the satellite animals were also subject to gross pathological observation and selected organs were weighed. The sponsor's summary of study design is shown below.

Group No.	Dose ppm	Sex	Number of Animals	Animal number
Main Groups				
1	0	male	50	1-50
2	50	male	50	51-100
3	100	male	50	101-150
4	200	male	50	151-200
5	0	female	50	201-250
6	50	female	50	251-300
7	100	female	50	301-350
8	200	female	50	351-400
Satellite Groups				
9	0	male	20	401-420
10	50	male	20	421-440
11	100	male	20	441-460
12	200	male	20	461-480
13	0	female	20	481-500
14	50	female	20	501-520
15	100	female	20	521-540
16	200	female	20	541-560

The sponsor's summary of observations is provided below:

Inspection of Animals:	
for Morbidity and Mortality	Twice daily, once daily on weekends and public holidays
Detailed Clinical Examinations	Weekly (for main groups from start of study and for satellite groups since March, 2008)
Determination of:	
Body Weights	Weekly until week 13, every 4 weeks thereafter (main groups)
Food Consumption	Weekly until week 13, every 4 weeks thereafter (main groups)
Water Consumption	Weekly until week 13, every 4 weeks thereafter (main groups)
Feeding Period:	Approximately 7 days
Total Feeding Period*	101 weeks (main groups)
Clinical Laboratory Investigation:	
Hematology	Week 51, 78, 103 (satellite groups)
Preparation of Blood Smears:	All moribund animals; All alive animals after 12, 18 months and near the end of study; see also Section 4.11
Necropsy:	Week 105-107 (main groups) Week 107 (satellite groups)

* number of days used for the calculation of cumulative intake values

Hematology parameters determined from peripheral blood: differential blood count, erythrocyte morphology, erythrocyte count, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, hematocrit, leucocyte count, reticulocyte count, thrombocyte count.

Blood was collected from animals dying ahead of scheduled euthanasia. Blood smears were prepared and differential blood counts performed.

Sponsor’s Necropsy Procedures Summary

Organs fixed Group No.	Organ weights 1-16	Histopathological evaluation			
		1	2	3	4
Abnormalities		X	X	X	X
Adrenals	X	X	X	X	X
Aorta		X	X	X	X
Brain (3 regions)	X	X	X	X	X
Cecum		X	X	X	X
Colon		X	X	X	X
Duodenum		X	X	X	X
Epididymides		X	X	X	X
Esophagus		X	X	X	X
Exorbital lacrimal glands		X	X	X	X
Eyes		X	X	X	X
Eyelids		X	X	X	X
Femur (with bone marrow/joint)		X	X	X	X
Gall bladder		X	X	X	X
Harderian glands			X	X	X
Heart			X	X	X
Ileum			X	X	X
Jejunum			X	X	X
Kidneys	X		X	X	X
Larynx			X	X	X
Liver	X		X	X	X
Lungs			X	X	X
Lymph node, mandibular			X	X	X
Lymph node, mesenteric			X	X	X
Lymph node, popliteal					
Ovaries / Oviducts			X	X	X
Optic nerves			X	X	X
Pancreas			X	X	X
Pharynx					
Pituitary gland			X	X	X
Prostate			X	X	X
Rectum			X	X	X
Salivary glands			X	X	X
Sublingual gland					
Submandibular gland					
Parotid gland					
Sciatic nerve			X	X	X
Seminal vesicles with Coagulation glands			X	X	X
Skeletal muscle			X	X	X
Skin/mammary region			X	X	X
Spinal cord 3x			X	X	X
Spleen	X		X	X	X
Sternum (with bone marrow)			X	X	X
Stomach			X	X	X
Testes	X		X	X	X
Thymus			X	X	X
Thyroid / Parathyroids			X	X	X
Tongue			X	X	X
Trachea			X	X	X
Uterus with Cervix			X	X	X
Ureters			X	X	X
Urinary bladder			X	X	X
Vagina			X	X	X
Zymbal glands			X	X	X
Physical identifier					

Blood samples were collected for toxicokinetics. The days and timepoints of sampling are summarized in the sponsor's table below.

Table 4-6: Frequency and Dates of Toxicokinetic Investigations

Toxicokinetics	
Blood Sampling time point:	day 3/4, week 53
Time Points per Day:	
Groups 10-12, 14-16:	6 (8:00, 13:00, 18:00, 23:00, 4:00, and 8:00 (CET))
Groups 9, 13 (controls):	3 (8:00, 23:00 and at 4:00 (CET))
Blood Sampling time point:	week 104
Time Points per Day:	
Groups 9-16:	1 (23:00 (CET))
Number of Animals per Time Point:	3 (scheduled)

Results

[Note: the CDER statistical review for the mouse and rat carcinogenicity studies is on file in DARRTs, filing date April 22, 2013]

Survival was affected in the male mice. The high dose male mice showed an increased mortality relative to all other dose groups. This was apparent early in the study. The CDER statistical reviewer confirmed that the effect was statistically significant. The sponsor proposed the increased gastrointestinal inflammation and secondary consequences as the cause of the increased mortality.

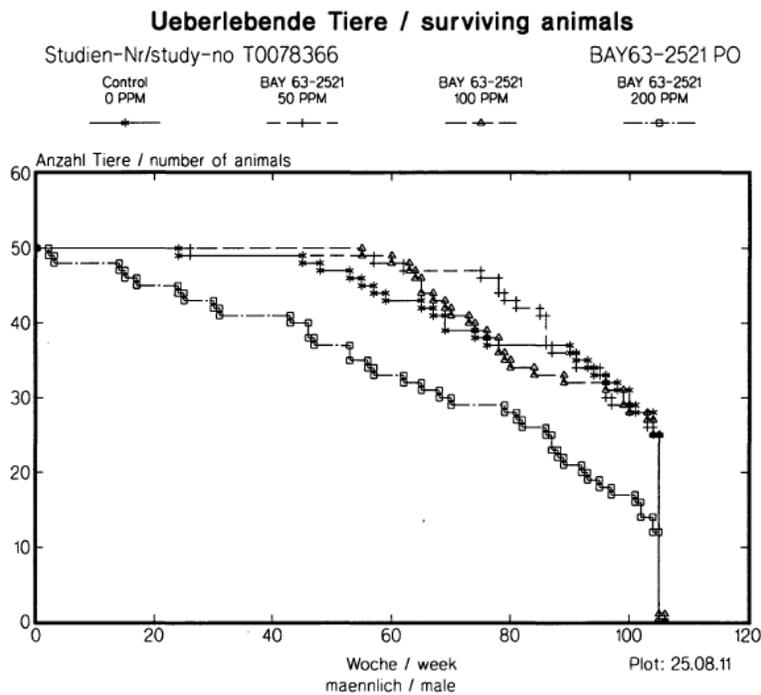
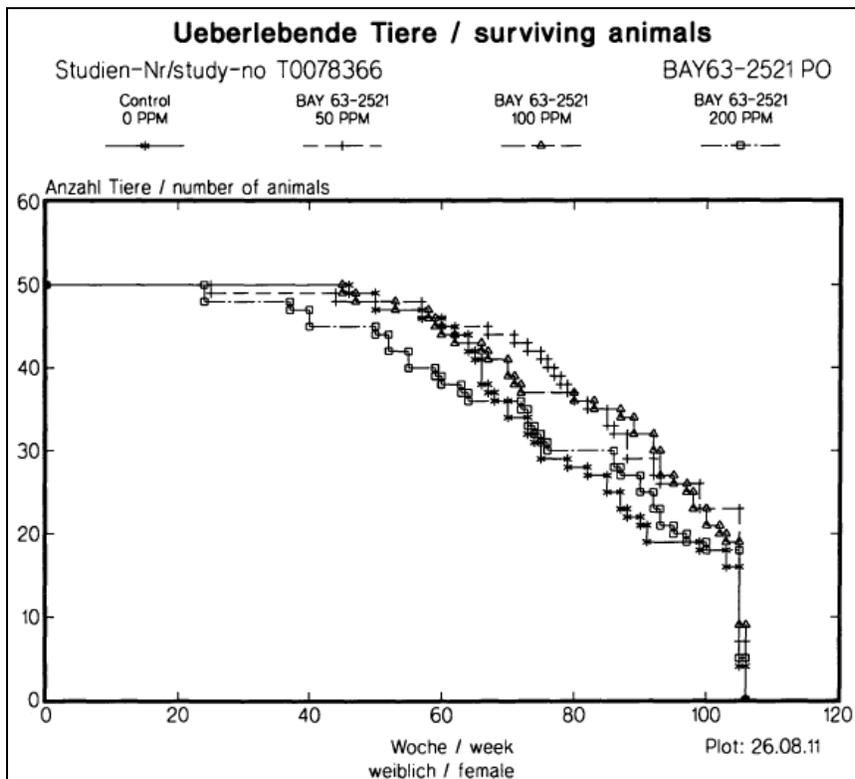


Table 5-1: Intercurrent Deaths (Main Groups)

Sex	m	m	m	m	f	f	f	f
Dose (ppm)	0	50	100	200	0	50	100	200
N	50	50	50	50	50	50	50	50
Week								
1-13	0	0	0	2	0	0	0	0
1-26	1	0	0	7	0	1	0	2
1-39	1	1	0	9	0	1	0	3
1-52	3	1	0	13	3	2	2	6
1-64	7	3	3	18	6	5	7	13
1-78	13	4	12	21	21	11	13	20
1-91	14	14	18	29	29	21	18	25
1-104	22	24	23	36	34	27	31	32
1-106	23	24	24	37	34	27	31	32

An early increase in mortality was also apparent for the high dose females.



The pattern of early mortality was repeated for the satellite groups.

Table 5-2: Intercurrent Deaths (Satellite Groups)

Sex Dose (ppm) N	m				f			
	0	50	100	200	0	50	100	200
Week	20	20	20	20	20	20	20	20
1-13	0	0	0	0	0	0	0	0
1-26	0	0	0	0	0	0	0	1
1-39	0	0	0	0	0	0	0	2
1-52	0	2	1	2	0	1	0	2
1-64	2	4	3	4	1	4	1	4
1-78	7	6	5	7	2	5	2	9
1-91	9	9	6	9	4	6	5	13
1-104	10	12	12	11	10	9	8	15
1-107	10	12	12	11	11	10	8	15

Clinical Observations

The reported clinical signs were non-specific.

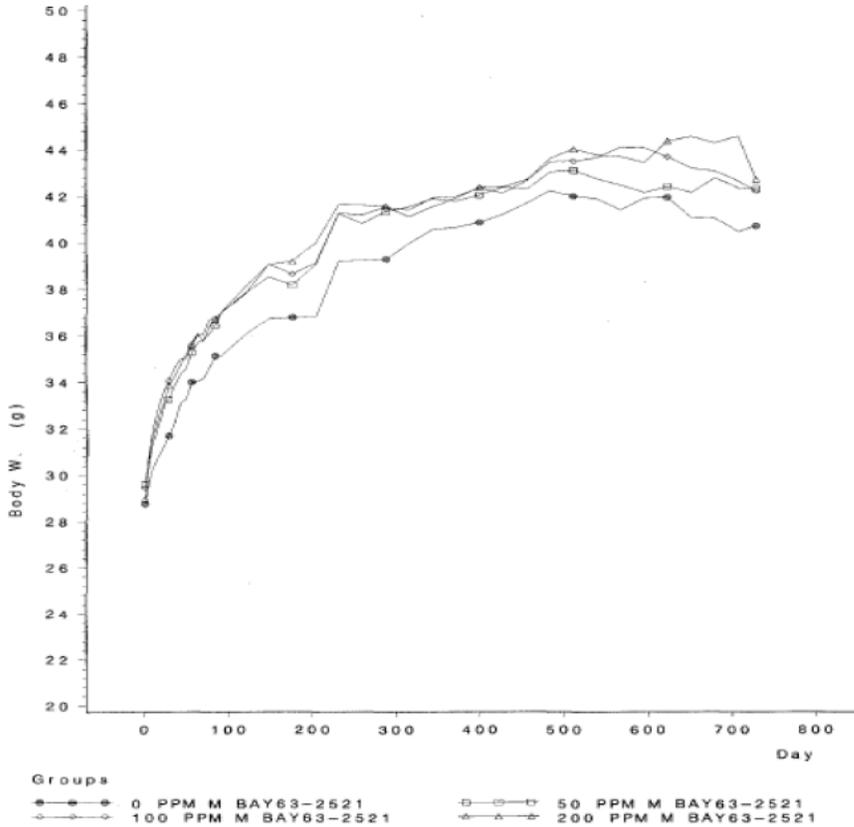
Sponsor’s Summary of Clinical Observations

	Males (mg/kg)				females (mg/kg)			
	Main study							
	0	6	12	25	0	8	16	32
piloerection	14	17	22	29				
pallor	29	34	29	46	33	34	34	42
Increased abdominal girth	16	24	23	41	17	23	29	35
squatting	3	3	7	18	6	6	4	8
	Satellite study							
piloerection					6	4	5	10
Increased girth	7	5	10	16				

Body weights: Males

Despite the increased mortality in the high dose group, the drug-treated males gained weight at a greater rate than did the control animals. This is summarized in the reviewer’s table below.

Figure 5-1: Body Weights (g) – Males - Main Groups



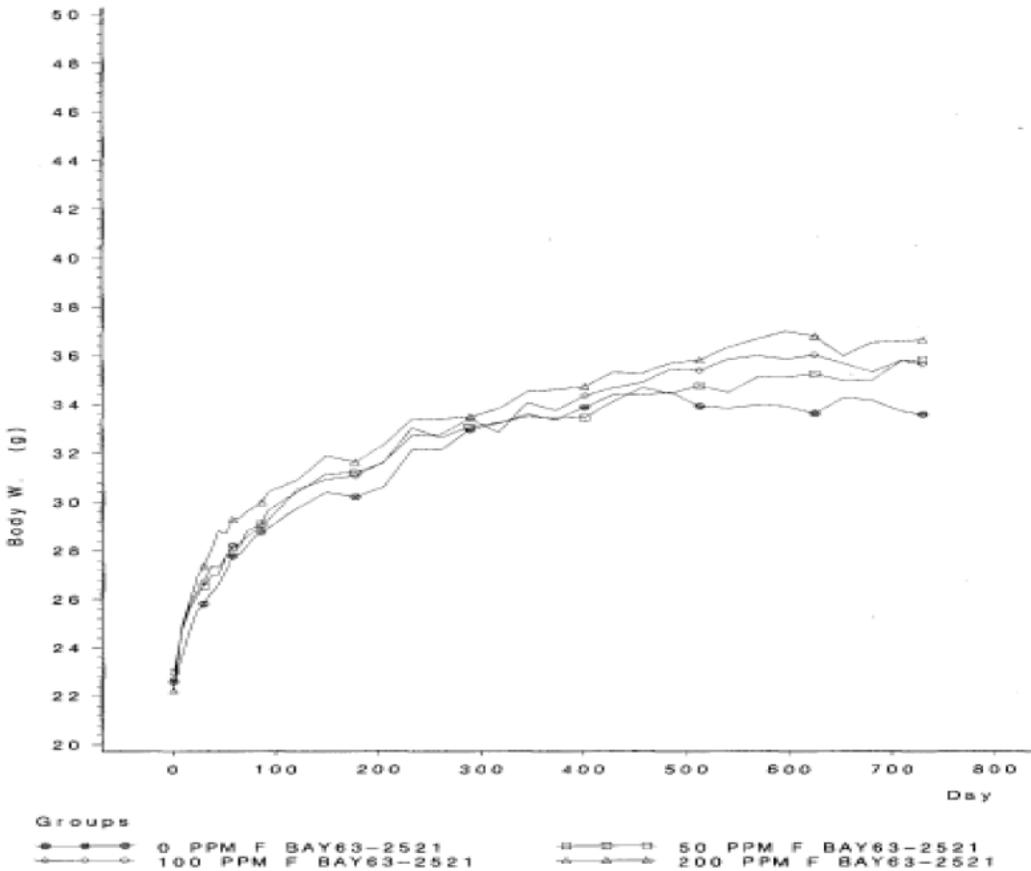
Mean BodyWeights (g) : Males

Dose (mg/kg/day)	0	6	12	25
Day 1	28.8	29.6	29.5	29.0
Day 729	40.7	42.3	42.2	42.7
Δ Day729-Day1 weight	11.9	12.7	12.7	13.7
% difference from control	NA	+7%	+7%	+15%

Bodyweights: Females

Similar to the males, drug-treated females gained weight at a greater rate than the control mice. This is summarized in the reviewer’s table below.

Figure 5-2: Body Weights (g) – Females - Main Groups



Mean BodyWeights (g) :Females

Dose (mg/kg/day)	0	8	16	32
Day 1	22.6	23.0	22.7	22.2
Day 729	33.6	35.8	35.6	36.6
Δ Day 729 weight –Day1 weight	11.0	12.8	12.9	14.4
% difference from control	NA	+16%	+17%	+31%

Food Consumption and Test Article Exposure

Food consumption did not differ significantly between the groups. In both sexes, the food consumption in the drug-treated groups was no greater than the consumption of the control animals.

Food and test substance exposure

There were no significant differences in food consumption between the groups. This is interesting in light of the body weight effects.

Table 5-4: Cumulative and Mean Daily Food Intake - Main Groups

Dose ppm	Days	Group means g/animal		g/kg body weight	
		total	per day	total	per day
Male					
0	707	3457	4.89	90199	127.58
50	707	3493	4.94	87776	124.14
100	707	3528	4.99	87498	123.76
200	707	3577	5.06	88071	124.57
Female					
0	708	3880	5.48	122859	173.53
50	708	3462	4.89	108218	152.85
100	708	3611	5.10	110873	156.60
200	708	3788	5.35	114958	162.37

The food administration resulted in the following mean test substance intake of 6, 12, and 25 mg/kg in males and 8, 16 and 32 mg/kg in females.

Table 5-5: Cumulative Test Substance Intake - Main Groups

Dose ppm	Days	Group means mg/animal		mg/kg body weight	
		total	per day	total	per day
Male					
50	707	177	0.25	4390	6.21
100	707	354	0.50	8753	12.38
200	707	714	1.01	17611	24.91
Female					
50	708	170	0.24	5409	7.64
100	708	361	0.51	11087	15.66
200	708	758	1.07	22989	32.47

Hematology: Females

The hematology indicates a dose-related anemia. By the end of the study, the apparent effect is mitigated in the low and middle dose groups but persists in the high dose. The anemia is normocytic, normochromic and regenerative according to the data presented. While mice typically have high reticulocyte counts, the drug-treated groups show higher mean levels than do the control animals.

Summary of Hematology for Females

	0 mg/kg	8 mg/kg	16 mg/kg	32 mg/kg
	ERY10E122/l			
Day364	8.86	8.48 (-4.2)	8.46	8.03 (-9%)
543	8.38	8.19 (-2)	7.91	7.32 (-13%)
718	7.55	7.50 (0)	7.73	6.85 (-9%)
	Hemoglobin g/l			
Day 364	140	131 (-6)	130	123 (-12%)
546	131	128	122	116 (-11%)
728	115	114	114	106 (-8%)
	HCT			
Day 364	0.453	0.428 (-6)	0.427	0.410* (-9%)
546	0.436	0.425 (-2)	0.408	0.387* (-11%)
728	0.390	0.393	0.389	0.358 (-8%)
	MCV fl			
Day 364	51.2	50.7	50.8	51.0
546	52.1	52.0	52.8	53.3
728	51.6	52.9	51.6	53.5
	MCHC g/l eryth			
Day 364	308	306	304	299
546	298	303	296	300
728	295	289	289	295
	Retic o/oo			
Day 364	24	32 (+33)	30	41 (+70%)
546	42	36	50	54 (+29%)
728	45	68 (+51)	70	68 (+51%)
	Thro 10e9/l			
Day 364	1332	1312	1207	1376
546	1402	1469	1261	1377
728	1095	1387	1156	1164

*p<0.05 Numbers in parentheses () are percent difference from the control value.

Hematology: Males

Differences from the control group appear primarily in the high dose group. A regenerative response is apparent.

Summary of Hematology for Males

	0 mg/kg	6mg/kg	12mg/kg	32mg/kg
	ERY10E122/l			
Day364	8.61	8.07 (-6)	8.02	7.83(-9)
543	8.33	8.19(-2)	7.70 (-8)	8.00 (-4)
718	8.24	9.05	7.45	7.32 (-11)
	Hemoglobin g/l			
Day 364	129	120 (-7)	120	115 (-11)
546	125	123	115	120
728	117	124	108	107
	HCT			
Day 364	0.425	0.397 (-7)	0.396	0.386 (-9)
546	0.421	0.413	0.391	0.404
728	0.404	0.421	0.372	0.375 (-7)
	MCV fl			
Day 364	49.4	49.4	49.6	49.7
546	50.7	50.6	50.9	50.8
728	49.4	48.0	50.0	52.1
	MCHC g/l eryth			
Day 364	304	301	302	298
546	298	297	294	296
728	291	292	290	282
	Retic o/oo			
Day 364	34	41	37	41(+21%)
546	35	32	41	45 (+29%)
728	44	34	51	85 (+93%)
	Thro 10e9/l			
Day 364	1660	1581	1622	1784
546	1971	1935	2079	1873
728	1902	1735	1711	1939

In mice, the lymphocyte is usually the predominant circulating white blood cell. This is apparent in the differential results shown below.

Table 5-8: Differential Blood Count - Satellite Groups

Dose ppm	LYM %	SEGM %	EOS %	MONO %	BAND %	ATYP LYM %	NUCL SH #/100 WBC
m Day 354/ 355							
0	67.9	26.0	1.5	1.3	0.2	3.2	54.8
50	59.1	33.5	0.7	2.1	0.1	4.5	50.9
100	65.1	28.2	1.1	2.0	0.1	3.4	56.5
200	55.5	35.0	0.4 *	3.6	0.8	4.8	38.3
m Day 543/ 544							
0	61.3	30.6	1.7	2.1	0.2	4.1	67.6
50	51.5	39.0	1.5	3.9	0.0	4.1	81.5
100	43.6	43.6	1.9	4.4	0.0	6.5	70.2
200	53.7	36.1	1.7	3.8	0.0	4.6	75.3
m Day 718/ 719							
0	50.4	39.4	1.4	0.9	0.1	7.7	41.6
50	42.9	48.6	1.5	0.4	0.7	5.9	51.7
100	41.1	45.7	1.6	2.6	1.1	8.0	41.4
200	56.3	34.3	1.6	0.7	0.3	6.8	59.0
f Day 354/ 355							
0	71.8	21.9	1.3	1.0	0.1	3.9	31.8
50	64.2	28.0	0.3	1.5	0.1	5.8	36.6
100	62.8	30.5	0.5	1.9	0.5	3.9	32.4
200	62.5	28.9	1.2	1.5	1.2	4.7	26.6
f Day 543/ 544							
0	68.8	24.9	1.6	2.1	0.0	2.6	28.3
50	69.4	25.3	0.7	3.1	0.2	1.2	36.1
100	62.4	30.6	0.7	3.5	0.5	2.3	48.9
200	63.4	30.3	1.0	2.6	0.1	2.5	41.2
f Day 718/ 719							
0	53.8	34.4	1.0	3.4	1.6	5.8	34.3
50	45.1	44.5	1.0	4.9	1.4	3.2 **	35.2
100	53.5	36.6	1.2	3.9	1.8	3.0 **	49.8
200	50.5	39.1	0.9	4.9	1.1	3.4 *	41.5

* significantly different at $p \leq 0.05$

** significantly different at $p \leq 0.01$

Organ weights

Spleen weight normalized to body weight was increased in both sexes at the high dose. In high dose males, normalized spleen weight was approximately doubled ($p < 0.05$) relative to control. In high dose females, spleen weight was increased by approximately 46% (n.s.) over control weight.

Table 5-10: Necropsy - Main Groups - Relative Organ Weights

Dose ppm	Body W. g	Brain mg/100g	Adrenals mg/100g	Liver mg/100g	Spleen mg/100g	Kidneys mg/100g	Testes mg/100g
m							
0	41	1252	20	5961	393	1973	546
50	42	1224	24	5580	403	1924	558
100	43	1212	16	5477	368	2066	517
200	43	1196	16	6248	820 *	2027	511
f							
0	33	1608	35	6103	828	1519	
50	37	1476	30	5843	714	1432	
100	36	1482	26 **	6438	751	1440	
200	36	1441 *	35	6348	1209	1530	

* significantly different at $p \leq 0.05$

** significantly different at $p \leq 0.01$

Non-Neoplastic Findings

The sponsor’s summary of non-neoplastic findings primarily indicate the effects on the gastrointestinal tract. The sponsor reports new observations of foamy macrophages in the epididymides and testes .

Table 5-13: Noteworthy Non-Neoplastic Changes

Sex Dose (ppm)	Male				Female					
	0	50	100	200	0	50	100	200		
Cecum - No. exam.	49	49	49	45	48	49	49	48		
- Inflammation	t*	1	3	8*	15**	t*	0	1	5*	14**
- Erosion/ Ulceration	t*	1	0	1	7*	t*	0	1	2	4
- Diverticulum/-i	t*	0	1	0	4*	t*	0	0	0	4
- Mucosal Hyperplasia		8	4	9	13	t*	2	3	8*	13**
Colon - No. exam.	49	50	49	45	49	49	49	49		
- Mucosal Hyperplasia		0	1	1	2		0	1	0	0
Rectum - No. exam	48	49	49	45	48	49	49	48		
- Mucosal Hyperplasia		0	0	1	2		1	0	0	0
Mesenteric lymph nodes – No. exam.	47	50	49	44	47	47	48	46		
- Increased cellularity /hyperplasia) diffuse		2	0	1	8*		4	1	3	5
- Hemopoiesis		2	3	4	4		2	7	3	14**
- Erythrophagocytosis or sinusoidal blood		6	13	10	11		5	15*	7	15**
- Dilated/cystic sinuses		0	3	2	3		3	6	6	11*
Liver – No. exam.	49	50	49	45	49	49	50	49		
- Foamy and enlarged macrophages	t*	8	9	10	23**	t*	7	11	15*	21**
- Inflammation		1	2	2	4		0	3	3	5*
- Hemopoiesis		2	7	3	15**		13	13	13	21
Spleen – No. exam.	49	50	49	45	49	49	50	49		
- Hemopoiesis		10	13	9	30**		16	16	20	33**
Kidneys – No. exam.	49	50	49	45	49	49	50	49		
- chronic progressive nephropathy		38	38	41	27		22	18	18	11*
Seminal vesicles – No. exam.	49	50	49	45						
- Increased and/or inhomogeneous secretion		20	25	19	5**					
Coagulation glands – No. exam.	49	50	49	44						
- Increased and/or inhomogeneous secretion		6	4	8	2					
Epididymides – No. exam.	49	50	49	45						
- Fluid in lumen		10	12	7	2*					
- Foamy macrophages		0	0	0	5*					
- Oligospermia		10	8	6	1**					
Testes – No. exam.	49	50	49	45						
- Diffuse atrophy/degeneration		10	12	9	3*					
- Foamy macrophages		1	0	1	13**					

* = p < 0.05 (one-tailed pairwise group comparison)

** = p < 0.01 (one-tailed pairwise group comparison)

t* = p < 0.05 (trend-test)

Reviewer’s Summary of Other Non-neoplastic Findings of Potential Interest

	males				females			
	0	50	100	200	0	50	100	200
Atrial dilation	0	0	0	1/45	2/49	2/49	2/48	5/48
Ventricular dilation	0	0	0	0	0	0	1/50	2/49

Neoplastic Findings

The pathologist's report indicated increased incidences of gastrointestinal inflammation and attributed the increased mortality in the drug-treated animals to these findings.

Histopathologically, increased incidences and severity of inflammatory changes of the large bowel (mainly cecum) were observed starting at 100 ppm males and females. Secondly to the aforementioned inflammation erosion/ulceration, mucosal hyperplasia and formation of diverticuli were found. Pathogenetically, the findings are seen as secondary changes to a reduced GI-tract motility: By the pharmacological mode of action, the stimulation of the soluble guanylate cyclase results in increased intracellular cGMP levels leading to smooth muscle cell relaxation (Carjaval et al., 2000; Young et al., 1996). As a consequence, disturbed/reduced GI-tract motility arises leading to the dilation of the cecum as observed at clinical observation (increased girth) or at necropsy. This chronic motility reduction results in chronic dysbiosis and subsequent mucosal degeneration and regeneration as well as inflammation (Ullman and Itzkowitz, 2011; Uronis et al. 2009; Yang and Pei, 2006.)

Since these changes are considered causative for the majority of prescheduled deaths, they were more frequently found in decedent animals.

As a consequence of the chronic degeneration and regeneration, reactive mucosal hyperplasia was seen.

In addition to the intestinal lesions, further non-neoplastic findings were observed which are considered as a consequence thereof:

- increased lymphoid hyperplasia of the cecum, colon or rectum (males at 200 ppm, females starting at 100 ppm);
- increased cellularity or hyperplasia of the mesenteric lymph node at 200 ppm (males and females);
- inflammatory changes (females 200 ppm) and reactive activation (foamy and enlarged macrophages, males and females at 200 ppm) of the Kupffer cells in the liver.

As a consequence of mode of action related smooth muscle cell relaxation, reduced GI-tract motility followed by altered intestinal bacterial homeostasis arose. As a consequence of this dysbiosis, chronic inflammation, associated with chronic mucosal degeneration and regeneration results in reactive mucosal hyperplasia associated with a higher cancer risk which is widely proven in colitis-associated mouse cancer models (Kanneganti et al., 2011; Taketo and Edelmann, 2009).

Thus, in summary, considering that BAY 63-2521 is non-genotoxic, the increase of intestinal tumors observed in mice is considered as a consequence of an exaggerated pharmacodynamic effect leading to chronic inflammatory bowel disease by reduction of GI-tract motility followed by excessive degeneration, regeneration, reactive hyperplasia and finally tumor formation.

The sponsor also speculates that the decrease in tumors in the male high dose group is due to the excessive mortality.

Table 5-16: Intestinal Neoplasms (Main Groups)

Sex Dose (ppm)	Male				Female			
	0	50	100	200	0	50	100	200
Cecum - No. examined	49	49	49	45	48	49	49	48
- Adenocarcinoma	0	0	0	0	0	0	2	0
Colon - No. examined	49	50	49	45	49	49	49	49
- Adenoma	0	0	0	1	0	0	0	0
- Adenocarcinoma	0	0	0	1	0	0	0	0

Testicular Leydig cell tumors were also noted in the rat study, although without an apparent dose-response.

	Males			
Leydig cell tumor	0/49	4/50	1/49	2/45

Toxicokinetics

Plasma level exposure to both parent drug and active metabolite was reported for each of the drug-treated groups. Approximately linear kinetics were demonstrated for the AUC₀₋₂₄ values. The high dose females showed somewhat great AUC values than the high dose males, but there are no obvious sex-related differences in metabolism.

Table 5-17: Toxicokinetics – Summary of Exposure in Week 53

Gender	Target dose [ppm]	male			female		
		50	100	200	50	100	200
Actual dose	[mg/kg]	6.1	12.49	25.49	6.84	14.22	29.72
BAY 63-2521							
AUC(0-24)	[µg·h/L]	917	2324	4308	1223	2925	6622
AUC(0-24)/norm	[kg·h/L]	0.150	0.186	0.169	0.179	0.206	0.223
C _{max}	[µg/L]	58.1	157	320	69.2	172	416
C _{max,norm}	[kg/L]	0.00952	0.0126	0.0126	0.0101	0.0121	0.0140
C _{min} /C _{max}	[%]	18.8	26.4	28.9	22.3	40.5	30.2
t _{max}	[h]	15.0	0.00	20.0	10.0	0.00	20.0
RA _{C_{max}}	[%]	61.4	67.7	67.2	53.4	79.2	93.1
RA _{AUC}	[%]	59.5	72.2	51.3	59.0	76.9	72.9
BAY 60-4552*							
AUC(0-24)	[µg·h/L]	134	331	717	109	259	791
AUC(0-24)/norm	[kg·h/L]	0.0226	0.0274	0.0291	0.0165	0.0188	0.0275
C _{max}	[µg/L]	8.82	22.7	62.2	5.81	17.8	56.0
C _{max,norm}	[kg/L]	0.00150	0.00188	0.00253	0.000878	0.00129	0.00195
C _{min} /C _{max}	[%]	32.6	37.1	26.2	52.1	40.8	25.6
t _{max}	[h]	20.0	0.00	20.0	20.0	0.00	20.0
RA _{C_{max}}	[%]	71.6	65.4	82.1	35.8	65.6	69.2
RA _{AUC}	[%]	55.5	64.1	48.5	40.6	51.1	54.2
MR _{AUC}	[%]	15.1	14.7	17.2	9.22	9.15	12.4

*Equivalent dose was used for the calculation of dose-normalized parameters of M-1 using a factor of 0.967.

The multiples of human exposure achieved are summarized in the reviewer’s tables below.

Reviewer’s Summary of Plasma Exposure to BAY63-2521 in Mice

	Male mg/kg/day			Female mg/kg/day		
Dose mg/kg	6	12	25	7	14	30
AUC ₀₋₂₄ µg.hr/l	917	2324	4308	1223	2925	6622
Multiple of human exposure	0.2X	0.6X	1X	0.3X	0.7X	1.6X
Human: 2.5 mg t.i.d. based on multiple dose study #12166 in pulmonary hypertension patients : AUC ₀₋₂₄ 4161µg hr/l						

Reviewer’s Summary of Plasma Exposure to BAY60-4552 in Mice

	Male mg/kg/day			Female mg/kg/day		
Dose mg/kg	6	12	25	7	14	30
AUC ₀₋₂₄ µg.hr/l	134	331	717	109	259	791
Multiple of human exposure	0.05X	0.13X	0.28X	0.04X	0.1X	0.3X
Human: 2.5 mg t.i.d. sponsor estimated based on multiple dose study #12166 in pulmonary hypertension patients AUC ₀₋₂₄ 2604 µg hr/l						

The CDER statistical reviewer concurred that there were no apparent neoplastic findings of statistical significance.

From the CDER Statistical Review

Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or Pairwise Comparisons of Treated Groups and Control in Mice

Sex	Organ Name	Tumor Name	Cont	Low	Med	High ...	P_Value			
			N=50	N=50	N=50	N=50	Dose Resp	C vs L	C vs M	C vs H
Male	LUNGS	Carcinoma bronchiolo-alveolar	4	6	11	4	0.4210	0.4014	0.0466	0.4244
Female	ADRENAL GLANDS	Tumor medullary benign	0	0	1	3	0.0145*	.	0.5385	0.1186

Based on the criteria of adjustment for multiple testing discussed in the rat data analysis section, the incidence of adrenal glands benign medullary tumor was considered to have statistically significant dose response relationship in female mice. The pairwise comparisons did not show statistically significant increased incidence in any observed tumor type in any treated group compared to their respective control in either sex.

The study was adequate. Despite somewhat increased mortality in the high dose groups, there was adequate survival for interpretation of results with no demonstration of new toxicities. The nonneoplastic toxicities reported are consistent with previous studies. The tables of neoplastic findings suggest background lesions with little indication of a superimposed drug-effect. Testicular Leydig cell tumors were also reported in rats with attendant Leydig cell hyperplasia, something not reported in the mice. While the incidence of Leydig cell tumors is not statistically significant by the sponsor's analysis of the Exact Fisher test, it is greater than both historical and concurrent control levels although without an apparent dose-response.

9. Reproductive and Developmental Toxicology

9.1 FERTILITY AND EARLY EMBRYONIC DEVELOPMENT

9.1.1 Study title: Study of fertility and early embryonic development tin rats after oral administration

Key study findings: Riociguat was administered in doses that caused overt effects. There was no substantive examination of the male reproductive tract or reasonable analysis of female cyclicity. This was discussed with the sponsor in a telecom at the time of submission.

Males lost weight (up to 43% vs control) in the pre-mating period while females gained significant, dose-related amounts (up to 10x more than the controls). There was a dose-related increase in mean time to insemination (1.7 days for control vs 3.9days for HD). It is not clear if this is secondary to decreased blood pressure or a primary effect. There were no obvious detrimental effects on fertility or early embryonic development.

Study no.: T8062956

Conducting laboratory and location:

Date of study initiation: August 26, 2003

GLP compliance: statement included

QA reports: yes (x) no ()

Drug, lot #, and % purity: Batch BX01BEP, 97.6% in a vehicle of 0.5% aqueous Tylopur® (methylhydroxyethylcellulose).

Methods

BAY63-2521 was given by oral gavage to Wistar rats at 0, 3, 10 and 30 mg/kg/day in a vehicle of 0.5% aqueous tylopur® (methylhydroxyethylcellulose). Male rats were treated for 4

weeks prior to mating and during the subsequent mating period up to the time of euthanasia. Female rats were treated for 2 weeks prior to mating, during the subsequent mating period and up to day 7 of gestation. Cesarean sections were performed on days 14 and 16 post-coitus. Animals were paired one-to-one. If females were not inseminated by the end of the two-week mating period they were then paired with a proven breeder.

Evaluated for:

- signs, mortality- daily
 - body weight- twice a week
 - food consumption- weekly
 - water consumption-daily visual inspection of water bottles
 - gross necropsy
 - Organs collected: testes, epididymides, prostate, seminal vesicles+coagulation glands,
 - uterus, vagina, ovaries, pituitary gland. Davidson's solution was used for testes
 - and epididymides. Formalin was used for all other organs.
 - Testicular weight
-
- Female cyclicity, # of corpora lutea, # of implantation sites, # live embryos in right and left uterine horns, # of resorptions, weight of ovaries.

Results

Mortality: 1 HD male was euthanized after it was given a pulmonary dose of drug. 1 control male was euthanized for broken upper incisors and deteriorating condition.

Clinical signs: reddening of the auricular pinnae was seen in both sexes at all doses. Salivation was seen in both sexes at HD and in MD males. Piloerection occurred transiently in MD males and HD males.

Food consumption: Mean food consumption was decreased in HD males in the first week and then recovered to the same levels as the other groups. HD females ate less in the first week than the other groups. Thereafter, the drug-treated females ate more than did the control group.

Table 6-2: Mean feed consumption (g/day) in the female animals during the pre-mating period and during gestation:

Dose (mg/kg bw/day)	week 1	week 2	days 0 – 7 p.c.	days 7 – 14 p.c.
0	17.1	17.4	20.7	22.8
3	17.3	18.3	21.9	23.9
10	17.2	19.3**	22.6*	25.1**
30	13.5**	19.0**	21.1	25.5**

Statistically significant difference to control * = p < 0.05
 Statistically significant difference to control ** = p < 0.01

Body weight: Despite the resumption of normal levels of food consumption, weight gain was depressed in all the drug-treated males during the first, second and last weeks of determination.

Table 6-3: Mean body weight gain (g) of the male animals during the pre-mating treatment period (day 1 to day 29):

Dose (mg/kg bw/day)	days 1 – 8	days 8 – 15	days 15 – 22	days 22 – 29
0	23.0	19.7	13.1	11.2
3	22.6	17.3	12.1	10.8
10	21.9	13.8**	12.6	10.8
30	3.3**	12.3**	13.1	9.0

Statistically significant difference to control ** = $p < 0.01$

Using the absolute numbers for the overall period of time, a dose-related decrease in weight gain becomes apparent.

Mean body weight changes in males during pre-mating (grams)

	0mg/kg	3mg/kg	10mg/kg	30mg/kg
Day 1-29	67±16	63±19 (-6%)	59±14 (-12%)	38**±16 (-43%)

** $p < 0.01$ by Dunnett's test. Numbers in parentheses are the percent difference from the control.

While the females ate more than the males, the weight gain did not show any consistent pattern in the sponsor's summary table.

Table 6-4: Body weight gain (g) of the female animals during the pre-mating treatment period (days 1 to 15) and during gestation (days 0 to 7 and 7 to 14 p.c.):

Dose (mg/kg bw/day)	days 1 – 8	days 8 – 15	0 – 7 p.c.	7 – 14 p.c.
0	-1.0	1.8	28.0	29.9
3	2.9	4.8	29.4	29.1
10	6.1**	1.3	25.9	30.7
30	2.0	7.4**	26.2	25.5*

Statistically significant difference to control * = $p < 0.05$

Statistically significant difference to control ** = $p < 0.01$

However, if one looks at the weight changes over the entire pre-mating period, more of a pattern is apparent.

Mean body weight changes in females during pre-mating (grams)

	0 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Day 1 to 15	0.8±7	7.7**±9	7.5*±6	9.3**±9
Mean body weight change during gestation (grams)				
Day 0-14	58±8	59±8	57±9	52±9

* $p < 0.05$, ** $p < 0.01$ by Dunnett's test

When corrected for differences in body weight, there were no significant differences in testicular weight.

Table 6-5: Mean absolute combined testes and ovaries weights (g) and mean testes and ovaries weights to body weights ratios (%):

Dose (mg/kg bw/day)	0	3	10	30
Testes	3.590 g	3.583 g	3.474 g	3.292 g**
Ovaries	0.105 g	0.104 g	0.116 g	0.117 g
Testes	0.8700 %	0.8866 %	0.8596 %	0.8793 %
Ovaries	0.0398 %	0.0380 %	0.0423 %	0.0433 %

Statistically significant difference to control ** = p < 0.01

All females were inseminated. There was no decrease in fertility index associated with drug treatment. Gestation index (viable embryos) was also unaffected.

The presentation of female cyclicity data was uninformative. The only value given was mean number of estruses without an explanation of what this signifies. There was no breakdown as to other phases of the cycle or duration of phases.

		MEAN NUMBER OF ESTRUSES			
		0 MG/KG	3 MG/KG	10 MG/KG	30 MG/KG
Pretreatment	MEAN	1.9 d	1.6	1.7	1.6
	S.D.	0.32	0.52	0.50	0.53
	N	10	10	9	9
	p-value	0.389			
During Treatment	MEAN	3.3 d	3.2	3.4	2.8
	S.D.	0.48	0.42	0.52	0.79
	N	10	10	10	10
	p-value	0.114			

Statistical key: d=Dunnett's-test

Mean time to insemination showed a dose-related increase with increasing dose.

		MEAN VALUES FOR TIME TO INSEMINATION			
		0 MG/KG	3 MG/KG	10 MG/KG	30 MG/KG
Mating Days until Day 0 pc	MEAN	1.7 d	2.3	2.5	3.9
	S.D.	1.43	2.74	3.43	3.90
	N	24	24	24	24
	p-value	0.082			

Statistical key: d=Dunnett's-test

There was one re-mated female in both the MD and HD groups.

Toxicokinetics: not found

Necropsy: gross observations only. There are no apparent drug-related findings.

9.2 EMBRYOFETAL DEVELOPMENT

9.2.1 Developmental toxicity study in rats after oral administration

Key study findings: Riociguat was given to the point of overt toxicity in the dams. The signs were more severe than in non-gravid animals. Postimplantation loss was significantly increased at doses of ≥ 5 mg/kg and early resorptions were increased at the high dose of 25 mg/kg. The incidence of ventricular septal defect was significantly increased at 25 mg/kg. The drug treated litters showed increases in incomplete ossification of several bones albeit non-dose related in most cases. While this may be a generalized developmental effect due to alterations in maternal nutrition, the combination of wavy ribs and incomplete ossification of sacral vertebral arches may indicate a drug-specific effect.

Study no.: T0073280

Conducting laboratory and location: Bayer, Wuppertal, Germany

Date of study initiation: February 20, 2004

GLP compliance: statement included

QA reports: yes (x) no ()

Drug, lot #, and % purity: batch BX01BEP, 98.4%, 0.5% tylose

Methods

Twenty-two inseminated Wistar rats were treated from GD6 to GD17 with daily oral gavage of BAY63-2521 at doses of 0, 1, 5, and 25 mg/kg/day. Five additional females per group were treated with doses of 1, 5 and 25 mg/kg/day from gd6 to gd17 for plasma level determination of test article. The satellite females were euthanized GD18 after the last blood sampling. In the main group, fetuses were delivered by Cesarean on GD20.

Results

Maternal signs @25 mg/kg:

Intermittent reddish vaginal discharge

Piloerection

Decreased feed consumption followed by increased feed intake after cessation of treatment

Increased water consumption/increased urination (PD/PU)

Maternal signs @ 5 mg/kg:

Transient decrease in feed intake at start of treatment

Some increase in feed intake at end of treatment

PU

Mortality (dams): no unscheduled mortality.

Body weight (dams):

Corrected body weight= after the uterine weight has been subtracted.

Dose [mg/kg bw/day]	0	1	5	25
mean body weight gain [g]				
days 6 - 17 p.c.	59.7	57.7	51.0*	34.2**
days 0 - 20 p.c.	121.2	116.9	106.3*	101.6**
Corrected body weight gain [g]				
days 0 - 20 p.c.	49.9	47.6	49.4	31.3**

Statistically significant difference to control * = $p < 0.05$

Statistically significant difference to control ** = $p < 0.01$

The percentage difference in weight gain compared to control from days 6-17 is : 3% (LD), 15% (MD), 43% (HD)

Food consumption (dams): Mean consumption decreased dose-dependently for the first 3 days then returned to control levels in the LD and MD groups but stayed depressed in the HD group until the end of the dosing phase. Feed consumption then increased in a dose-related manner in all drug-treated groups.

Toxicokinetics: blood collected day 17 after 12 daily doses at 1, 3, 7 and 24 hours after administration.

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

There were greater numbers of post-implantation losses and early resorptions in the drug-treated groups.

	0 MG/KG	1 MG/KG	5 MG/KG	25 MG/KG
Number of females				
with implantations (a)	19	19	21	19
with viable fetuses (b)	19	19	21	17
Postimplantation Loss				
total per group	10 f	6	24*	31**
% of implantations	4.2	2.6	10.3	12.6
p-value	0.000	1.000	0.036	0.003
per female: mean	0.5 k	0.3	1.1	1.6
st. dev.	0.77	0.48	1.46	3.39
n (a)	19	19	21	19
p-value	0.120			
No. of females affected	8	6	11	11
Early Resorptions				
per group	0 f	0	0	18**
% of implantations	0.0	0.0	0.0	7.3
p-value	0.000			0.000
per female: mean	0.0 k	0.0	0.0	0.9
st. dev.	0.00	0.00	0.00	3.47
n (a)	19	19	21	19
p-value	0.098			
No. of females affected	0	0	0	2

Weight of live fetuses also showed a dose-related decrease. Both sexes of fetuses were affected.

	0 MG/KG	1 MG/KG	5 MG/KG	25 MG/KG
Number of females				
with implantations (a)	19	19	21	19
with viable fetuses (b)	19	19	21	17
Weight of Live Fetuses				
Litter Basis				
total fetuses				
mean (g)	3.82 d	3.76	3.63	3.45**
st. dev.	0.357	0.186	0.309	0.286
n (litters)	19	19	21	17
p-value	0.002	0.842	0.108	0.001

Reviewer's Summary of Malformations

		Dose mg/kg			
		0	1	5	25
Number of fetuses per group		230	224	209	215
# of litters per group		19	19	21	17
Ventricular septal defect		5(5)	4(2)	5(4)	22**(10)
Hairline ventricular septal defect		0(0)	2(2)	2(1)	1(1)
Sponsor's Summary					
		0 MG/KG	1 MG/KG	5 MG/KG	25 MG/KG
TOTAL MALFORMATIONS					
Fetal Incidence	N	8 f	15	11	26**
	%	3.5	6.7	5.3	12.1
	p-value	0.003	0.411	1.000	0.002
Litter Incidence	N	7 f	9	7	11
	%	36.8	47.4	33.3	64.7
	p-value	0.223			
Statistical key: f=Fisher's Exact ** = p<0.01					

Numbers in parentheses () are litter incidence

Summary of Fetal Skeletal Observations

		Dose riociguat mg/kg/day							
		0		1		5		25	
1 st distal phalanx incompletely ossified:right		62: 51%		75:64%		73*:67%		74:67%	
1 st distal phalanx incompletely ossified:left		64:53%		75:64%		74:68%		80**:72%	
		0 MG/KG		1 MG/KG		5 MG/KG		25 MG/KG	
Number of litters		19		19		21		17	
		Number %		Number %		Number %		Number %	

STERNEBRA(E)									

-INCOMPLETELY OSSIFIED									
1st	1	5.3	6	31.6	2	9.5	4	23.5	
2nd	14	73.7	17	89.5	10	47.6	16	94.1	
3rd	3	15.8	1	5.3	1	4.8	0	0.0	
4th	2	10.5	1	5.3	2	9.5	3	17.6	
5th	11	57.9	13	68.4	10	47.6	11	64.7	
6th	6	31.6	7	36.8	10	47.6	15**	88.2	
-UNOSSIFIED									
2nd	0	0.0	1	5.3	0	0.0	0	0.0	
3rd	0	0.0	0	0.0	1	4.8	0	0.0	
4th	0	0.0	0	0.0	1	4.8	0	0.0	
5th	0	0.0	0	0.0	2	9.5	0	0.0	
6th	1	5.3	0	0.0	3	14.3	6	35.3	
Ribs									
-WAVY SUM									
	4	21.1	8	42.1	11	52.4	6	35.3	

SACRAL VERTEBRAL ARCH(ES)									

-INCOMPLETELY OSSIFIED									
3rd	right	0	0.0	1	5.3	2	9.5	0	0.0
4th	right	2	10.5	7	36.8	9	42.9	10*	58.8
-INCOMPLETELY OSSIFIED									
3rd	left	0	0.0	1	5.3	2	9.5	0	0.0
4th	left	4	21.1	7	36.8	11	52.4	9	52.9

* p<0.05, **p<0.01 by f= Fisher's exact Numbers in parentheses () indicate litter incidence.

Toxicokinetics summary of results

Dose	[mg/kg]	1.0		5.0		25	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
Time	[h]	geom.	geom.	geom.	geom.	geom.	geom.
AUC(0-24)	[µg·h/L]	627	1.24	2197	1.21	10626	1.30
AUC(0-24) _{norm}	[kg·h/L]	0.627	1.24	0.439	1.21	0.425	1.30
C _{max}	[µg/L]	100	1.23	284	1.09	959	1.34
C _{max, norm}	[kg/L]	0.100	1.23	0.0568	1.09	0.0384	1.34
C(24) / C _{max}	[%]	n.c.	n.c.	1.16	2.18	9.43	1.78
t _{max}	[h]	1.25	1.63	1.00	1.00	2.18	2.90

T0073280_63-2521.xls \ Summary_short \ PHOPF \ 27.10.04

n.c. = not calculated

9.2.2 T1073920 Developmental Toxicity Study in Rabbits after oral administration

Key study findings: The rabbits were very sensitive to the pharmacology and extended pharmacology of the drug. While all females mated, incidence of abortions increased with increased dose. Postimplantation loss and late resorptions were significantly (p<0.01) increased in the HD group. Placental weight was significantly increased at the HD(22% greater than control, p<0.05). There was no clear signal for any kind of increase in variations or terata.

Report number: PH-34628

Study location:

GLP: statement included

QA: yes

Study initiated: March 15, 2004

Test substance: Bay63-2521 batch BX01BEP, 98.4%

Twenty female Himalayan rabbits per group were treated with daily oral dosing of BAY63-2521 (micronized) in 0.5% methylhydroxyethylcellulose.

- Doses: 0. 0.5, 1.5, 5 mg/kg body weight per day.
- Dosing period: GD6 to GD20
- Three additional females per group were added to each group for the toxicokinetic analysis (1, 2, 4, 7 and 24 hours after administration on GD6 and GD20).
- Females were delivered by Caesarean section on GD29.

Parameters studied: body weights, feed and water intake, TK
 Post-mortem: corpora lutea and implantations, uterine weights, placental weights, number and type of resorption, viability and sex of fetuses, external findings in fetuses, internal findings (including brain), skeletal findings

Results

The sponsor felt that all doses showed maternal toxicity.

- Abortions: 0 (control), 0 (LD), 3/20 (MD), 11/20 (HD)
- Females in all drug-treated groups were reported to show cold ears, ostensibly due to decreased blood pressure.
- Food intake in the HD group was greatly decreased in the first 3 days of treatment then returned to at least the control levels.

Summary of clinical signs (frequency/animals)

	0 mg/kg	0.5 mg/kg	1.5 mg/kg	5 mg/kg
Cold to touch ears	6/5	36/11	24/12	72/17
Reduced feces	6/5	37/11	27/13	103/19
Soft feces	14/7	8/5	30/9	132/18
Decreased urination	3/2	4/2	2/2	17/6
Increased urination	8/2	19/6	51/8	30/12
abortion	0	0	3/3?	11/11?
Increased water consumption	17/6	31/7	69/14	48/14
Decreased water consumption	10/6	15/7	10/4	32/9

Abortions were increased and live fetuses decreased with increased dose.

		FERTILITY AND GESTATION INDEX			
		0 MG/KG	0.5 MG/KG	1.5 MG/KG	5 MG/KG
Females Mated	N	20	20	21	20
Females Excluded	N	0	1	1	0
Females Evaluated	N	20	19	20	20
Females with Implantations	N	19 f	19	19	19
Fertility Index	%	95.0	100.0	95.0	95.0
	p-value	0.804			
Females with Abortions	N	0	0	3	11
Females with Total Resorptions	N	0	0	0	2
Females with Viable Fetuses	N	19 f	19	16	6**
Gestation Index	%	100.0	100.0	84.2	31.6
	p-value	0.000	1.000	0.689	0.000

Statistical key: f=fisher's Exact ** = p<0.01

Table 6-1: Mean Feed Intake

Dose (mg/kg b.w./day)	0	0.5	1.5	5
mean feed intakes (g/animal/day)				
days 0 - 3 p.c.	102.6	104.2	101.9	112.1
days 3 - 6 p.c.	103.8	99.5	106.1	109.3
days 6 - 9 p.c.	102.5	91.7	93.5	56.1**
days 9 - 12 p.c.	99.3	89.5	93.7	90.2
days 12 - 15 p.c.	86.3	78.1	88.4	91.2
days 15 - 18 p.c.	94.9	75.7*	99.4	98.3
days 18 - 20 p.c.	90.4	82.0	92.9	108.6
days 20 - 21 p.c.	79.7	72.7	84.8	90.7
days 21 - 24 p.c.	83.9	74.6	83.9	90.9
days 24 - 27 p.c.	82.5	77.1	86.6	102.6**
days 27 - 29 p.c.	88.3	84.2	89.4	101.5

Statistically significant difference to control * = $p < 0.05$

Statistically significant difference to control ** = $p < 0.01$

The body weight gains do not show consistent patterns.

Table 6-2: Mean Body Weight Gain

Dose (mg/kg b.w./day)	0	0.5	1.5	5
absolute body weight gain (g) days 6 - 20 p.c.				
	133.8	103.1	136.4	128.0
absolute body weight gain (g) days 0 - 29 p.c.				
	398.1	296.4	359.4	332.3
corrected body weight gain (g) days 0 - 29 p.c.				
	-30.9	-110.2*	-47.6	12.0

Statistically significant difference to control * = $p < 0.05$

Weight of the gravid uterus was decreased in all drug-treated groups with an increase in the standard deviation (SD).

Mean uterine weights and corrected body weight changes

	0 mg/kg	0.5 mg/kg	1.5 mg/kg	5 mg/kg
Gravid uterus	429±47.81	406.5±53.03	407.0±98.60	320.3**±125.14
carcass	2392±150	2447±182	2485±218	2355±139

Carcass weight = day 29 body weight - uterine weight

The HD group showed 10% fewer implantations per group than did the control group. Postimplantation loss and late resorptions were significantly increased in the HD group.

Summary of gestation and intrauterine development parameters

	0 mg/kg	0.5 mg/kg	1.5 mg/kg	5 mg/kg
Fertility index(females w/ implantations)	95% 19	100 19	95 19	19 95
Gestation index (females w/ viable fetuses)	100 19	100 19	84 16	32 6**
Implantations: % corpora lutea	97.0	90.5	95.3	87.9
Postimplantation loss: % of implantations	5±0	4.6±1.0	2.5±1.00	31.4±0.00**
Late resorptions: % of implantations	5±0	4.6±1.0	2.5±1.00	31.4±0.00**
Preimplantation loss:% of corpora lutea	3.0	9.5	4.7	12.1
Placental weight in g	4.16	4.28	4.79*	5.09*

*p<0.05 , **p<0.01 compared to control

Despite the toxicity and overt clinical signs seen in each dose group, there was no strong signal for teratogenicity. The sponsor's summary is shown below.

Table 6-6: Fetal Malformations

Malformation	Dose (mg/kg b.w./day)			
	0	0.5	1.5	5
malposition of forelimb(s)	2 (2)	3 (3)	4 (2)	-
cardiac ventricular septal defect	2 (2)	-	2 (2)	-
malformation of the heart and major vessels	1	-	-	-
fusion of ribs in the cartilaginous part	1	2 (2)	-	-
7 th lumbar vertebral arch left looks like the 1 st	1	-	-	-
sacral vertebral arch, pelvis left shift to cranial	1	-	-	-
malformations of lumbar vertebrae with 13 th ribs	-	-	1	-
malformations of cervical vertebrae with 1 st rib left shortened	-	2 (2)	1	-
fusion of caudal vertebral bodies	1	-	-	-
bipartite caudal vertebral bodies	-	-	-	-
number of fetuses per group	151	145	118	35
number of fetuses with malformations	6	7	8	0
malformed fetuses per group (%)	4.0	4.8	6.8	0.0
number of litters per group	19	19	16	6
number of litters with malformations	4	7	5	0
malformed litters per group (%)	21.1	36.8	31.3	0.0

() number of litters affected

Toxicokinetics

Plasma concentrations of BAY63-2521 and of the metabolite M-1 (BAY60-4552) were determined. On GD6 and GD20 blood samples were collected at predose, 1, 2, 4, 7, and 24 hours after administration.

Dose	[mg/kg]	0.5		1.5		5.0	
		BAY 63-2521	BAY 60-4552*	BAY 63-2521	BAY 60-4552*	BAY 63-2521	BAY 60-4552*
AUC(0-24)	[µg·h/L]	5064	1327	18898	6014	62467	17210
AUC(0-24) _{norm}	[kg·h/L]	10.1	2.75	12.6	4.15	12.5	3.56
C _{max}	[µg/L]	558	100	1775	350	4776	960
C _{max, norm}	[kg/L]	1.12	0.208	1.18	0.242	0.955	0.199
C(24) / C _{max}	[%]	17.1	24.3	26.8	54.9	27.5	50.2
t _{max}	[h]	1.59	3.17	1.59	3.17	1.26	4.00
R _{A1}	[%]	115	161	146	192	97.4	118
R _{A2}	[%]	142	448	143	222	109	153
R _{A3}	[%]	127	152	162	225	102	140
MR	[%]		27.1		32.9		28.5

T1073920_Tabs_M1.xls \ Summary Bericht 63-2521 60-4552 \ Kch \ 08.02.06

R_{A1} = ratio of accumulation regarding C_{max} Day 20 p.c. / C_{max} Day 6 p.c. in [%]R_{A2} = ratio of accumulation regarding C(24) Day 20 p.c. / C(24) Day 6 p.c. in [%]R_{A3} = ratio of accumulation regarding AUC(0-24) Day 20 p.c. / AUC(0-24) Day 6 p.c. in [%]MR = metabolic ratio of metabolite AUC(0-24)_{norm} / BAY 63-2521 AUC(0-24)_{norm}

*Equivalent doses were used for calculation of dose-normalized parameters of M-1 using a factor of 0.9669.

9.3 POST-NATAL DEVELOPMENT

9.3.1 Pre- and Post-Natal Development Study in Rats

Report number: PH36162

Study number: T0076755

Study location: Bayer Schering Pharma Ag, Wuppertal, Germany

GLP: statement included

QA: yes

Experimental start date: February 5, 2008

Animals: Wistar rats (Hsd Cpb:WU)

Test article: BAY 63-2521, batch BX028BL, purity 96.9%

Before the start of treatment, the stability of the formulations were confirmed by the analysis of concentration, homogeneity and stability of the dosage forms.

Stability in the vehicle was confirmed before the start of the study. Analyses during the study verified that the test item content was within ±10 % of the nominal concentration. Homogeneity (low and high dose investigated, only) was within ±5% of the nominal value.

Vehicle: 0.5% methylhydroxycellulose (Tylose®)

The doses were selected based on a dose-ranging study that examined doses of 16 and 25 mg/kg/day. At both doses, the females showed poor clinical condition and decreased body

weight gain. Pup mortality was increased and rearing index decreased. Based on the clinical findings at both doses, the sponsor chose the doses listed below.

Twenty-four timed pregnant Wistar rats were given daily oral gavage of BAY63-2521 from gestation days (GD) 6 to post-partum day (PP) 21. The doses of test article were 0, 1.5, 5 and 15 mg/kg/day. The females were allowed to deliver and rear their pups. As far as possible, one male and one female per litter were reared to maturity for fertility testing.

Observations	Frequency/Comments
Body weights	Daily from GD6 to day of delivery and then days 0, 4,7,11,14,17 and 21pp
Food intake	Days 0-6, 6-11, 11-16, 16-21 post-coitum and days 0-7 and 7-14 pp
Water consumption	Once weekly by visual estimation
F0 females	Fertility index, gestation index, rearing index, duration of gestation, course of birth Lactation behavior #implantation sites at end of rearing period (10% ammonium sulfide staining)
F0 necropsy	Gross pathology Livers were collected in formalin although histology was not performed

F1 Pups

observation	Frequency/comment
General behavior and mortality	Twice daily
External malformations, variations	Not specified
Pup weights	After first milk intake, days 4, 7, 14, and 21 after birth
Litter size and viability	Birth, days 4,7,14 and 21
Sex ratio of pups	Birth and days 4,7,14 and 21
Gross pathology	Up to/at the end of the rearing period id pups were not cannabilized or autolyzed
Fore and hindlimbs collected in formalin	All pup euthanized after PP4 and all pups used for motor activity testing. Histology not performed.

F1 Pups : Developmental Landmark Evaluation

Observation	Frequency/comment
Time of pinnae detachment from head	
Time of fur development	
Time of eruption of all incisors	
Time at which eyes open	
Time at which normal gait was observed	
Time of balanopreputial separation and body weight at this time	Only male pups selected for F1 mating
Time of opening of vagina and body weight at this time	Only female pups selected for F1 mating

F1 Reflex and Behavioral Testing

Observation	Frequency/comment
Surface righting	From day PP1 to not later than day PP5
Negative geotaxis	From PP5 to not later than PP9
Papillary reflex at end of rearing period	Reflex had to be triggered in both eyes. Test performed on all pups except those with presumed ocular malformations. How presumptions were made was not defined.
Hearing ability	At end of rearing period. Both ears tested using Preyer's reflex (pinna twitch reflex) for response to five stimuli from an electronic tone generator
Motor activity	Day PP22, one male and one female per litter were randomly selected for spontaneous motor activity testing using infrared photocell boxes.
Learning and memory test (Water-M-maze test)	In the 5 th and 6 th weeks of life (1 st and 2 nd test respectively) one male and one female pup per litter were randomly selected. A positive was finding its way out of the maze immediately.

F1 Fertility Testing

24 pups of each sex, one male and one female per litter, were selected from each study group using a computer generated randomization list. The animals were reared to at least 14 weeks of age. Avoiding sibling matings, one female was placed overnight with one male. A maximum of eight pairings was allowed, each female with the same male. Females with positive evidence of mating were not mated further. Females with no positive evidence of copulation after 8 matings were paired 4 more times with another male from the same dose group which had already inseminated a female. The females were allowed to litter. After evaluation of the F2 pups at birth, these pups were euthanized.

F1 fertility observations

Observation	Frequency/Comment
Signs and behavior during pre mating, mating and gestation	Twice daily except weekends and holidays (once daily)
Water intake and excretory products	Visual assessment once daily
Feed intake	Weekly during pre mating and gestation
Body weight development	Once a week from 5 to 14 weeks
Body weights inseminated females	Days 0, 7, 14 and 21 post-coitum
Gross pathology	At end of fertility testing. Females were euthanized after the last female had delivered.
Insemination index	# of inseminated females becoming pregnant
Gestation index	# of pregnant females which littered
Fertility index	# of inseminated females with implantation sites
Implantation sites	10% ammonium sulfide staining of uterus
Duration of gestation	
Course of birth	
Viability of F2 pups at birth	
Sex distribution of F2 pups at birth	
Appearance and behavior of F2 pups at birth	Including notation of malformations
Individual F2 pup weights	

Females without implantation sites or without viable litters were excluded from statistical evaluation, except for fertility index, delivery and litter data and prenatal loss.

Differences between the control and BAY 63-2521-treated groups were considered significant when $p < 0.05$. Significant differences from the control are indicated with * for $p < 0.05$, ** for $p < 0.01$.

Results

No treatment related signs were reported.

However, in both sexes, at doses ≥ 5 mg/kg, light colored feces were reported with increasing frequency with increasing dose.

Body weight gain: F_0

A dose-related decrease in weight gain was apparent from day 0-7. The HD group gained on average 32% less than the control group. Weight gain was also depressed in the drug treated groups from days 7-21. In the last 2 days of the study the drug-treated animals lost weight. The HD group is reported to have lost on average 12% ($p < 0.01$) of the Day 21 weight.

(F0-GENERATION)					
MEAN BODY WEIGHTS DURING GESTATION (GRAMS)					
		0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG
DAY 0	MEAN	223.4 d	228.6	227.8	231.7
	S.D.	10.68	12.05	10.01	9.63
	N	21	21	22	21
	p-value	0.094			
DAY 6	MEAN	250.0 d	250.5	250.7	254.7
	S.D.	10.00	11.71	10.97	10.24
	N	21	22	22	21
	p-value	0.467			
DAY 7	MEAN	254.9 d	253.0	251.7	253.1
	S.D.	10.42	11.17	10.78	11.52
	N	21	22	22	21
	p-value	0.817			
DAY 21	MEAN	366.2 d	357.3	360.3	352.9
	S.D.	14.48	22.20	15.72	20.49
	N	21	22	22	21
	p-value	0.135			
DAY 22	MEAN	367.4 d	340.1	345.3	310.0**
	S.D.	10.41	35.05	32.63	35.63
	N	7	15	14	20
	p-value	0.001	0.158	0.302	0.001

Statistical key: d=Dunnett's-test * = $p < 0.05$ ** = $p < 0.01$

Feed intake was somewhat depressed in the dug-treated groups during the gestation period, consistent with decreased rate of gain.

Table 5-2: Mean Feed Intake of the F0 females

Dose [mg/kg bw/day]	0	[g/female/day]		
		1.5	5	15
days 0 - 6 p.c.	21.4	20.4	21.5	21.9
days 6 - 11 p.c.	23.5	21.8*	21.6**	17.6**
days 11 - 16 p.c.	25.1	23.5*	23.9	22.0**
days 16 - 21 p.c.	26.6	25.8	26.3	24.6*
days 0 - 7 p.p.	48.6	46.9	46.3	48.4
days 7 - 14 p.p.	66.1	65.3	62.3	65.7

Statistically significant difference to control * = p < 0.05 ** = p < 0.01

The HD group gained on average ~3% more than the control group during the lactation period.

		(F0-GENERATION) MEAN BODY WEIGHTS DURING LACTATION (GRAMS)			
		0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG
DAY 0	MEAN	264.0 d	259.5	260.5	254.5
	S.D.	13.59	14.17	12.86	13.31
	N	21	22	22	21
	p-value	0.156			
DAY 4	MEAN	288.3 d	282.0	276.5*	273.2**
	S.D.	11.87	13.79	12.99	16.21
	N	21	22	22	21
	p-value	0.004	0.313	0.017	0.002
DAY 7	MEAN	291.9 d	286.8	285.0	283.1
	S.D.	9.57	13.04	12.24	16.80
	N	21	22	22	21
	p-value	0.173			
DAY 11	MEAN	303.2 d	296.5	294.4	292.0
	S.D.	10.86	15.38	12.17	17.79
	N	21	22	22	21
	p-value	0.071			
DAY 14	MEAN	304.0 d	300.3	295.7	293.9
	S.D.	11.12	15.75	11.53	18.76
	N	21	22	22	21
	p-value	0.114			
DAY 17	MEAN	304.7 d	298.5	298.9	296.0
	S.D.	11.69	14.32	13.89	18.48
	N	21	22	22	21
	p-value	0.281			
DAY 21	MEAN	295.5 d	286.9	289.0	289.1
	S.D.	12.96	16.22	10.63	19.05
	N	21	22	22	21
	p-value	0.274			

Statistical key: d=Dunnett's-test * = p<0.05 ** = p<0.01

There were no apparent effects in the F₀ fertility and gestation indices.

		(FO-GENERATION) FERTILITY AND GESTATION INDEX			
		0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG
Females Inseminated	N	24	24	24	24
Females Excluded	N	0	0	0	0
Females Evaluated	N	24	24	24	24
Females with Implantations	N	21 f	22	22	21
Fertility Index	%	87.5	91.7	91.7	87.5
	p-value	0.930			
Females with Total Resorptions	N	0	0	0	0
Females with Viable Pups	N	21 f	22	22	21
Gestation Index	%	100.0	100.0	100.0	100.0
	p-value	1.000			

Statistical key: f=Fisher's Exact

Increased numbers of stillborn pups were seen in the MD and HD groups. Duration of gestation was statistically significantly increased in the LD and HD groups, a not unexpected effect of the pharmacology of a drug that causes smooth muscle cell relaxation and decreased blood pressure.

		(FO-GENERATION) NATURAL DELIVERY DATA AND LITTER DATA -- SUMMARY			
		0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG
Females Inseminated	N	24	24	24	24
Females with Implantations	N	21 f	22	22	21
Fertility Index	%	87.5	91.7	91.7	87.5
	p-value	0.930			
Females Completing Delivery	N	21 f	22	22	21
Gestation Index	%	100.0	100.0	100.0	100.0
	p-value	1.000			
with Stillborn Pups	N	0 f	0	5	3
	%	0.0	0.0	22.7	14.3
	p-value	0.021		0.145	0.695
with all Stillborn	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
	p-value	1.000			
Females with Liveborn	N	21 f	22	22	21
	%	100.0	100.0	100.0	100.0
	p-value	1.000			
Females Completing Rearing	N	21 f	22	22	21
Rearing Index	%	100.0	100.0	100.0	100.0
	p-value	1.000			
Litters with Liveborn, but no Pups Alive					
day 4	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
	p-value	1.000			
day 21	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
	p-value	1.000			
Duration of Gestation	MEAN	22.33 d	22.68*	22.59	22.95**
	S.D.	0.483	0.477	0.503	0.218
	N	21	22	22	21
	p-value	0.000	0.029	0.140	0.000

Statistical key: d=Dunnett's-test f=Fisher's Exact * = p<0.05 ** = p<0.01

There were no findings of toxicological significance in the natural delivery and litter summary data.

(FO-GENERATION)					
NATURAL DELIVERY DATA AND LITTER DATA -- SUMMARY					
		0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG
Litters with Liveborn Pups	N	21	22	22	21
Pups Delivered (total)	N	287	287	305	293
	MEAN	13.67 d	13.05	13.86	13.95
	S.D.	1.826	2.886	1.246	2.559
	p-value	0.533			
Liveborn Pups	N	287	287	299	286
Live Birth Index	MEAN%	100.00 d	100.00	97.97*	97.89*
	S.D.	0.000	0.000	4.049	3.593
	p-value	0.008	1.000	0.043	0.036
Stillborn	N	0 f	0	6	3
	%	0.0	0.0	2.0	1.0
	p-value	0.015		0.093	0.746
Uncertain	N	0	0	0	4
Culled day 4	N	113	111	120	111
Pups Died, Missing, Killed and/or Cannibalized day 0	N	3 f	0	0	4
	%	1.0	0.0	0.0	1.4
	p-value	0.058			
days 1-4	N	3 f	3	3	3
	%	1.0	1.0	1.0	1.0
	p-value	1.000			
days 5-21	N	3 f	1	0	0
	%	1.0	0.3	0.0	0.0
	p-value	0.106			
days 0-4	N	6 f	3	3	7
	%	2.1	1.0	1.0	2.4
	p-value	0.407			
days 0-21	N	9 f	4	3	7
	%	3.1	1.4	1.0	2.4
	p-value	0.234			
Pups Surviving 4 days Viability Index	MEAN%	98.15 d	99.24	99.08	97.82
	S.D.	3.671	3.553	2.378	4.675
	p-value	0.508			
Pups Surviving 21 days Lactation Index	MEAN%	98.21 d	99.43	100.00	100.00
	S.D.	4.482	2.665	0.000	0.000
	p-value	0.091			

Statistical key: d=Dunnett's-test f=Fisher's Exact * = p<0.05

(FO-GENERATION)
NATURAL DELIVERY DATA AND LITTER DATA -- SUMMARY

		0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG
Implantation Sites per Litter	N	309	309	321	308
	MEAN	14.71 d	14.05	14.59	14.67
	S.D.	1.384	2.380	1.297	2.477
	p-value	0.653			
Prenatal Loss per Litter	N	22	22	16	15
	MEAN	1.05 d	1.00	0.73	0.71
	S.D.	0.865	1.195	0.935	0.956
	p-value	0.575			
Live Pups/Litter day 0	MEAN	13.67 d	13.05	13.59	13.62
	S.D.	1.826	2.886	1.436	2.355
	N	21	22	22	21
	p-value	0.765			
day 4 preculling	MEAN	13.38 d	12.91	13.45	13.29
	S.D.	1.596	2.706	1.335	2.194
	N	21	22	22	21
	p-value	0.817			
day 4 postculling	MEAN	8.00 d	7.86	8.00	8.00
	S.D.	0.000	0.468	0.000	0.000
	N	21	22	22	21
	p-value	0.151			
day 7	MEAN	7.95 d	7.86	8.00	8.00
	S.D.	0.218	0.468	0.000	0.000
	N	21	22	22	21
	p-value	0.268			
day 14	MEAN	7.86 d	7.86	8.00	8.00
	S.D.	0.359	0.468	0.000	0.000
	N	21	22	22	21
	p-value	0.196			
day 21	MEAN	7.86 d	7.82	8.00	8.00
	S.D.	0.359	0.501	0.000	0.000
	N	21	22	22	21
	p-value	0.114			
Sex Ratio - Male Pups:Total Pups day 0	MEAN%	51.57 d	52.16	47.52	45.90
	S.D.	14.525	17.260	12.764	13.387
	p-value	0.426			
day 21	MEAN%	49.74 d	53.11	50.00	47.62
	S.D.	3.924	9.476	0.000	7.520
	p-value	0.049	0.206	0.998	0.570

Statistical key: d=Dunnett's-test

There were no apparent effects in pup weight up to day 7.

		(FO-GENERATION) SUMMARY OF PUP WEIGHTS (GRAMS)				
			0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG
day 0	males	MEAN	6.26 d	6.48	6.35	6.40
		S.D.	0.560	0.690	0.672	0.584
		N	21	22	22	21
		p-value	0.704			
0	females	MEAN	5.99 d	6.12	6.05	6.03
		S.D.	0.597	0.711	0.584	0.564
		N	21	22	22	21
		p-value	0.914			
0	males+females	MEAN	6.13 d	6.31	6.20	6.21
		S.D.	0.573	0.700	0.610	0.554
		N	21	22	22	21
		p-value	0.809			
day 4	males preculding	MEAN	10.36 d	10.67	10.22	10.43
		S.D.	1.086	1.500	1.226	1.318
		N	21	22	22	21
		p-value	0.713			
4	females preculding	MEAN	9.90 d	10.13	9.85	9.75
		S.D.	1.192	1.498	1.071	1.348
		N	21	22	22	21
		p-value	0.798			
4	males+females preculding	MEAN	10.13 d	10.42	10.02	10.09
		S.D.	1.134	1.490	1.124	1.280
		N	21	22	22	21
		p-value	0.734			
day 4	males postculding	MEAN	10.38 d	10.64	10.33	10.49
		S.D.	1.113	1.504	1.216	1.227
		N	21	22	22	21
		p-value	0.856			
4	females postculding	MEAN	10.00 d	10.15	9.87	9.81
		S.D.	1.232	1.539	1.067	1.341
		N	21	22	22	21
		p-value	0.825			
4	males+females postculding	MEAN	10.19 d	10.42	10.10	10.14
		S.D.	1.156	1.505	1.126	1.232
		N	21	22	22	21
		p-value	0.840			
day 7	males	MEAN	17.10 d	17.59	17.04	17.15
		S.D.	1.472	1.973	1.619	1.609
		N	21	22	22	21
		p-value	0.697			
7	females	MEAN	16.47 d	16.69	16.41	16.05
		S.D.	1.394	2.231	1.365	1.906
		N	21	22	22	21
		p-value	0.699			
7	males+females	MEAN	16.78 d	17.17	16.73	16.57
		S.D.	1.382	2.071	1.461	1.662
		N	21	22	22	21
		p-value	0.688			

Statistical key: d=Dunnett's-test

There were non-significant decreases in pup weights of both sexes: 4% in HD male pups and 6% in HD female pups relative to the control groups.

		(F0-GENERATION) SUMMARY OF PUP WEIGHTS (GRAMS)				
		0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG	
day 14	males	MEAN	33.14 d	33.34	32.36	32.01
		S.D.	1.973	2.748	1.954	1.649
		N	21	22	22	21
		p-value	0.139			
14	females	MEAN	32.30 d	32.07	31.33	30.52
		S.D.	2.142	2.797	2.186	1.994
		N	21	22	22	21
		p-value	0.060			
14	males+females	MEAN	32.71 d	32.74	31.84	31.24
		S.D.	1.967	2.757	2.028	1.607
		N	21	22	22	21
		p-value	0.069			
day 21	males	MEAN	50.69 d	51.00	49.18	48.63
		S.D.	4.045	4.704	3.144	2.734
		N	21	22	22	21
		p-value	0.116			
21	females	MEAN	49.20 d	48.99	47.90	46.41
		S.D.	4.069	4.113	2.760	3.608
		N	21	22	22	21
		p-value	0.060			
21	males+females	MEAN	49.92 d	50.07	48.54	47.50
		S.D.	3.927	4.424	2.866	3.021
		N	21	22	22	21
		p-value	0.072			

Statistical key: d=Dunnett's-test

		(F0-GENERATION)			
		SUMMARY OF PUP BODY WEIGHT CHANGES -- GRAMS			
		0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG
day 0- 4 males	MEAN	4.10 d	4.18	3.87	4.03
	S.D.	0.679	0.941	0.687	0.833
	N	21	22	22	21
	p-value	0.605			
females	MEAN	3.91 d	4.01	3.79	3.73
	S.D.	0.723	0.954	0.633	0.839
	N	21	22	22	21
	p-value	0.650			
males+females	MEAN	4.00 d	4.11	3.82	3.88
	S.D.	0.690	0.935	0.645	0.799
	N	21	22	22	21
	p-value	0.621			
day 4- 7 males	MEAN	6.72 d	6.95	6.71	6.66
	S.D.	0.625	0.835	0.818	0.753
	N	21	22	22	21
	p-value	0.616			
females	MEAN	6.47 d	6.53	6.54	6.25
	S.D.	0.501	0.991	0.737	0.939
	N	21	22	22	21
	p-value	0.613			
males+females	MEAN	6.59 d	6.75	6.63	6.44
	S.D.	0.512	0.890	0.757	0.808
	N	21	22	22	21
	p-value	0.608			
day 7-14 males	MEAN	16.04 d	15.75	15.31	14.86*
	S.D.	0.928	1.346	1.358	1.437
	N	21	22	22	21
	p-value	0.021	0.795	0.166	0.011
females	MEAN	15.83 d	15.38	14.91	14.47**
	S.D.	1.238	1.241	1.512	1.181
	N	21	22	22	21
	p-value	0.007	0.532	0.061	0.003
males+females	MEAN	15.93 d	15.58	15.11	14.66**
	S.D.	1.029	1.281	1.376	1.229
	N	21	22	22	21
	p-value	0.008	0.673	0.088	0.004
day 14-21 males	MEAN	17.55 d	17.67	16.82	16.62
	S.D.	2.359	2.347	1.759	2.032
	N	21	22	22	21
	p-value	0.286			
females	MEAN	16.90 d	16.92	16.58	15.88
	S.D.	2.081	1.783	1.331	2.172
	N	21	22	22	21
	p-value	0.242			
males+females	MEAN	17.21 d	17.33	16.70	16.26
	S.D.	2.147	2.068	1.431	2.056
	N	21	22	22	21
	p-value	0.258			

Statistical key: d=Dunnett's-test * = p<0.05 ** = p<0.01

Pinnae detachment occurred statistically earlier in LD and HD pups. Incisor eruption and development of fur also occurred slightly earlier than in the control group. The lack of dose dependence suggests normal variability.

(F0-GENERATION)					
MEAN AGE IN DAYS OF PUPS REACHING CRITERION -- SUMMARY					
		0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG
PINNAE DETACHMENT	MEAN	2.60 d	2.18*	2.31	2.02**
	S.D.	0.534	0.501	0.548	0.418
	N	21	22	22	21
	p-value	0.003	0.023	0.160	0.001
	pups reaching criterion, %	100.0	100.0	100.0	100.0
DEVELOPMENT OF FUR	MEAN	9.58 d	9.30	9.37	9.45
	S.D.	0.531	0.507	0.619	0.557
	N	21	22	22	21
	p-value	0.388	100.0	100.0	100.0
	pups reaching criterion, %	100.0	100.0	100.0	100.0
INCISOR ERUPTION	MEAN	10.44 d	10.06	10.06	9.88
	S.D.	0.944	0.577	0.624	0.673
	N	21	22	22	21
	p-value	0.079	100.0	100.0	100.0
	pups reaching criterion, %	100.0	100.0	100.0	100.0
EYES OPENED	MEAN	16.81 d	16.32	17.03	16.79
	S.D.	0.768	0.883	0.536	0.750
	N	21	22	22	21
	p-value	0.018	0.081	0.662	0.999
	pups reaching criterion, %	100.0	99.4	98.9	100.0
NORMAL GAIT	MEAN	16.45 d	15.89	16.82	16.54
	S.D.	0.699	0.879	0.852	0.826
	N	21	22	22	21
	p-value	0.003	0.075	0.316	0.971
	pups reaching criterion, %	100.0	100.0	100.0	100.0

Statistical key: d=Dunnett's-test * = p<0.05 ** = p<0.01

Balanopreputial separation and vaginal opening occurred at the same time across groups. However, the mean weight of the drug-treated groups was less than that of the control groups at the time of reaching the developmental landmark.

		(F1-GENERATION) MEAN VALUES OF SEXUAL MATURATION			
		0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG
Males showing balanopreputial separation					
MEAN AGE (DAYS)		43.6 d	43.2	43.8	43.8
	S.D.	2.24	1.56	3.46	2.43
	N	24	24	24	24
	p-value	0.819			
BODY WEIGHT ON COMPLETION (G)		206.7 d	203.8	200.2	202.1
	S.D.	21.24	13.93	23.05	23.47
	N	24	24	24	24
	p-value	0.736			
Females showing vaginal opening					
MEAN AGE (DAYS)		32.2 d	32.3	33.0	32.8
	S.D.	1.53	1.97	1.90	2.15
	N	24	24	24	24
	p-value	0.487			
BODY WEIGHT ON COMPLETION (G)		102.0 d	100.2	105.6	100.9
	S.D.	11.16	11.97	15.35	12.42
	N	24	24	24	24
	p-value	0.468			

Statistical key: d=Dunnett's-test

There were no apparent significant findings on the summary of reflexes and hearing ability.

		(F0-GENERATION) SUMMARY OF REFLEXES AND HEARING ABILITY IN PUPS			
		0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG
SURFACE RIGHTING	MEAN	1.00 d	1.01	1.02	1.00
	S.D.	0.000	0.024	0.050	0.016
	N	21	22	22	21
	p-value	0.233			
	pups reaching criterion, %	100.0	100.0	100.0	100.0
NEGATIVE GEOTAXIS	MEAN	5.04 d	5.00	5.00	5.02
	S.D.	0.070	0.000	0.000	0.109
	N	21	22	22	21
	p-value	0.176			
	pups reaching criterion, %	100.0	100.0	100.0	100.0
HEARING TEST	MEAN%	100.00 k	100.00	100.00	100.00
	S.D.	0.000	0.000	0.000	0.000
	N	21	22	22	21
	p-value	1.000			
PUPILLARY REFLEX	MEAN%	100.00 k	100.00	100.00	100.00
	S.D.	0.000	0.000	0.000	0.000
	N	21	22	22	21
	p-value	1.000			

Statistical key: d=Dunnett's-test k=Kruskal-Wallis

There were no obvious trends in the summary of horizontal activity for the male pups.

		(F0-GENERATION) SUMMARY OF MOTOR ACTIVITY DATA HORIZONTAL ACTIVITY (count)			
Male Pups		day 22 p.p.			
		0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG
Minutes of Testing					
Number tested	N	21	22	22	21
1- 2	MEAN	697.6 d	774.5	730.8	728.0
	S.D.	223.93	167.41	100.43	202.15
	p-value	0.570			
3- 4	MEAN	515.0 d	518.3	510.3	521.3
	S.D.	103.20	129.67	129.58	136.80
	p-value	0.993			
5- 6	MEAN	400.0 d	438.9	421.0	442.0
	S.D.	119.84	129.11	108.12	105.81
	p-value	0.625			
7- 8	MEAN	413.3 d	385.5	360.2	385.7
	S.D.	124.62	154.31	121.94	163.38
	p-value	0.683			
9-10	MEAN	350.2 d	372.4	330.3	332.4
	S.D.	137.77	106.19	86.62	113.19
	p-value	0.577			
1-10	MEAN	2376.1 d	2489.5	2352.5	2409.4
	S.D.	416.83	369.57	404.92	446.00
	p-value	0.706			
11-20	MEAN	1612.4 d	1500.6	1488.0	1568.5
	S.D.	363.52	509.09	518.02	326.78
	p-value	0.767			
21-30	MEAN	1590.2 d	1386.8	1312.2	1375.5
	S.D.	486.93	679.97	542.07	537.07
	p-value	0.416			
31-40	MEAN	1525.4 d	1111.7	1278.7	1529.6
	S.D.	541.35	775.82	573.08	580.57
	p-value	0.086			
41-50	MEAN	1462.6 d	1141.8	1163.6	1536.0
	S.D.	559.31	726.82	719.22	787.46
	p-value	0.158			
51-60	MEAN	1365.1 d	1453.9	1170.2	1351.6
	S.D.	551.59	728.81	748.53	724.71
	p-value	0.588			

Statistical key: d=Dunnett's-test

There were no obvious trends in the summary of horizontal activity for the female pups.

Female Pups		(FO-GENERATION) SUMMARY OF MOTOR ACTIVITY DATA HORIZONTAL ACTIVITY (count)				day 22 p.p.
		0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG	
Minutes of Testing						
Number tested	N	21	22	22	21	
1- 2	MEAN	650.4 d	715.5	751.8	651.9	
	S.D.	261.36	122.86	129.61	198.76	
	p-value	0.206				
3- 4	MEAN	529.0 d	522.6	518.8	541.5	
	S.D.	101.86	94.37	108.94	124.40	
	p-value	0.909				
5- 6	MEAN	442.6 d	443.7	404.5	437.8	
	S.D.	103.65	120.46	110.36	129.60	
	p-value	0.644				
7- 8	MEAN	398.2 d	380.2	364.0	404.7	
	S.D.	118.29	95.98	122.10	117.09	
	p-value	0.642				
9-10	MEAN	315.4 d	378.7	360.4	417.8	
	S.D.	115.03	149.85	107.81	144.98	
	p-value	0.091				
1-10	MEAN	2335.6 d	2440.7	2399.6	2453.6	
	S.D.	445.28	391.91	398.21	453.64	
	p-value	0.801				
11-20	MEAN	1733.3 d	1594.3	1590.1	1737.1	
	S.D.	409.85	538.19	540.84	489.38	
	p-value	0.623				
21-30	MEAN	1615.4 d	1506.9	1468.1	1765.0	
	S.D.	507.42	583.21	705.61	636.28	
	p-value	0.395				
31-40	MEAN	1606.3 d	1172.8*	1457.7	1688.5	
	S.D.	456.04	714.09	591.14	575.36	
	p-value	0.029	0.049	0.746	0.944	
41-50	MEAN	1509.0 d	1259.4	1460.0	1525.2	
	S.D.	687.87	778.77	538.07	580.16	
	p-value	0.518				
51-60	MEAN	1571.1 d	1203.5	1433.7	1676.9	
	S.D.	782.12	872.79	432.62	740.63	
	p-value	0.171				

Statistical key: d=Dunnett's-test * = p<0.05

There were no obvious trends in the total distance traveled by males.

(F0-GENERATION) SUMMARY OF MOTOR ACTIVITY DATA TOTAL DISTANCE (cm)					
Male Pups		day 22 p.p.			
		0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG

Minutes of Testing					
Number tested	N	21	22	22	21
1- 2	MEAN	343.8 d	396.1	370.7	359.3
	S.D.	144.26	128.12	88.91	119.98
	p-value	0.550			
3- 4	MEAN	186.7 d	193.0	184.4	200.0
	S.D.	63.85	83.36	85.32	88.23
	p-value	0.923			
5- 6	MEAN	120.3 d	156.6	142.9	155.0
	S.D.	69.83	67.93	66.22	60.40
	p-value	0.261			
7- 8	MEAN	143.4 d	131.0	115.6	123.3
	S.D.	76.26	84.62	70.11	89.31
	p-value	0.706			
9-10	MEAN	114.0 d	119.2	95.9	89.0
	S.D.	78.77	60.70	54.69	53.68
	p-value	0.340			
1-10	MEAN	908.2 d	996.0	909.5	926.6
	S.D.	239.06	222.28	255.93	251.88
	p-value	0.592			
11-20	MEAN	481.2 d	449.3	454.7	487.2
	S.D.	170.47	223.46	271.02	156.39
	p-value	0.915			
21-30	MEAN	538.3 d	485.0	408.5	440.2
	S.D.	258.63	314.06	226.14	254.62
	p-value	0.413			
31-40	MEAN	521.5 d	397.0	418.0	551.4
	S.D.	239.90	344.66	256.25	296.49
	p-value	0.221			
41-50	MEAN	545.5 d	425.0	408.3	574.7
	S.D.	288.65	342.47	310.00	404.01
	p-value	0.274			
51-60	MEAN	472.2 d	567.0	456.0	499.8
	S.D.	301.98	320.47	353.22	339.33
	p-value	0.695			

Statistical key: d=Dunnett's-test

There were no obvious trends in the total distance traveled by females.

		(F0-GENERATION) SUMMARY OF MOTOR ACTIVITY DATA TOTAL DISTANCE (cm)			
Female Pups		day 22 p.p.			
		0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG
Minutes of Testing					
Number tested	N	21	22	22	21
1- 2	MEAN	315.9 d	349.3	374.1	304.7
	S.D.	152.43	93.18	104.09	126.46
	p-value	0.224			
3- 4	MEAN	202.8 d	197.1	199.1	207.5
	S.D.	77.07	49.39	73.31	88.25
	p-value	0.969			
5- 6	MEAN	165.0 d	154.1	135.4	138.9
	S.D.	82.34	70.49	58.31	77.68
	p-value	0.516			
7- 8	MEAN	129.9 d	124.0	114.0	122.1
	S.D.	65.08	55.81	68.13	54.12
	p-value	0.862			
9-10	MEAN	81.9 d	124.4	121.5	144.1
	S.D.	58.87	80.45	69.54	80.42
	p-value	0.052			
1-10	MEAN	895.5 d	949.0	944.1	917.4
	S.D.	302.41	239.16	243.95	241.83
	p-value	0.896			
11-20	MEAN	535.9 d	490.6	490.0	543.1
	S.D.	204.90	266.97	223.92	213.36
	p-value	0.801			
21-30	MEAN	543.7 d	500.1	497.5	605.3
	S.D.	256.87	277.73	330.81	315.09
	p-value	0.606			
31-40	MEAN	546.7 d	429.6	488.4	582.0
	S.D.	270.33	323.18	255.30	301.06
	p-value	0.331			
41-50	MEAN	567.9 d	493.3	503.7	539.4
	S.D.	335.41	380.12	280.35	284.99
	p-value	0.869			
51-60	MEAN	610.7 d	470.7	520.4	709.1
	S.D.	392.91	452.17	197.81	447.64
	p-value	0.198			

Statistical key: d=Dunnett's-test

There were no obvious trends in the movement time for male pups.

		(FO-GENERATION) SUMMARY OF MOTOR ACTIVITY DATA MOVEMENT TIME (sec)			
Male Pups		day 22 p.p.			
		0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG
Minutes of Testing					
Number tested	N	21	22	22	21
1- 2	MEAN	37.4 d	40.4	41.6	38.0
	S.D.	14.52	11.01	7.84	11.89
	p-value	0.588			
3- 4	MEAN	20.4 d	20.3	20.3	19.7
	S.D.	7.23	8.30	9.56	8.27
	p-value	0.991			
5- 6	MEAN	12.2 d	16.6	14.7	15.5
	S.D.	6.93	8.32	6.18	4.85
	p-value	0.183			
7- 8	MEAN	14.1 d	12.6	11.2	13.2
	S.D.	7.60	8.65	6.15	9.20
	p-value	0.670			
9-10	MEAN	11.3 d	11.0	10.0	9.0
	S.D.	6.61	5.83	5.08	5.30
	p-value	0.548			
1-10	MEAN	95.5 d	101.0	97.8	95.4
	S.D.	23.33	22.20	21.64	22.11
	p-value	0.831			
11-20	MEAN	46.3 d	45.0	44.8	48.6
	S.D.	13.94	22.52	24.23	15.51
	p-value	0.918			
21-30	MEAN	49.0 d	44.3	40.3	39.9
	S.D.	20.47	26.88	22.13	20.68
	p-value	0.536			
31-40	MEAN	47.1 d	34.3	38.0	49.5
	S.D.	23.37	28.59	22.00	23.04
	p-value	0.138			
41-50	MEAN	47.0 d	35.6	35.0	48.0
	S.D.	22.53	26.74	26.72	30.53
	p-value	0.227			
51-60	MEAN	40.6 d	47.4	37.4	41.8
	S.D.	21.43	25.27	27.06	26.39
	p-value	0.615			

Statistical key: d=Dunnett's-test

There were no obvious trends in the movement time for female pups.

Female Pups		(FO-GENERATION) SUMMARY OF MOTOR ACTIVITY DATA MOVEMENT TIME (sec)				
		0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG	
Minutes of Testing						
	Number tested	N	21	22	22	21
1- 2	MEAN		33.0 d	37.3	40.5	33.1
	S.D.		14.89	8.24	11.07	13.83
	p-value		0.135			
3- 4	MEAN		19.9 d	20.7	19.9	20.6
	S.D.		7.07	4.83	6.49	8.89
	p-value		0.966			
5- 6	MEAN		15.2 d	16.4	13.6	14.9
	S.D.		6.56	8.19	6.00	7.91
	p-value		0.662			
7- 8	MEAN		12.5 d	12.1	11.1	13.2
	S.D.		6.87	4.93	5.52	6.37
	p-value		0.723			
9-10	MEAN		7.9 d	12.4	12.3	13.0
	S.D.		5.47	7.96	7.40	6.71
	p-value		0.071			
1-10	MEAN		88.5 d	98.9	97.5	94.9
	S.D.		26.64	20.52	22.01	27.47
	p-value		0.518			
11-20	MEAN		51.7 d	46.8	48.8	50.5
	S.D.		21.41	21.88	21.84	19.25
	p-value		0.884			
21-30	MEAN		49.7 d	45.4	43.8	53.7
	S.D.		21.72	23.79	26.28	25.14
	p-value		0.538			
31-40	MEAN		49.4 d	35.7	42.7	50.6
	S.D.		19.04	27.69	21.11	22.69
	p-value		0.131			
41-50	MEAN		48.6 d	41.0	43.3	46.2
	S.D.		24.67	29.78	22.33	21.96
	p-value		0.772			
51-60	MEAN		49.3 d	36.4	43.7	53.7
	S.D.		31.16	30.66	16.17	30.28
	p-value		0.204			

Statistical key: d=Dunnett's-test

Increased vertical activity was apparent in the HD males at some but not all points of determination.

		(F0-GENERATION) SUMMARY OF MOTOR ACTIVITY DATA VERTICAL ACTIVITY (count)			
Male Pups		day 22 p.p.			
		0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG
Minutes of Testing					
Number tested	N	21	22	22	21
1- 2	MEAN	36.0 d	34.8	41.4	45.6
	S.D.	20.57	20.87	14.88	20.85
	p-value	0.246			
3- 4	MEAN	31.2 d	30.5	31.8	26.0
	S.D.	13.86	15.28	14.56	10.92
	p-value	0.502			
5- 6	MEAN	16.5 d	18.0	24.2	31.8**
	S.D.	14.55	13.15	13.61	20.13
	p-value	0.008	0.977	0.247	0.006
7- 8	MEAN	18.0 d	18.2	17.2	17.5
	S.D.	13.18	14.81	11.33	14.47
	p-value	0.995			
9-10	MEAN	15.0 d	17.2	19.7	17.0
	S.D.	16.33	13.68	20.30	12.21
	p-value	0.813			
1-10	MEAN	116.7 d	118.7	134.4	137.8
	S.D.	60.16	42.79	41.49	47.61
	p-value	0.374			
11-20	MEAN	75.8 d	66.5	68.6	83.7
	S.D.	51.15	43.59	39.57	42.20
	p-value	0.578			
21-30	MEAN	66.0 d	67.5	66.9	63.6
	S.D.	28.89	56.14	42.99	46.66
	p-value	0.993			
31-40	MEAN	65.6 d	48.9	56.7	76.1
	S.D.	43.66	50.52	45.44	59.74
	p-value	0.326			
41-50	MEAN	63.1 d	53.1	57.1	77.7
	S.D.	38.58	45.77	55.73	67.16
	p-value	0.453			
51-60	MEAN	65.6 d	72.9	60.3	71.2
	S.D.	53.07	50.11	53.05	48.70
	p-value	0.844			

Statistical key: d=Dunnett's-test ** = p<0.01

Increased vertical activity was seen in the female HD points at some points of determination.

		(F0-GENERATION) SUMMARY OF MOTOR ACTIVITY DATA VERTICAL ACTIVITY (count)			
Female Pups		day 22 p.p.			
		0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG
Minutes of Testing					
Number tested	N	21	22	22	21
1- 2	MEAN	37.6 d	39.0	35.4	32.8
	S.D.	22.71	23.95	17.38	19.40
	p-value	0.788			
3- 4	MEAN	27.2 d	32.6	31.1	28.3
	S.D.	16.95	19.06	13.96	15.81
	p-value	0.696			
5- 6	MEAN	22.4 d	20.5	20.3	23.0
	S.D.	12.27	13.53	9.84	16.08
	p-value	0.878			
7- 8	MEAN	16.3 d	12.8	17.1	19.0
	S.D.	10.83	8.60	11.26	11.23
	p-value	0.282			
9-10	MEAN	14.4 d	16.8	18.4	28.2
	S.D.	10.71	12.82	13.17	30.14
	p-value	0.081			
1-10	MEAN	117.9 d	121.7	122.3	131.3
	S.D.	52.99	56.91	42.35	60.76
	p-value	0.869			
11-20	MEAN	94.4 d	70.4	69.3	81.0
	S.D.	60.51	42.65	41.63	53.25
	p-value	0.325			
21-30	MEAN	76.8 d	66.5	70.4	87.2
	S.D.	49.01	37.00	51.08	47.62
	p-value	0.490			
31-40	MEAN	90.3 d	55.6	66.9	75.7
	S.D.	53.04	60.08	46.50	57.19
	p-value	0.208			
41-50	MEAN	71.5 d	57.1	54.7	68.4
	S.D.	43.23	53.37	38.45	41.58
	p-value	0.532			
51-60	MEAN	84.9 d	60.0	70.5	90.5
	S.D.	56.74	64.77	42.56	70.81
	p-value	0.329			

Statistical key: d=Dunnett's-test

There were no apparent trends in the watermaze data for the combined male and female data.

SEXES COMBINED		(F0-GENERATION) SUMMARY OF WATER MAZE EVALUATION (Number and Percent of Animals Reaching Criteria)			
		0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG
LEARNING 1					
TRIAL 1	N	10 f	22*	15	9
	%	23.8	50.0	34.1	21.4
	p-value	0.019	0.044	1.000	1.000
TRIAL 2	N	26 f	29	23	24
	%	61.9	65.9	52.3	57.1
	p-value	0.594			
TRIAL 3	N	35 f	37	32	29
	%	83.3	84.1	72.7	69.0
	p-value	0.242			
TRIAL 4	N	38 f	38	40	39
	%	90.5	86.4	90.9	92.9
	p-value	0.780			
TRIAL 5	N	37 f	38	37	36
	%	88.1	86.4	84.1	85.7
	p-value	0.961			
TRIAL 6	N	35 f	35	42	38
	%	83.3	79.5	95.5	90.5
	p-value	0.113			
MEMORY					
	N	35 f	39	41	36
	%	83.3	88.6	93.2	83.7
	p-value	0.469			
LEARNING 2					
TRIAL 1	N	2 f	2	1	2
	%	4.8	4.5	2.3	4.7
	p-value	0.923			
TRIAL 2	N	6 f	8	8	6
	%	14.3	18.2	18.2	14.0
	p-value	0.913			
TRIAL 3	N	14 f	17	17	21
	%	33.3	38.6	38.6	48.8
	p-value	0.523			
TRIAL 4	N	18 f	21	17	28
	%	42.9	47.7	38.6	65.1
	p-value	0.072			
TRIAL 5	N	26 f	21	21	25
	%	61.9	47.7	47.7	58.1
	p-value	0.433			
TRIAL 6	N	26 f	27	19	28
	%	61.9	61.4	43.2	65.1
	p-value	0.150			

Statistical key: f=Fisher's Exact * = p<0.05					

There were no findings of toxicological significance in the pup necropsy observations.

(F0-GENERATION) SUMMARY OF PUP NECROPSY OBSERVATIONS					
		0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG
Litters Evaluated	N	21	22	22	21
Pups Evaluated	N	234	236	249	233
Live	N	234	236	249	233
Stillborn	N	0	0	0	0
GROSS EXAM					
AUTOLYSIS					
Pup Incidence	N	0 f	0	1	2
	%	0.0	0.0	0.4	0.9
	p-value	0.292			
Litter Incidence	N	0 f	0	1	1
	%	0.0	0.0	4.5	4.8
	p-value	0.562			
Affected Pups/Litter	MEAN%	0.00 k	0.00	0.32	0.87
	S.D.	0.000	0.000	1.523	3.968
	p-value	0.567			
CLOSED UMBILICAL HERNIA					
Pup Incidence	N	1 f	0	0	0
	%	0.4	0.0	0.0	0.0
	p-value	0.381			
Litter Incidence	N	1 f	0	0	0
	%	4.8	0.0	0.0	0.0
	p-value	0.372			
Affected Pups/Litter	MEAN%	0.43 k	0.00	0.00	0.00
	S.D.	1.984	0.000	0.000	0.000
	p-value	0.377			
ABDOMINAL WALL THIN					
Pup Incidence	N	1 f	0	0	0
	%	0.4	0.0	0.0	0.0
	p-value	0.381			
Litter Incidence	N	1 f	0	0	0
	%	4.8	0.0	0.0	0.0
	p-value	0.372			
Affected Pups/Litter	MEAN%	0.43 k	0.00	0.00	0.00
	S.D.	1.984	0.000	0.000	0.000
	p-value	0.377			

Statistical key: f=Fisher's Exact k=Kruskal-Wallis					

There were no significant findings in the pup necropsy observations.

		(F0-GENERATION) SUMMARY OF PUP NECROPSY OBSERVATIONS			
		0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG
Litters Evaluated	N	21	22	22	21
Pups Evaluated	N	234	236	249	233
Live	N	234	236	249	233
Stillborn	N	0	0	0	0
EYES					

EYE BALL REDUCED IN SIZE					
Pup Incidence	N	1 f	0	1	0
	%	0.4	0.0	0.4	0.0
	p-value	0.583			
Litter Incidence	N	1 f	0	1	0
	%	4.8	0.0	4.5	0.0
	p-value	0.562			
Affected Pups/Litter	MEAN%	0.43 k	0.00	0.38	0.00
	S.D.	1.984	0.000	1.777	0.000
	p-value	0.567			
KIDNEY					

DILATION OF RENAL PELVIS					
Pup Incidence	N	0 f	1	0	0
	%	0.0	0.4	0.0	0.0
	p-value	0.386			
Litter Incidence	N	0 f	1	0	0
	%	0.0	4.5	0.0	0.0
	p-value	0.400			
Affected Pups/Litter	MEAN%	0.00 k	0.32	0.00	0.00
	S.D.	0.000	1.523	0.000	0.000
	p-value	0.406			
TOTAL PUP NECROPSY OBSERVATIONS					
Pup Incidence	N	2 f	1	2	2
	%	0.9	0.4	0.8	0.9
	p-value	0.935			
Litter Incidence	N	2 f	1	2	1
	%	9.5	4.5	9.1	4.8
	p-value	0.868			

Statistical key: f=Fisher's Exact k=Kruskal-Wallis					

A dose-related decrease in body weight gain was observed by week 6 of life. The HD males showed 6% ($p < 0.05$) less mean body weight than the control group. By week 21 no significant differences were apparent. It is not clear when the differences resolved, but by week 10, it appears that the weekly rate of body weight gain in the HD group exceeded that of the control group (sponsor's summary table shown next page).

MALES		(F1-GENERATION)			
		MEAN BODY WEIGHTS DURING PREMATING AND MATING (GRAMS)			
		0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG

WEEK AFTER BIRTH					
WEEK 6	MEAN	136.9 d	136.0	132.2	128.1*
	S.D.	11.78	12.24	10.25	12.38
	N	24	24	24	24
	p-value	0.042	0.988	0.376	0.029

WEEK 21	MEAN	481.0 d	477.5	488.3	482.0
	S.D.	32.26	26.11	26.27	50.55
	N	4	4	3	4
	p-value	0.983			

 Statistical key: d=Dunnett's-test

The MD group showed a lower rate of weight gain than the other groups. The lack of dose response suggests that this is normal variability.

MALES		(F1-GENERATION)			
		MEAN BODY WEIGHTS CHANGES DURING PREMATING (GRAMS)			
		0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG

WEEK AFTER BIRTH					
WEEK 6 TO 7	MEAN	55.5 d	54.8	50.4*	52.9
	S.D.	5.48	5.57	7.29	5.51
	N	24	24	24	24
	p-value	0.021	0.949	0.012	0.317
WEEK 7 TO 8	MEAN	56.3 d	55.8	51.5**	52.8
	S.D.	5.45	5.64	5.45	4.12
	N	24	24	24	24
	p-value	0.004	0.969	0.005	0.054
WEEK 8 TO 9	MEAN	50.9 d	50.5	47.8	50.6
	S.D.	5.71	5.44	5.48	4.80
	N	24	24	24	24
	p-value	0.153			
WEEK 9 TO 10	MEAN	36.1 d	37.3	34.6	37.7
	S.D.	4.99	5.72	4.94	4.66
	N	24	24	24	24
	p-value	0.153			
WEEK 10 TO 11	MEAN	28.7 d	29.0	26.5	29.7
	S.D.	3.93	4.97	5.96	3.69
	N	24	24	24	24
	p-value	0.127			
WEEK 11 TO 12	MEAN	22.9 d	23.8	21.3	24.1
	S.D.	4.14	4.55	5.76	3.79
	N	24	24	24	24
	p-value	0.164			
WEEK 12 TO 13	MEAN	20.0 d	20.0	17.9	20.0
	S.D.	4.80	3.54	5.17	4.22
	N	24	24	24	24
	p-value	0.285			
WEEK 13 TO 14	MEAN	16.3 d	17.4	16.8	17.8
	S.D.	5.90	3.45	5.56	4.23
	N	24	24	24	24
	p-value	0.718			
WEEK 14 TO 15	MEAN	16.3 d	14.7	16.5	16.0
	S.D.	5.39	5.06	4.12	4.85
	N	24	24	24	24
	p-value	0.545			
WEEK 6 TO 15	MEAN	303.0 d	303.1	283.4**	301.5
	S.D.	22.01	18.57	27.26	17.94
	N	24	24	24	24
	p-value	0.005	1.000	0.007	0.991

 Statistical key: d=Dunnett's-test * = p<0.05 ** = p<0.01

The female HD groups weighed on average 5% (n.s.) less than the control group.

FEMALES		(F1-GENERATION) MEAN BODY WEIGHTS DURING PREMATING AND MATING (GRAMS)				
		0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG	

WEEK AFTER BIRTH						
WEEK		MEAN	115.1 d	112.8	115.0	109.0
		S.D.	9.01	9.12	9.59	10.66
		N	24	24	24	24
		p-value	0.103			
WEEK	21	MEAN	289.5 d	271.8	303.0	285.8
		S.D.	29.44	10.05	19.08	16.78
		N	4	4	3	4
		p-value	0.291			

Statistical key: d=Dunnett's-test						

FEMALES		(F1-GENERATION) MEAN BODY WEIGHTS CHANGES DURING PREMATING (GRAMS)				
		0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG	

WEEK AFTER BIRTH						
WEEK	6 TO 7	MEAN	31.0 d	31.3	31.3	31.6
		S.D.	5.40	5.26	4.12	4.97
		N	24	24	24	24
		p-value	0.986			
WEEK	7 TO 8	MEAN	25.5 d	23.7	24.9	25.0
		S.D.	4.62	3.55	4.89	3.63
		N	24	24	24	24
		p-value	0.480			
WEEK	8 TO 9	MEAN	23.2 d	21.9	22.4	23.6
		S.D.	6.39	5.37	6.21	4.26
		N	24	24	24	24
		p-value	0.705			
WEEK	9 TO 10	MEAN	19.5 d	19.2	18.4	17.9
		S.D.	7.84	5.22	4.71	5.42
		N	24	24	24	24
		p-value	0.760			
WEEK	10 TO 11	MEAN	14.3 d	13.9	14.5	15.4
		S.D.	4.84	5.75	5.52	4.74
		N	24	24	24	24
		p-value	0.784			
WEEK	11 TO 12	MEAN	11.6 d	12.3	10.9	11.6
		S.D.	4.85	5.50	4.76	3.91
		N	24	24	24	24
		p-value	0.774			
WEEK	12 TO 13	MEAN	10.3 d	8.8	11.1	8.7
		S.D.	6.40	4.11	5.59	4.45
		N	24	24	24	24
		p-value	0.320			
WEEK	13 TO 14	MEAN	9.0 d	9.3	8.8	9.4
		S.D.	5.09	4.42	3.50	4.50
		N	24	24	24	24
		p-value	0.961			
WEEK	14 TO 15	MEAN	6.4 d	6.1	7.2	5.6
		S.D.	6.34	4.84	4.88	4.63
		N	24	24	24	24
		p-value	0.764			
WEEK	6 TO 15	MEAN	150.7 d	146.5	149.4	148.8
		S.D.	17.40	9.51	17.39	15.69
		N	24	24	24	24
		p-value	0.815			

Statistical key: d=Dunnett's-test						

The HD females gained approximately 4% (n.s.) less than the control group during gestation.

		(F1-GENERATION) MEAN BODY WEIGHTS DURING GESTATION (GRAMS)			
		0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG
DAY 0	MEAN	282.4 d	271.1	280.7	273.0
	S.D.	18.91	14.50	28.94	19.83
	N	23	21	20	20
	p-value	0.220			
DAY 7	MEAN	309.3 d	299.2	306.6	299.0
	S.D.	18.72	14.03	28.04	21.47
	N	23	21	20	20
	p-value	0.275			
DAY 14	MEAN	334.7 d	322.6	334.4	324.5
	S.D.	21.42	15.20	27.32	23.26
	N	23	21	20	20
	p-value	0.163			
DAY 21	MEAN	416.3 d	404.3	410.9	398.5
	S.D.	28.11	16.82	26.62	28.59
	N	23	21	20	20
	p-value	0.123			

Statistical key: d=Dunnett's-test

There were no significant trends in the necropsy observations for either sex of the F1 generation.

		(F1-GENERATION) SUMMARY OF NECROPSY OBSERVATIONS			
		0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG
MALES	N	24	24	24	24
EYE	N	0	1	1	0
EYE BALL REDUCED IN SIZE	N	0	1	1	0
NO REMARKABLE OBSERVATIONS	N	24	23	23	24

		(F1-GENERATION) SUMMARY OF NECROPSY OBSERVATIONS			
		0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG
FEMALES	N	24	24	24	24
EYE	N	0	0	2	0
EYE BALL REDUCED IN SIZE	N	0	0	1	0
EYE BALL ENLARGED IN SIZE	N	0	0	1	0
NO REMARKABLE OBSERVATIONS	N	24	24	22	24

There were no significant differences in time to insemination for the F1 animals used in the mating study.

		(F1-GENERATION) SUMMARY OF MATING RESULTS (F1-Generation)			
		0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG
Females Placed with Males	N	24	24	24	24
Inseminated	N	24 f	22	23	23
Insemination Index	%	100.0	91.7	95.8	95.8
	p-value	0.555			
Females Excluded	N	0	2	1	2
Females Evaluated	N	24	22	23	22
With Implantation Sites	N	23 f	21	20	20
Fertility Index	%	95.8	95.5	87.0	90.9
	p-value	0.634			
With Viable Pups	N	23 f	21	20	20
Gestation Index	%	100.0	100.0	100.0	100.0
	p-value	1.000			

Statistical key: f=Fisher's Exact

As seen in the previous generation, there was an increase in the number of litters with stillborn pups.

(F1-GENERATION) NATURAL DELIVERY DATA AND LITTER DATA -- SUMMARY					
		0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG
Females Inseminated	N	24	22	23	23
Females with Implantations	N	23 f	21	20	20
Fertility Index	%	95.8	95.5	87.0	87.0
	p-value	0.533			
Females Completing Delivery	N	23 f	21	20	20
Gestation Index	%	100.0	100.0	100.0	100.0
	p-value	1.000			
with Stillborn Pups	N	1 f	0	4	5
	%	4.3	0.0	20.0	25.0
	p-value	0.035	1.000	0.500	0.244
with all Stillborn	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
	p-value	1.000			
Females with Liveborn	N	23 f	21	20	20
	%	100.0	100.0	100.0	100.0
	p-value	1.000			
Duration of Gestation	MEAN	22.61 d	22.81	22.60	22.75
	S.D.	0.499	0.402	0.503	0.444
	N	23	21	20	20
	p-value	0.370			

Statistical key: d=Dunnett's-test f=Fisher's Exact

(F1-GENERATION) NATURAL DELIVERY DATA AND LITTER DATA -- SUMMARY					
		0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG
Litters with Liveborn Pups	N	23	21	20	20
Pups Delivered (total)	N	311	294	258	251
	MEAN	13.52 d	14.00	12.90	12.55
	S.D.	1.880	2.168	2.882	2.395
	p-value	0.204			
Liveborn Pups	N	309	294	253	242
Live Birth Index	MEAN%	99.33 d	100.00	98.23	96.89
	S.D.	3.208	0.000	3.861	6.496
	p-value	0.080			
Stillborn	N	2 f	0	5	8
	%	0.6	0.0	1.9	3.2
	p-value	0.007	1.000	0.761	0.145
Uncertain	N	0	0	0	1
Pups Died, Missing, Killed and/or Cannibalized day 0	N	0 f	2	2	0
	%	0.0	0.7	0.8	0.0
	p-value	0.252			

Statistical key: d=Dunnett's-test f=Fisher's Exact

		(F1-GENERATION) NATURAL DELIVERY DATA AND LITTER DATA -- SUMMARY			
		0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG
Implantation Sites per Litter	N	338	313	291	278
	MEAN	14.70 d	14.90	14.55	13.90
	S.D.	2.225	2.022	2.395	2.337
	p-value	0.519			
Prenatal Loss per Litter	N	27	19	33	27
	MEAN	1.17 d	0.90	1.65	1.35
	S.D.	0.984	0.944	1.565	1.137
	p-value	0.232			
Live Pups/Litter day 0	MEAN	13.43 d	14.00	12.65	12.10
	S.D.	1.950	2.168	2.796	2.174
	N	23	21	20	20
	p-value	0.046	0.754	0.548	0.148
Sex Ratio - Male Pups:Total Pups day 0	MEAN%	48.65 d	51.88	52.55	44.68
	S.D.	11.714	11.585	15.603	12.024
	p-value	0.195			

 Statistical key: d=Dunnett's-test

There was a slight, approximately 4% (n.s.) increase in the HD pup body weight relative to the control group.

		(F1-GENERATION) SUMMARY OF PUP WEIGHTS (GRAMS)			
		0 MG/KG	1.5 MG/KG	5 MG/KG	15 MG/KG
day 0 males	MEAN	6.58 d	6.55	6.67	6.82
	S.D.	0.512	0.469	0.694	0.608
	N	23	21	20	20
	p-value	0.438			
0 females	MEAN	6.26 d	6.23	6.26	6.50
	S.D.	0.506	0.492	0.661	0.602
	N	23	21	20	20
	p-value	0.398			
0 males+females	MEAN	6.41 d	6.40	6.46	6.64
	S.D.	0.508	0.472	0.660	0.618
	N	23	21	20	20
	p-value	0.501			

 Statistical key: d=Dunnett's-test

Fetal drug exposure (i.e., toxicokinetics) was not assessed. Therefore, it is not clear if absence of significant effects was due to lack of toxicity or lack of fetal exposure to drug.

9.3.2 T0081434 Pilot study in neonatal rats

Report number: PH36257

Study number: T0081434

Study location: Bayer Schering Pharma AG, Wuppertal, Germany

Experimental start date: February 2, 2010

GLP: statement included

QA: yes

Test article: BAY63-2521, batch BX02RKK, purity 98.4% . Before the start of dosing, the stability and homogeneity of the formulations in the dose range and volume were analytically confirmed. Concentrations were within $\pm 5\%$ of nominal.

Formulation: suspension in 0.5% tylose

Animals: Wistar (Hsd Cpb:WU)

BAY 63-2521 was administered daily by gavage to 5 male and 5 female Wistar rats per group at doses of 0, 3, 10 or 30 mg/kg per day in 0.5% aqueous Tylose starting at postnatal day (PND) 6 over a treatment period of 14 days (main groups 1-4). By PND 19, animals were necropsied. Organs were weighed and/or fixed. Histopathology was done in the femur and tibia only.

In addition, 6 male and 6 female animals per group (kinetic I groups 9-12) were treated once and euthanized PND 6 after blood sampling for toxicokinetics. Another 12 male and 12 female animals, per group (kinetic II groups 5-8) were treated for 14 days and then euthanized after blood sampling for toxicokinetics at presumed steady state (PND 19). Three males and three females were run as control kinetic groups I and II.

Doses were chosen based on the adult toxicology studies where doses of 30 mg/kg correspond to approximately 10 fold the clinical AUC.

F₀ dams were allowed to deliver their pups. On PND 5, litters were reduced randomly, albeit, as follows:

Litters with more males than females were taken for the male study groups and litters with more females than males were used for the female study groups as far as possible.

Eight litters reduced to 5 male or 5 female pups were used as main groups.

For treated kinetic groups I and II each twelve litters were reduced to 6 male and twelve litters to 6 female pups. Two litters were reduced to 3 male and 3 female pups and used for kinetic control groups I and II.

Inspection of Animals:	
Morbidity and Mortality	Twice daily, once daily on weekends and public holidays
General Clinical Observations (in-cage)	Daily directly after administration (groups 1-4)
Detailed Clinical Observations	Prior to first administration and then weekly before dosing (groups 1-4)
Determination of Body Weights:	Daily
Necropsy:	Day 15 of study (groups 1-4)

Organ weights (prior to fixation) : brain, heart, liver, spleen, kidneys

Histology: right femur and tibia. Gross observation only for all other organs.

Tissues collected in 10% formalin: abnormalities, adrenals, brain, femur, heart, 1/3 right kidney, liver, lungs, ovaries, pituitary, prostate, spleen, thymus, uterus/cervix and vagina.

Tissues collected in Davidson's solution: eyes, testes, left and 2/3 of the right kidney.

Toxicokinetics	
Days of Blood Sampling:	day 1 (= PND 6) day 14 (= PND 19)
Time Points per Day:	4 (0.5, 2, 4 and 7 hours after administration)
Number of Treated Animals per Dose, Sex and Time Point:	3
Number of Controls per Sex and Time Point (2h)	3

Results

Two males in the HD toxicokinetic group died on PND17 (study day 12) with no reported clinical signs. Necropsy was not performed and a cause of death was not determined.

No clinical signs were reported for the animals surviving to scheduled necropsy.

Body Weights

A dose related decrease in body weight gain was seen in the data for both sexes. The high dose male pups gained on average 40% less than the control males. The high dose females gained on average 21% less than the control group.

Sponsor's Summary of Body Weight Gain (g) Main Groups

Dose (mg/kg) Day	Sex	m	m	m	m	f	f	f	f
		0	3	10	30	0	3	10	30
1		13.72	15.12 **	14.72 *	15.82 **	15.44	14.88	14.52	13.18 **
8		35.34	33.60	31.54 **	28.88 **	35.30	32.10 **	30.60 **	27.64 **
14		56.18	48.26 **	47.26 **	41.50 **	53.34	48.56 **	46.70 **	43.08 **

Organ Weights

Both sexes of pups showed increases in brain weight normalized to body weight. The HD males showed 123% (p<0.01) normalized brain weight relative to the control group. The HD females showed 114% (p<0.01) normalized brain weight relative to the controls. Heart, spleen and kidney weight were also increased in both sexes while normalized liver weights were decreased. These changes are summarized in the reviewer's table below.

Table 5-4: Necropsy - Relative Organ Weights

Dose mg/kg	TBW	Brain	Heart	Liver	Kidneys	Spleen
	g	% of Terminal Body Weight				
m						
0	59.22	2.5358	0.6386	3.9387	1.1565	0.3787
3	50.68	2.8314 **	0.6960	3.7622	1.1375	0.3530
10	50.68	2.6722	0.6474	3.7679	1.1061	0.4584 *
30	43.96	3.1232 **	0.9758 *	3.5942	1.2597	0.5222 **
f						
0	56.76	2.4875	0.6489	4.0391	1.1679	0.4045
3	51.28	2.7814 **	0.6255	4.0414	1.2349	0.4439
10	48.64	2.8265 **	0.6927	3.7292 *	1.2504	0.3782
30	45.22	2.8412 **	0.7511	3.6676 **	1.2207	0.4441

Organ weights normalized to body weight: Percent change from control group

	brain	heart	liver	kidneys	spleen
HD males	+123% **	+53% *	-9%	+9%	+38% **
HD females	+114%**	+16%	-9% **	+5%	+10%

*p<0.05, **p<0.01

The histopathology of the femur showed changes including disorganization of the epiphyseal bone and marrow cavity with thickening of the trabecular bone and resultant decrease in the marrow cavity and marrow cells. Activated (undefined) osteoblasts and multinucleated osteoclasts were detectable. Hyperostosis and remodeling were noted in the metaphyseal and diaphyseal bone in both sexes. I do not agree with the sponsor's interpretation that the bone effects begin at 10 mg/kg, because some effects were noted at the low dose of 3 mg/kg. No NOAEL was identified in this study. For facilitated understanding of the histology, several representative photomicrographs of normal histology are provided in Appendix I.

Dose mg/kg	Males (n=5)				Females (n=5)			
	0	3	10	30	0	3	10	30
Disorganized bone / bone marrow cavity epiphysis		1	4	5			5	5
Grade 1		1	2					
Grade 2			2				1	
Grade 3							3	
Grade 4				5			1	5
Reduced epiphyseal bone marrow cells		1	5	5			5	5
Grade 1		1	1					
Grade 2			1					
Grade 3			2	1			2	
Grade 4			1	4			3	
Grade 5								5
Hyperostosis metaphysis			1	5			4	5
Grade 1			1					
Grade 2							3	1
Grade 3							1	4
Grade 4				5				
Hyperostosis diaphysis				5			1	1
Grade 1							1	
Grade 2								1
Grade 3				2				
Grade 4				3				

Toxicokinetics

Plasma level exposure to both parent drug and active metabolite was confirmed.

The increase in AUC was relatively linear from LD to MD but less than proportional from MD to HD.

BAY 63-2521				
	Dose[mg/kg]	3	10	30
AUC(0-7)	[µg·h/L]	1660	5025	10681
AUC(0-7) _{norm}	[kg·h/L]	0.553	0.502	0.356
C _{max}	[µg/L]	371	915	2944
C _{max, norm}	[kg/L]	0.124	0.0915	0.0981
C(7)/C _{max}	[%]	32.7	68.8	31.8
t _{max}	[h]	0.500	2.00	2.00
RA _{Cmax}	[%]	72.6	78.3	136
RA _{AUC}	[%]	69.0	89.3	93.6
BAY 60-4552				
AUC(0-7)	[µg·h/L]	207	651	1301
AUC(0-7) _{norm}	[kg·h/L]	0.0713	0.0673	0.0449
C _{max}	[µg/L]	37.7	123	364
C _{max, norm}	[kg/L]	0.0130	0.0128	0.0125
C(7)/C _{max}	[%]	58.8	84.5	39.9
t _{max}	[h]	2.00	4.00	2.00
RA _{Cmax}	[%]	55.0	61.5	75.7
RA _{AUC}	[%]	63.1	72.3	74.9
MR _{AUC}	[%]	12.9	13.4	12.6

9.3.3 Juvenile Animal Study: Repeat Dose Systemic Toxicity Study in Neonatal Rats Followed by a Drug-Free Recovery Period

Report number: PH36659

Study numbers: T6081458, T8082404, T4082428

Study location: Bayer Pharma AG, Wuppertal, Germany

GLP: yes

QA: yes

Test article: BAY63-2521, batch BX02RKK, purity 98.4% by HPLC analysis. Formulated in a vehicle of 0.5% aqueous tylose. Samples of the dosing preparations were analyzed for concentration. Homogeneity and stability data covering the concentration range used were generated prior to the start of the study, referenced study F1011973. The concentration analysis showed that the formulations were within ±10% of the nominal concentrations. In addition, the report states that test item concentration and homogeneity were checked three (T6081458) or four (T8082404/T4082428) times at the beginning, during the course of the study and near termination.

Formulations used for T8082404 were used also for T4082428

Animals: Wistar rats, Hsd Cpb:WU

Dosing was initiated at 6 days of age. In each of the studies listed below, 12 male and 12 female neonatal rats per dose group received BAY63-2521 by daily gavage at doses of 0, 0.3, 1 or 3 mg/kg for a period as indicated in the reviewer's table below, starting on PND 6. Satellite animals were used for toxicokinetic evaluation. The doses were selected on the basis of a pilot study (filed in DARRTS May 25, 2011). The dose 3 mg/kg caused a decrease in weight gain with concurrent reduction in brain, kidney and liver weights. Doses greater than or equal to 10 mg/kg caused bone remodeling in both sexes.

Study number	Dosing period	Recovery	Study initiated
T6081458	14 weeks Satellite animals for tk	No recovery	Nov 24, 2011
T4082428	4 weeks	4 weeks	Mar 10, 2011
T4082428	4 weeks	No recovery	
T8082404	13 weeks	8 weeks	Jan 25, 2011

Observations

Assessments were conducted with awareness of treatment groups (unblinded).

Inspection of Animals:	
for Morbidity and Mortality	Twice daily
Clinical Observations (in-cage)	Daily about 30 minutes after administration or in the late morning
Detailed Clinical Observations	Weekly (T6081458) or daily (T4082428 and T8082404)
Open Field Observations	At weaning and weekly thereafter
Determination of:	
Body Weight	Daily during dosing and weekly thereafter
Ophthalmological Investigation:	Prior to study start and near termination
Clinical Laboratory Investigation*:	
T6081458 Hematology	Day 92/93 of Dosing
Clinical Chemistry	Day 33/34 and 92/93 of Dosing
Urinalysis	Day 92/93 of Dosing
T8082404 Hematology	Day 53/54 of Recovery
Clinical Chemistry	Day 53/54 of Recovery
Urinalysis	Day 53/54 of Recovery
T4082428 Hematology	Day 23/24 of Dosing Day 28/29 of Recovery
Clinical Chemistry	Day 23/24 of Dosing Day 28/29 of Recovery
T6081458 Learn and Memory Test:	Day 35-37, 42-44 of dosing
T6081458 Functional Observation Battery:	Day 83/84 and 88/89 of Dosing (relative)
T8082404 Functional Observation Battery:	Day 38/39 and 42/43 of Recovery (relative) = day 129/130 and 133/134 of study
T6081458 Motor/Locomotor Activity:	Day 84/85 and 89/90 of Dosing = week 12/13 of study
T8082404 Motor/Locomotor Activity:	Day 43 until 45 of Recovery = week 20 of study
T6081458 Necropsy / Organ Weights:	Day 97 until 99 of Dosing
T8082404 Necropsy / Organ Weights:	Day 59 until 61 of Recovery
T4082428 Necropsy / Organ Weights:	Day 29 until 31 of Dosing 30 until 32 Recovery
T6081458 Toxicokinetics:	See Table 3-8

*in the first ten living animals per group and sex at study start and also near termination of studies T6081458 and T8082404

The following hematological parameters were determined in peripheral blood:

Red blood	Blood coagulation	White Blood
Erythrocyte count	Thrombocyte count	Leukocyte count
Hemoglobin concentration	Thromboplastin time (Hepato-Quick) [§]	Differential blood count
Hematocrit		
Mean corpuscular hemoglobin		
Mean corpuscular hemoglobin concentration		
Mean corpuscular volume		
Reticulocyte count		
Erythrocyte morphology		

[§] Study T6081458 and T8082404 only

The following parameters were determined in studies T6081458 and T8082404 each near terminal sacrifice.

Quantitatively	Qualitatively
Density	Blood
Volume	Bilirubin
Protein concentration	Glucose
Protein excretion	Ketone bodies
Creatinine concentration	Urobilinogen
Creatinine excretion	Microscopy of sediment
Urea concentration	pH
Urea excretion	
Protein/creatinine ratio	

During the time of collection water was offered *ad libitum*.

The following parameters were determined:

Blood enzyme	Blood substrates	Blood electrolytes
Alanine aminotransferase	Glucose [§]	Chloride [§]
Aspartate aminotransferase	Cholesterol [§]	Calcium [§]
Alkaline phosphatase	Triglyceride [§]	Inorganic phosphate [§]
Glutamate dehydrogenase	Creatinine [§]	Potassium [§]
Lactate dehydrogenase [§]	Urea [§]	Sodium [§]
Gamma glutamyl transferase	Total bilirubin [§]	Calcium in plasma [§]
Creatine kinase [§]	Total protein [§]	Magnesium [§]
	Albumin [§]	

[§] determination after 13 weeks administration and in week 8 of recovery only

[§] study T4082428 in week 4 of administration and after 4 weeks recovery only

The length of the right femur and humerus was determined in the first 10 living animals per group and sex in each study.

Table 3-7: Organ Weights, Tissue Preservation and Histopathological Evaluation

Organs	Organ weights	Tissue Preservation	Histopathological Evaluation															
			All Studies	T6081458				T4082428				T8082404						
				All Groups	1	2	3	4	1	2	3	4	1	2	3	4		
Group Number																		
Abnormalities		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Adrenals	X	X	X		X													
Aorta		X	X		X													
Brain (3 regions)	X	X	X		X													
Cecum		X	X		X													
Colon		X	X		X													
Duodenum		X	X		X													
Epididymides	X	X	X		X													
Esophagus		X	X		X													
Extraorbital lacrimal glands		X	X		X													
Eyes		X	X		X													
Femur, left (with bone marrow/joint)		X	X		X	X	X	X	X									
Gall bladder																		
Harderian glands		X	X		X													
Head with skull cap		X																
Heart	X	X	X		X													
Ileum		X	X		X													
Jejunum		X	X		X													
Kidneys	X	X	X		X													
Larynx		X	X		X													
Liver	X	X	X		X													
Lungs		X	X		X													
Lymph node, mandibular		X	X		X								X				X	
Lymph node, mesenteric		X	X		X								X	X	X	X	X	
Lymph node, popliteal		X	X		X								X				X	
Mesentery		X	X	X	X													
Nasal cavities		X																
Ovaries / Oviducts		X	X		X													
Optic nerves		X	X		X													
Pancreas		X	X		X													
Peyer's patches		X	X		X													
Pharynx		X																
Pituitary gland		X	X		X													
Prostate		X	X		X													
Rectum		X	X		X													

Organs	Organ weights	Tissue Preservation	Histopathological Evaluation															
			All Studies	T6081458				T4082428				T8082404						
				All Groups	1	2	3	4	1	2	3	4	1	2	3	4		
Group Number																		
Salivary glands		X	X		X													
Sublingual gland																		
Submandibular gland																		
Parotid gland																		
Sciatic nerve		X	X		X													
Seminal vesicles with Coagulation glands		X	X		X													
Skeletal muscle		X	X		X													
Skin/mammary region		X	X		X													
Spinal cord 3x		X	X		X													
Spleen	X	X	X		X								X				X	
Sternum (with bone marrow)		X	X		X	X	X	X	X									
Stomach		X	X		X													
Testes	X	X	X		X													
Thymus	X	X	X		X								X				X	
Thyroid / Parathyroids		X	X		X													
Tongue		X	X		X													
Trachea		X	X		X													
Uterus	X	X	X		X													
Urethra		X																
Ureters		X	X		X													
Urinary bladder		X	X		X													
Vagina		X	X		X													
Zymbal glands		X																
Physical Identifier		X																

Toxicokinetics	
Days of Blood Sampling:	Day 1/2 (PND 6/7) Day 22/23 (PND 27/28) Day 69/70 (PND 74/75)
Time Points :	
- treated animals	At 0.5, 2, 7 and 24 hrs after administration
- control animals	At 2 hrs after vehicle administration
Number of Animals per Time Point:	5 (control) and 3 per dose group

Results

There was no unscheduled mortality in studies T6081458 and T4082428. In study T8082404, one male at 0 mg/kg died (dosing day 5) and one female at 3 mg/kg died on day 5 of dosing.

The fact that in six females of one 3 mg/kg litter of the recovery group tremor on the whole body was noted from day 10 to 12/18 of dosing is considered as an incidental finding as in none of the other 3 mg/kg litter this symptom was recorded.

No other clinical signs were reported for any of the groups.

Body Weights

Relative body weight changes did not consistently indicate dose-related patterns across the three studies. In T6081458, the control group gained on average the most of the different groups. The drug-treated males gained less than the control group, but without a dose response. The drug-treated females gained on average more than the control group, but without apparent dose response.

Table 4-2: Mean Body Weights (g) and Change to Control Mean - T6081458

Sex Dose mg/kg	Males				Females			
	0	0.3	1	3	0	0.3	1	3
Day	Dosing Phase							
1	16.3	14.5**	17.5*	15.6	15.1	16.1	14.3	15.3
% Change		-12%	+7%	-4%		+7%	-5%	-
28	136.0	125.2**	141.2**	125.8**	114.3	124.6**	112.9	114.1
% Change		-8%	+1%	-7%		+9%	-1%	-
50	313.6	282.4**	309.1	292.3**	210.3	230.8**	208.8	212.3
% Change		-10%	-2%	-7%		+10%	-	+1%
91	465.8	419.3**	450.8	437.6	274.1	299.3	281.6	279.3
% Change		-10%	-3%	-6%		+9%	+3%	+2%

Figure 4-1 Body Weights during 14 Weeks Dosing Phase Study T6081458

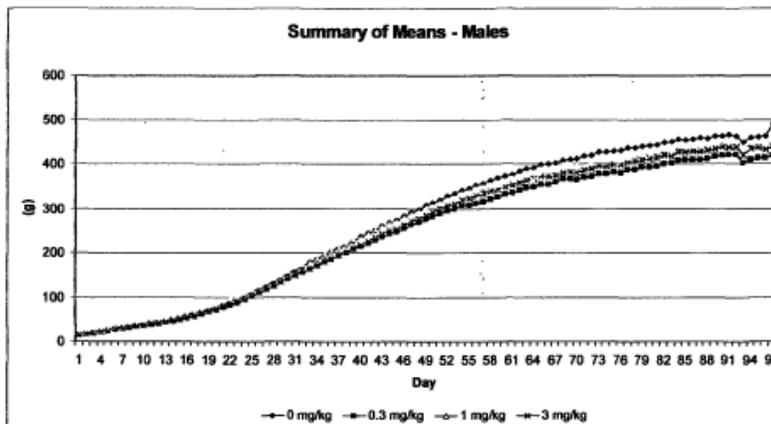
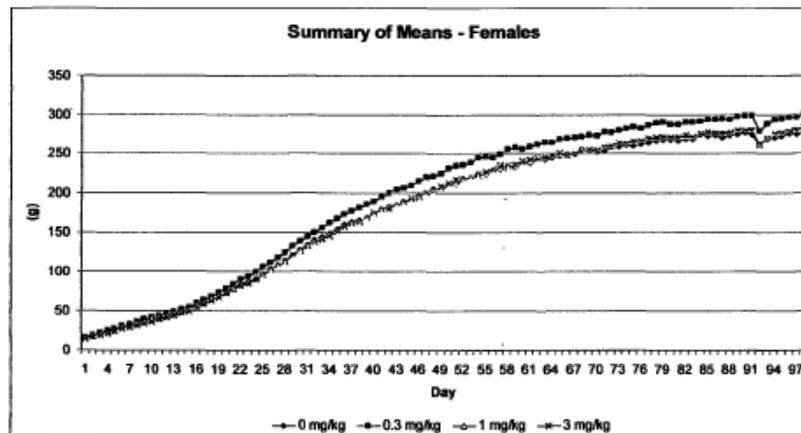


Figure 4-2 Body Weights during 14 Weeks Dosing Phase Study T6081458

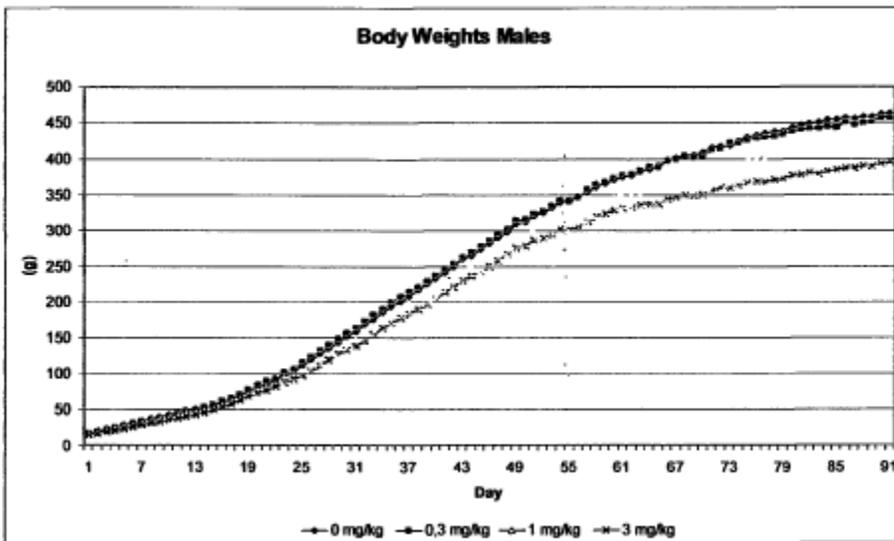


In T8082404, the drug-treated males also gained on average less than the control group, and the low dose and mid-dose females gained more than the control group, similar to T6081458. The high dose females gained less than the control group, different than the previous study.

Table 4-3: Mean Body Weights (g) and Change to Control Mean - T8082404

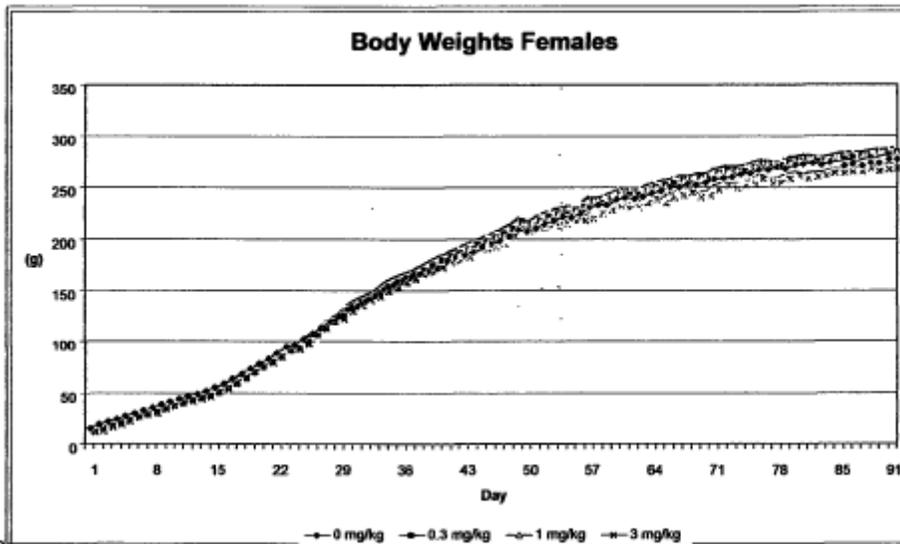
Sex Dose mg/kg	Males				Females			
	0	0.3	1	3	0	0.3	1	3
Day	Dosing Phase							
1	16.37	16.24	14.63**	14.23**	16.09	16.72	14.91**	16.27
% Change		-	-11%	-13%		+4%	-7%	+1%
28	134.92	139.37	126.96*	118.33**	119.97	123.62	118.96	123.25
% Change		+3%	-6%	-12%		+3%	-1%	+3%
50	311.25	314.08	294.88*	276.64**	207.53	216.80	219.14	210.18
% Change		+1%	-5%	-11%		+4%	+8%	+1%
91	463.61	456.56	433.11*	395.99**	281.41	288.63	289.55	272.24
% Change		-2%	-7%	-15%		+3%	+3%	-3%
Day	Recovery Phase							
57	532.25	510.59	486.42**	459.97**	305.62	310.40	306.69	289.28
% Change		-4%	-9%	-14%		+2%	-	-5%

Figure 4-3 Body Weights during 13 Weeks Dosing Phase Study T8082404



The weight curve for the high dose males suggests a lower rate of gain than the control group.

Figure 4-4 Body Weights during 13 Weeks Dosing Phase Study T8082404



There is no obvious dose-relationship in the weight gain data for the females. The lines are parallel with variability becoming more apparent (spreading of the lines) over the course of the study.

The study with the shortest dosing period, T4082428, showed the same weight effects in males as the preceding two studies. The drug-treated males gained on average less than the control group. The results in drug-treated females were similar to T8082404 in that two groups gained on average more than the controls and 1 group gained less.

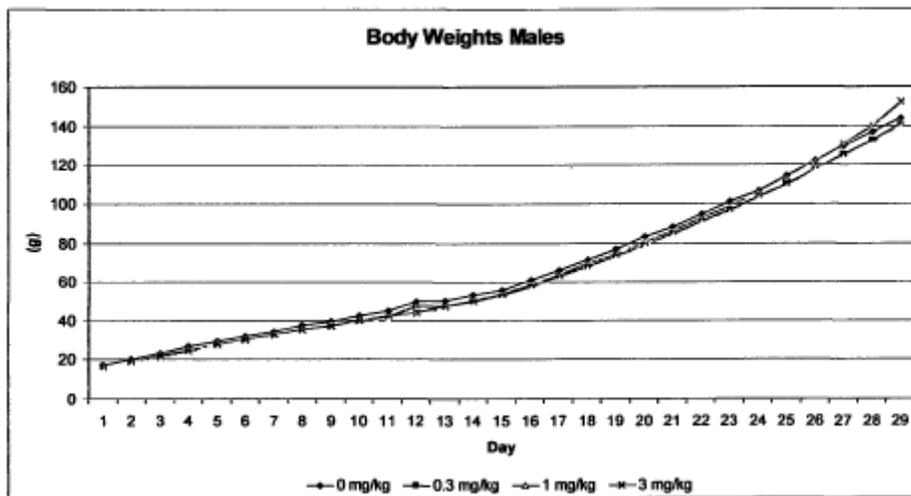
Table 4-4: Mean Body Weights (g) and Change to Control Mean - T4082428

Sex Dose mg/kg	Males				Females			
	0	0.3	1	3	0	0.3	1	3
Dosing Phase Main Groups								
Day 1	17.39	16.76	16.13**	16.88	15.29	16.72*	16.15	14.84
% Change		-4%	-7%	-3%		+9%	+6%	-3%
Day 28	136.95	132.58	130.83	139.82	126.85	130.81	134.63*	125.67
% Change		-3%	-4%	+2%		+3%	+6%	-1%
Dosing Phase Recovery Groups								
Day 1	16.84	16.44	17.71*	17.43	16.12	16.00	16.10	17.30**
% Change		-2%	+5%	+3%		-	-	+7%
Day 28	146.72	137.85**	141.88	131.34**	121.35	121.98	118.91	126.44*
% Change		-6%	-3%	-3%		+1%	-2%	+4%
Recovery Phase Recovery Groups								
Day 29	362.98	342.21*	339.53**	325.44**	224.33	222.48	217.32	221.28
% Change		-6%	-7%	-10%		-1%	-3%	-1%

* = p ≤ 0.05

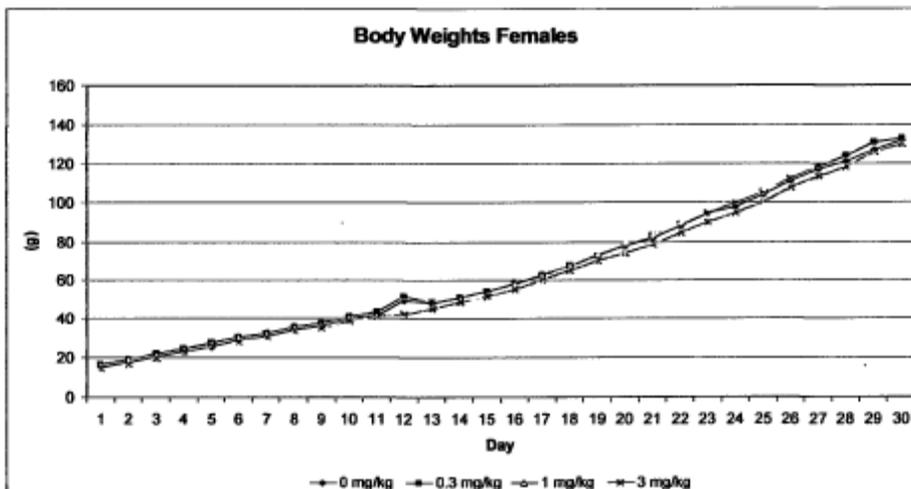
** = p ≤ 0.01

Figure 4-5 Body Weights during 4 Weeks Dosing Phase Study T4082428



The weight curves indicate similar rates of gain between the groups.

Figure 4-6 Body Weights during 4 Weeks Dosing Phase Study T4082428



The weight curves suggest similar rates of gain between the groups.

Hematology

There were no obvious findings of toxicological significance in the red cell data.

Differential Data

Table 4-7: Differential Blood Count - T6081458 and T8082404

Dose mg/kg	LEUCO G/l	LYM G/l	NEUTRO G/l	Baso-phils G/l	EOS G/l	MONO G/l	Atypical G/l
End of 14 Weeks Dosing Phase T6081458							
Males Day 93 of Dosing							
0	9.252	7.797	1.002	0.054	0.128	0.182	0.092
0.3	10.788*	8.775	1.358	0.064	0.170	0.307**	0.113
1	9.860	7.917	1.364*	0.059	0.120	0.296**	0.110
3	9.458	7.885	1.025	0.053	0.130	0.258**	0.110
Females Day 92 of Dosing							
0	7.203	6.064	0.783	0.035	0.121	0.146	0.054
0.3	8.144	6.852	0.925	0.045	0.113	0.149	0.065
1	9.122*	7.485*	1.178	0.057**	0.132	0.201	0.071
3	8.189	7.098	0.758	0.045	0.102	0.123	0.063
End of 8 Weeks Recovery Phase T8082404							
Males Day 53 of Recovery							
0	9.122	7.539	1.141	0.043	0.127	0.209	0.061
0.3	9.566	7.914	1.177	0.059	0.147	0.197	0.071
1	8.334	7.150	0.853	0.034	0.089	0.159*	0.050
3	8.607	7.265	0.955	0.039	0.101	0.189	0.056
Females Day 54 of Recovery							
0	6.492	5.270	0.872	0.026	0.114	0.159	0.049
0.3	6.536	5.028	1.183	0.025	0.103	0.152	0.043
1	5.177	4.259	0.689	0.020	0.084	0.097*	0.031
3	4.150**	3.438**	0.543	0.013*	0.055*	0.077*	0.020**

The data indicates an increase in lymphocytes (17%, n.s.) in high dose females at the end of the dosing period and a 35% (p<0.01) decrease in lymphocytes at the end of the recovery period. Monocytes were significantly increased in the drug-treated males at the end of the dosing of T6081458 and decreased at the end of dosing in T8082404.

Table 4-8: Differential Blood Count - T4082428

Dose mg/kg	LEUCO G/l	LYM G/l	NEUTRO G/l	Baso-phils G/l	EOS G/l	MONO G/l	Atypical G/l
End of 4 Weeks Dosing Phase (Main groups)							
Males Day 23 of Dosing							
0	7.034	5.794	0.799	0.048	0.141	0.219	0.037
0.3	7.394	6.037	0.872	0.056	0.162	0.210	0.049
1	6.422	5.256	0.741	0.059	0.153	0.166	0.047
3	6.320	5.282	0.607	0.048	0.110	0.161	0.114
Females Day 24 of Dosing							
0	8.219	6.936	0.737	0.059	0.243	0.202	0.043
0.3	9.469	7.885	0.875	0.074	0.323	0.245	0.068*
1	7.321	6.054	0.736	0.067	0.224	0.164	0.075
3	6.693	5.442	0.721	0.053	0.226	0.188	0.065

In study T4082428, the high dose females showed a decrease in lymphocytes at both the end of the dosing period (-22%, n.s.) and the end of the recovery period (-12%, n.s.). Thus there is little consistency between the two studies for the observation of lymphocyte behavior.

Table 4-11: Clinical Chemistry: Substrates - T6081458 and T8082404

Dose mg/kg	GLUCOSE mmol/l	CHOL mmol/l	TRIGL mmol/l	CREA μmol/l	UREA mmol/l	Bili-t μmol/l	Protein g/l	Albumin g/l
End of 14 Weeks Dosing Phase T6081458								
Males Day 93 of Dosing								
0	4.394	1.874	0.745	60.6	6.046	0.42	65.75	35.48
0.3	4.165	2.065	0.619	59.1	5.436*	0.59	66.51	36.64
1	4.121	1.614	0.811	53.6**	5.642	0.84**	64.92	35.84
3	3.888**	1.930	0.591	51.2**	5.461	0.79*	65.36	35.55
Females Day 92 of Dosing								
0	4.486	1.888	0.535	65.0	5.988	0.34	66.46	38.44
0.3	4.591	1.563*	0.471	68.3	6.217	0.55	66.83	37.73
1	4.395	2.099	0.519	64.7	6.594	0.59**	66.62	38.08
3	4.568	1.646	0.508	60.1*	6.277	0.84**	67.58	39.22
End of 8 Weeks Recovery Phase T8082404								
Males Day 53 of Recovery								
0	3.979	2.257	0.994	61.6	5.713	0.63	63.59	34.30
0.3	4.331*	2.389	1.245	61.7	5.420	0.40	68.75**	34.86
1	4.141	2.108	0.888	59.4	5.092*	0.71	66.14*	35.22
3	4.167	2.004	0.982	57.6**	5.601	0.51	67.22**	36.07*
Females Day 54 of Recovery								
0	4.223	2.045	0.720	67.2	5.770	0.97	68.71	38.46
0.3	4.124	2.289	0.536*	69.4	6.661*	0.81	70.47	38.38
1	4.156	1.998	0.538*	69.6	6.334	0.88	73.10*	40.65
3	4.225	1.898	0.453**	67.4	6.065	0.76	70.16	38.67

* = p ≤ 0.05

** = p ≤ 0.01

During the dosing phase, serum glucose decreased in males by 12% ($p < 0.01$) but was unaffected in females. During the recovery period, serum glucose increased in males to above the levels of the control group. Serum creatinine decreased in males by 16% ($p < 0.01$) relative to controls during the dosing phase and to -6% ($p < 0.05$) during the recovery period. Creatinine was also decreased in females during the dosing period, -5% ($p < 0.05$) relative to control. Total bilirubin was increased in both sexes during the dosing period, suggesting either an alteration in liver function or mild hemolysis.

Table 4-10: Clinical Chemistry: Enzymes - T4082428

Dose mg/kg	ALAT U/l	ASAT U/l	APh U/l	GLDH U/l	gamma- GT U/l
End of 4 Weeks Dosing Phase (Main groups)					
Males Day 23 of Dosing					
0	73.87	91.32	635.9	6.69	1.29
0.3	70.41	88.30	627.5	6.29	1.54
1	76.15	87.40	583.8	6.40	1.32
3	85.84**	88.16	622.7	6.71	1.57
Females Day 24 of Dosing					
0	68.97	91.27	494.4	7.74	1.20
0.3	70.73	95.06	597.6**	8.20	1.11
1	81.75**	90.45	538.6	7.55	1.03
3	77.89*	88.90	560.0	6.56*	1.55
End of 4 Weeks Recovery Phase (Recovery Groups)					
Males Day 28 of Recovery					
0	82.08	76.54	397.4	6.33	0.92
0.3	78.31	78.08	363.1*	6.18	0.80
1	83.35	75.29	405.2	7.62	1.13
3	85.76	72.01	387.7	8.74	1.16
Females Day 29 of Recovery					
0	68.73	77.63	278.1	9.39	1.23
0.3	74.75	75.38	263.9	4.72	0.74*
1	68.84	70.87	281.5	4.39	1.10
3	71.85	70.97	232.6**	7.43	0.56**

The slight increase in AL(A)T was not repeated in the other studies.

The sponsor notes the changes in serum electrolytes present in both sexes and attributes those changes to the pharmacology of the drug without further explanation. Possible explanations include the pharmacologic effect on the bone or effects on renal tubular absorption, the parathyroids or muscle.

Table 4-13: Clinical Chemistry: Electrolytes - T4082428

Dose mg/kg	CA-PLAS mmol/l	CA-PLAS mmol/l
Males	Day 23 of Dosing	Day 28 of Recovery
0	2.982	2.994
0.3	3.079	3.020
1	3.011	3.058
3	3.119*	3.072*
Females	Day 24 of Dosing	Day 29 of Recovery
0	2.893	2.929
0.3	2.869	2.987
1	2.836	2.983
3	2.882	2.954

* = $p \leq 0.05$ ** = $p \leq 0.01$ **Table 4-12: Clinical Chemistry: Electrolytes - T6081458 and T8082404**

Dose mg/kg	Na mmol/l	K mmol/l	Cl mmol/l	Ca mmol/l	P mmol/l	Mg mmol/l
End of 14 Weeks Dosing Phase T6081458						
Males	Day 93 of Dosing					
0	145.6	5.84	98.3	2.695	3.279	1.214
0.3	145.8	6.70*	99.4	2.801**	3.425	1.280
1	144.9	6.59*	98.3	2.799**	3.119	1.135
3	144.2**	6.96**	98.7	2.860**	3.120	1.036**
Females	Day 92 of Dosing					
0	144.0	6.48	99.6	2.706	3.122	1.373
0.3	143.9	6.61	99.7	2.758	2.835**	1.307
1	143.9	6.68	100.4	2.776	3.005	1.261*
3	143.8	6.87	100.0	2.866**	2.905	1.230**
End of 8 Weeks Recovery Phase T8082404						
Males	Day 53 of Recovery					
0	147.5	6.40	99.9	2.653	3.139	1.118
0.3	147.3	6.74	99.2	2.785**	2.943	1.157
1	146.0**	7.04*	100.6	2.804**	2.918	1.054
3	145.5**	6.94	101.3	2.823**	2.859*	1.056
Females	Day 54 of Recovery					
0	146.7	6.52	101.4	2.680	2.510	1.212
0.3	145.6*	6.59	102.4	2.726	2.389	1.195
1	146.1	7.04	101.8	2.866**	2.404	1.214
3	145.3**	6.70	103.0	2.771*	2.303	1.167

The determination of blood electrolyte concentration at the end of the 13 weeks dose phase revealed a dose-independent increase in potassium and calcium concentration in males starting at 0.3 mg/kg. Calcium increased also in 3 mg/kg females and in 3 mg/kg males receiving the test substance for 4 weeks. Magnesium decreased slightly in males and females starting at 1 mg/kg. These changes were shown to be reversible except the calcium concentrations in males receiving the test substance for 13 or 4 weeks.

These changes were slight to moderate and are attributed to the mode of pharmacological action of the test substance.

Urinalysis

At the end of the dosing period and the end of the recovery period, drug-treated animals excreted less urea. This was statistically significant in the high dose males at the end of recovery.

Table 4-14: Urinalysis - T6081458

Dose mg/kg	Volume ml	Density kg/l	PROT-q g/l	PROT* UVOL mg	CREA mmol/l	CRE* UVOL μ mol	Prot/ Crea mg/mmol	Urea-u mmol/l	Urea* UVOL mmol
End of 14 Weeks Dosing Phase T6081458									
Males Day 93 of Dosing									
0	9.55	1.0307	1.425	11.79	10.535	87.4	144.9	620.4	5.11
0.3	12.39	1.0205	0.911	10.11	7.232	80.1	126.4	426.4	4.77
1	9.01	1.0297	1.572	13.03	9.670	81.5	164.8	542.6	4.55
3	10.90	1.0267	1.154	12.27	8.544	85.0	141.9	493.8	4.96
Females Day 92 of Dosing									
0	6.35	1.0331	0.271	1.41	9.967	52.3	26.9	765.1	3.95
0.3	6.03	1.0291	0.283	1.60	8.842	51.7	31.4	620.4	3.68
1	6.50	1.0333	0.289	1.59	11.007	58.3	27.1	717.1	3.82
3	6.96	1.0300	0.266	1.45	9.478	52.2	27.5	644.3	3.58

Table 4-15: Urinalysis - T8082404

Dose mg/kg	Volume ml	Density kg/l	PROT-q g/l	PROT* UVOL mg	CREA mmol/l	CRE* UVOL μ mol	Prot/ Crea mg/mmol	Urea-u mmol/l	Urea* UVOL mmol
End of 8 Weeks Recovery Phase T8082404									
Males Day 53 of Recovery									
0	8.63	1.0353	2.123	20.15	15.040	115.3	164.0	725.5	5.69
0.3	9.09	1.0367	3.088	23.71	15.231	114.0	206.9	795.0	5.95
1	5.85	1.0478	3.218	17.34	20.301	105.7	161.7	992.2	5.17
3	5.93	1.0367	2.175	12.83	15.336	85.7**	145.9	799.7	4.51*
Females Day 54 of Dosing									
0	12.07	1.0178	0.173	1.67	6.507	62.9	26.4	419.4	4.33
0.3	9.48	1.0226	0.242	1.56	8.461	59.0	25.8	546.4	3.91
1	4.39**	1.0402**	0.510**	2.13	14.660**	58.3	35.7	905.3**	3.59
3	7.10	1.0287	0.267	1.68	10.477	57.5	27.0	678.4	3.87
pH									

Learning and Memory Test in Weanlings (Watermaze evaluation) T6081458: There were no obvious effects in the data as presented.

Functional Observational Battery: The results showed no biologically significant differences between any of the groups for any parameter for either sex.

Motor Activity: The summary tables provided in the appendix did not show discernible patterns or trends for locomotor activity. There was an inconsistent tendency to greater activity in the drug treated animals, but without statistical significance.

The other studies also showed inconsistent levels of motor and locomotor activity. There was a tendency for greater activity in the drug-treated versus control groups, but without dose dependence or statistical significance. One summary table is shown below as an example.

BAYER HealthCare AG Table 4 Week 20 - Week 20 - (Z01)						
BAY 63-2521 Summary Session LOCOMOTOR Activity for Male Rats Study Number T8082404						
Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 mg/kg	60 ± 17 (10)	32 ± 15 (10)	25 ± 7 (10)	15 ± 7 (10)	9 ± 9 (8)	5 ± 6 (6)
0.3 mg/kg	50 ± 7 (10)	28 ± 7 (10)	17 ± 7 (10)	14 ± 9 (10)	11 ± 7 (9)	10 ± 3 (5)
1 mg/kg	59 ± 11 (10)	26 ± 12 (10)	18 ± 7 (10)	14 ± 8 (10)	12 ± 8 (10)	11 ± 6 (8)
3 mg/kg	74 ± 25 (10)	31 ± 14 (10)	21 ± 17 (10)	25 ± 14 (10)	16 ± 9 (10)	12 ± 8 (9)

*Statistically significant differences (Dunnett's method $p \leq 0.05$, ANOVA)
 MEAN +/- S.D. (n) of 10 - Minute Intervals
 Nominal Day 1 = 2011-02-07
 Print Date: 2011-06-29

Developmental Milestones: There were no apparent effects on developmental milestones for females. There was a 1.5 day delay in balanopreputial separation.

Table 4-1: Developmental Milestones - T6081458

Dose mg/kg	0	0.3	1	3
Mean age of balano-preputial separation in days	40.6	40.2	40.5	42.1
Mean age of vaginal opening in days	33.6	33.3	33.1	32.7

Necropsy

Study T6081458

The control and high dose groups of each sex were examined. Atrophy of the testes and epididymides and aspermia of both organs were each listed for 1 HD male.

Several males in the mid- dose and high dose groups showed swollen (0-0-2-1) and enlarged (0-0-2-3) livers. The sponsor reported that there was no corresponding histopathology.

In rats receiving BAY 63-2521 for 14 weeks mesenteric veins showed dilated spaces in the vessel wall ("plexiform change") with mildly increasing incidences at 3 mg/kg in males. A treatment effect possibly due to the mode of action of BAY 63-2521, which was also present in some rats investigated after a 8 week recovery period, can not be excluded for this finding.

Because of bone effects seen prior to this, the histopathology of the bones examined is included here. No effects are noted.

DOSE GROUP:	01		02		03		04	
SEX :	M	F	M	F	M	F	M	F
NO. ANIMALS:	12	12	12	12	12	12	12	12
BONE, FEMUR :	11	11	-	-	-	-	12	10
BONE MARROW, FEMUR :	11	11	-	-	-	-	12	10
BONE, STERNUM :	12	12	-	-	-	-	12	12
BONE MARROW, STERNUM :	12	12	-	-	-	-	12	12
SPINAL CORD :	12	12	-	-	-	-	12	12

Study T8082404

The two animals who died ahead of schedule are noted in this pathology report. The high dose rat was reported to have severe bronchopneumonia and also hypoplasia/atrophy of the thymus gland with overall poor general condition. The cause of death of the control animal was uncertain due to autolysis of the tissues. The findings were regarded as “incidental and spontaneous.”

This study collected the femorotibial joint, skull and nasal cavity, and sternum. Very few findings are listed. The bones were not mentioned at all.

As in study T6081458, there was an increase in the dilated spaces in mesenteric vessel walls (“plexiform change”) in males with an incidence of 2-3-2-4. The sponsor notes that because this has been seen in several studies it can’t be excluded as treatment related.

Study T4082428

T4082428: the femorotibial joint was collected, along with the skull and nasal cavity, and sternum. The pathologist’s summary specifically states “no treatment-related findings in the region of the femur and the sternum.” One high dose animal (male) was reported to have erythroid hypoplasia of the bone marrow. This result was found in the individual animal data. No findings of toxicological significance were reported in the summary tables.

Femur and Humerus Length

There were no significant differences in length of femur or humerus during the dosing period in either sex in either study.

Table 4-16: Femur and Humerus Length in mm - T6081458 and T8082404

Dose mg/kg	14 Weeks Treatment		8 Weeks Recovery	
	Femur length	Humerus length	Femur length	Humerus length
M				
0	35.22	28.96	37.90	30.57
0.3	34.45	28.41	37.76	30.34
1	34.96	28.88	37.68	30.18
3	34.55	28.18	36.37	29.16
F				
0	31.38	25.54	33.08	25.95
0.3	31.74	25.93	33.52	26.68
1	31.33	25.61	32.80	26.43
3	31.23	25.73	32.49	26.12

Table 4-17: Femur and Humerus Length in mm - T4082428

Dose mg/kg	4 Week Treatment		4 Week Recovery	
	Femur length	Humerus length	Femur length	Humerus length
M				
0	23.43	20.50	32.80	26.69
0.3	24.11	19.62	31.60	25.72
1	24.45	19.67	32.57	26.10
3	23.89	19.71	32.64	25.88
F				
0	23.53	19.23	29.83	23.87
0.3	23.81	19.37	29.75	24.00
1	23.69	19.36	29.15	23.78
3	23.38	19.00	29.47	23.84

*Gross Observations and Organ Weights*T6081458

The gross observations for T6081458 noted:

Necropsy revealed swollen and enlarged livers in male animals of the intermediate (1 mg/kg) and high dose group (3 mg/kg). These findings did not correspond to any of the observed histopathological alterations.

Femur, sternum, skull and nasal cavity were the bony tissues collected.

Both high dose males and females had increased liver weight as a percentage of body weight compared to the control groups: males (+11%, $p < 0.01$) and females (+5%, n.s.). Normalized kidney weight was increased in high dose males (+7%, n.s.) and decreased in mid-dose and high dose females (-6%, $p < 0.05$). There was no apparent effect on spleen weight, thus not supporting the suggestion of hemolysis. The weight of the testes showed statistically non-significant increases over the control group, reaching a maximum of 17%.

Table 4-19: Organ Weights in % of TBW - 14 Weeks Treatment T6081458

Sex	Dose mg/kg	TBW (g)	BRAIN	ADRENAL GLANDS	HEART	LIVER	KIDNEYS	SPLEEN
Males	0	466.1	0.4636	0.0127	0.3473	3.8483	0.6461	0.1762
	0.3	417.9**	0.5118**	0.0149	0.3494	4.0199	0.6594	0.1926
	1	455.9	0.4641	0.0137	0.3483	3.9335	0.6805	0.1837
	3	442.8	0.4770	0.0150	0.3652	4.2623**	0.6938	0.1781
Females	0	280.1	0.6927	0.0275	0.3928	3.7609	0.7105	0.2191
	0.3	302.9 *	0.6570	0.0299	0.4147	3.7236	0.6790	0.2123
	1	287.7	0.6641	0.0247	0.3775	3.7837	0.6595 *	0.2059
	3	283.0	0.6644	0.0261	0.3694	3.9534	0.6645 *	0.2028
			THYMUS	TESTES	EPIDIDY- MIDES	UTERUS		
Males	0	0.1203	0.7629	0.3377				
	0.3	0.1258	0.7844	0.3391				
	1	0.1307	0.8986	0.3706				
	3	0.1239	0.7946	0.3573				
Females	0	0.1773			0.2716			
	0.3	0.1554			0.2842			
	1	0.1487			0.2792			
	3	0.1632			0.2744			

* = p ≤ 0.05

** = p ≤ 0.01

In T8082404, the recovery animals showed increased normalized spleen weight: +6% (n.s.) in males and +10% (n.s.) in females. The testes showed a dose-related (+19%, p<0.01) increase as did the epididymides (+17%, p<0.05).

Table 4-21: Organ Weights in % of TBW - 8 Weeks Recovery T8082404

Sex	Dose mg/kg	TBW (g)	BRAIN	ADRENAL GLANDS	HEART	LIVER	KIDNEYS	SPLEEN
Males	0	541.0	0.4064	0.0106	0.3108	3.8697	0.6049	0.1568
	0.3	522.0	0.4318	0.0109	0.3114	3.7788	0.6358	0.1612
	1	491.9**	0.4340	0.0119	0.3372	3.6737	0.6194	0.1664
	3	465.5**	0.4325	0.0114	0.3191	3.8152	0.6067	0.1662
Females	0	310.7	0.6355	0.0273	0.3736	3.4698	0.6481	0.1856
	0.3	318.3	0.6417	0.0251	0.3659	3.5428	0.6237	0.1974
	1	312.9	0.6472	0.0244	0.3570	3.3171	0.6021	0.1851
	3	296.6	0.6532	0.0267	0.3743	3.6248	0.6436	0.2035
			THYMUS	TESTES	EPIDIDY- MIDES	UTERUS		
Males	0	0.0865	0.6904	0.3302				
	0.3	0.0790	0.7323	0.3311				
	1	0.0850	0.7992**	0.3768**				
	3	0.0954	0.8224**	0.3852**				
Females	0	0.1234			0.3450			
	0.3	0.1161			0.3910			
	1	0.1096			0.2555			
	3	0.1045 *			0.3120			

* = p ≤ 0.05

** = p ≤ 0.01

In T4082428, the drug-treated females showed increased liver weight (+13%, $p < 0.01$) and spleen weight (+27%, $p < 0.01$). The testes showed a non-dose related increase (12%, $p \leq 0.01$) while the weight of epididymides were decreased at the high dose (15%, $p \leq 0.05$).

Table 4-23: Organ Weights in % of TBW - 4 Week Treatment T4082428

Sex	Dose mg/kg	TBW (g)	BRAIN	ADRENAL GLANDS	HEART	LIVER	KIDNEYS	SPLEEN
Males	0	149.3	1.1378	0.0207	0.4508	5.2112	0.8486	0.3197
	0.3	145.1	1.2004	0.0208	0.4944	5.0206	0.8595	0.3418
	1	144.3	1.1997	0.0217	0.4582	5.0504	0.8961	0.3167
	3	153.7	1.1048	0.0191	0.4591	5.3052	0.8718	0.3332
Females	0	138.8	1.2077	0.0232	0.5429	4.8343	0.9012	0.2624
	0.3	140.2	1.2370	0.0241	0.5002	5.0958 *	0.8765	0.3075**
	1	145.8	1.1734	0.0223	0.4793*	5.5632**	0.9911**	0.3132**
	3	138.3	1.1976	0.0235	0.5069	5.4675**	0.8843	0.3330**
		THYMUS	TESTES	EPIDIDY- MIDES	UTERUS			
Males	0	0.3301	0.8651	0.1379				
	0.3	0.3388	0.9680**	0.1372				
	1	0.3283	0.9252	0.1349				
	3	0.2967	0.8820	0.1170 *				
Females	0	0.3478			0.2460			
	0.3	0.3387			0.2750			
	1	0.3755			0.2000			
	3	0.3370			0.2511			

* = $p \leq 0.05$

** = $p \leq 0.01$

The end of the recovery period also reported increases in testes weight (16%, $p \leq 0.01$) while the epididymides showed a 9% (ns) increase.

Table 4-25: Organ Weights in % of TBW - 4 week Recovery T4082428

Dose mg/kg	Dose mg/kg	TBW (g)	BRAIN	ADRENAL GLANDS	HEART	LIVER	KIDNEYS	SPLEEN
Males	0	374.8	0.5634	0.0158	0.3921	4.8939	0.7037	0.1999
	0.3	351.9 *	0.5788	0.0160	0.4192	5.0311	0.7462	0.2281**
	1	350.0 *	0.5938	0.0160	0.3790	4.7319	0.7864**	0.2135
	3	337.4**	0.5992 *	0.0170	0.4016	4.9174	0.7429	0.1998
Females	0	232.8	0.8178	0.0297	0.4311	4.7031	0.7690	0.2239
	0.3	227.3	0.8227	0.0295	0.4316	4.5063	0.7429	0.2321
	1	225.3	0.8434	0.0326	0.4046	4.4803	0.7543	0.2322
	3	229.0	0.8187	0.0311	0.4097	4.4872	0.7659	0.2365
		THYMUS	TESTES	EPIDIDY- MIDES	UTERUS			
Males	0	0.1904	0.8608	0.2633				
	0.3	0.2162 *	0.9546**	0.2654				
	1	0.1665**	0.9967**	0.2877				
	3	0.1807	0.9468**	0.2815				
Females	0	0.2319			0.2878			
	0.3	0.2289			0.2830			
	1	0.2030 *			0.2962			
	3	0.2070			0.2559			

* = $p \leq 0.05$

** = $p \leq 0.01$

Looking across the three studies at the organ weight data, there is some consistency in effects on the testes and epididymides, but not necessarily a dose-relatedness. The spleen, liver and kidney weights were inconsistently affected. With the available data set, it is not possible to determine if the organ weight effects are due to normal variability or age specific drug effects.

Toxicokinetics show plasma levels of parent drug for each dose group. The values reported are consistent to those reported for similar doses given to mature animals. The toxicokinetic parameters for the pilot study were calculated for a different time interval than for the definitive study.

Summary of Toxicokinetics for Definitive Juvenile Animal Study

	male			female		
Dose of BAY63-2521 (mg/kg)	0.3	1	3	0.3	1	3
Juvenile animal study day 1: AUC _{0-t}	358	1140	3470	269	940	4400
Juvenile animal study day 69: AUC _{0-t}	70	206	573	79	275	794
AUC _{0-last} single oral dose	79.6	363	1524			
Standard toxicology studies				10 mg/kg	30mg/kg	
AUC0-24 steady state day 91			956	3699	10388	
Human AUC						
* single oral doses, PH33998, PH-34490, PH33934*, males and females combined						

Summary of toxicokinetics from the neonatal rat pilot study (T0081434) with values for sexes combined

Dose of BAY63-2521 (mg/kg)	3	10	30
AUC ₀₋₇ µg.h/l	1660	5025	10681
Multiplied by 3	4980	15073	32043
Plasma levels of BAY60-4552 (M1 metabolite)			
AUC ₀₋₇ µg.h/l	207	651	1301
Multiplied by 3	621	1953	3903

10 Special Toxicology Studies

Potential phototoxicity was investigated because riociguat showed some absorption of light in the range between 290 nm and 720 nm wavelength. Under the conditions of the studies minimal phototoxic potential was demonstrated.

Potential immunotoxicity was investigated in mice (LLNA/IMDS), a 4 week rat study (PH33408), and 13-week rat study (PH34674). The investigations included :

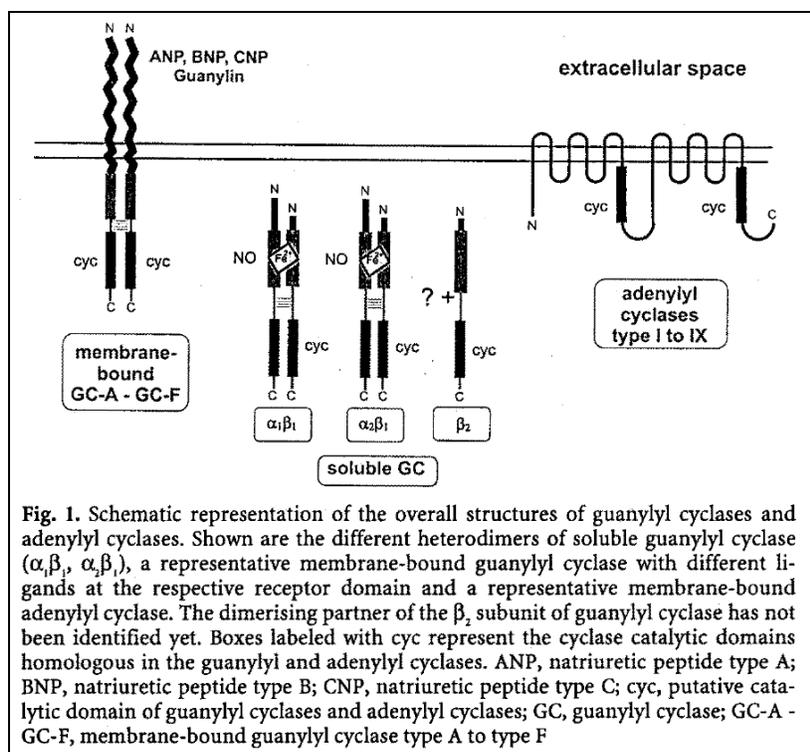
- determination of splenic cell counts; FACScan analyses on a per animal basis to determine subpopulations of the spleen cells
- determination on a per animal basis of serum antibody (IgG, IgM, IgA) titers using a sandwich ELISA
- plaque cell forming assay (PCFA) using spleens of satellite animals

Under the conditions of the studies, immunotoxicity was not apparent.

11 Integrated Summary and Safety Evaluation

BAY63-2521 is an activator of soluble guanylate cyclase, catalyzing the formation of the signaling molecule cGMP. Cyclic GMP is a critical factor in the regulation of cellular functions such as vascular tone, cellular proliferation, fibrosis and inflammation. Soluble guanylate cyclase is ubiquitously expressed and may also be found in platelets and cells of bone marrow lineage such as osteoblasts.

Guanylyl cyclases (GC) exist as soluble (cytosolic) or particulate (membrane associated) enzymes in the same cell, and catalyze the conversion of GTP to cGMP (Waldman and Murad, 1987). Several forms of the membrane-associated enzymes have been identified. (Tremblay et al, 2002). Natriuretic peptides activate several isoforms of membrane bound guanylyl cyclases, while nitric oxide is the only physiologically occurring activator of the soluble isoforms known to date. The designations for the particulate guanylyl cyclases, e.g. GC-B for the type B guanylyl cyclase, appear to be interchangeable with natriuretic peptide receptor, e.g. natriuretic receptor B (NPR-B). The degree of structural similarity between cytosolic and transmembrane GCs is of some interest as an indicator of possible secondary pharmacologic or toxicologic off-target effects.



From Koesling and Friebe, 1999.

In vitro, riociguat has been shown to cause smooth muscle relaxation of arteries, coronary arteries, veins, and corpus cavernosum. This effect has also been demonstrated in arteries taken from nitrate tolerant rabbits. In a Langendorff heart preparation, riociguat decreased coronary perfusion pressure.

In vivo studies have been conducted in anesthetized, normotensive rats, conscious spontaneously hypertensive and normotensive rats and anesthetized and conscious dogs. In these studies, riociguat produced a dose-related decrease in blood pressure. Sensitization or tachyphylaxis was not apparent. In the dog studies, coronary vasodilation was not measured per se. However, coronary blood flow was assessed and the data suggested an increase with riociguat administration.

The testing in models of pulmonary hypertension was unique in that the drug was not administered concurrent with the development of the lung pathology. Rather, hypoxia-induced pulmonary hypertension in mice and monocrotaline-induced pulmonary hypertension in rats was allowed to develop prior to administration of drug.

Riociguat was evaluated for secondary affinities in a battery of radioligand binding assays. Interaction with PDE isoforms was also examined as were particulate guanylate cyclase assays.

The safety pharmacology studies indicated that a single oral dose of riociguat produced a decrease in blood pressure accompanied by a decrease in excreted urine volume and sodium,

decreased gastrointestinal motility, decreased serum glucose and gastric irritation. These effects were apparent in the toxicology studies also. While the degree of effect varied, it is possible that these different phenomena are all manifestations of smooth muscle relaxation with subsequent effects on different organ systems.

An increased time to nocifensive response was also apparent. While a direct neurologic effect can't be ruled out, decreased muscle tone and reduced blood pressure may also cause this apparent effect.

The bone findings may be explained in light of the eNOS-NO-sGC-cGMP-PGK pathway and its involvement in regulating bone homeostasis (Wimalawansa, 2010; Rangaswami et al 2012; Rangaswami et al 2010; Rangaswami et al 2009; Marathe et al 2012). It cannot be determined from the available data if the parathyroid gland is affected

NONCLINICAL TOXICITIES: OVERVIEW

Genetic Toxicology

Under the conditions of the assays there was little evidence of a genetic toxicology associated with BAY63-2521. The assays conducted are summarized in the reviewer's table below.

Reviewer's Summary of Genetic Toxicity Testing for BAY63-2521

Assay	Test system	results
Ames assay (T2071789)	S. typhimurium ± S9 TA1535, TA100, TA1537, TA98, TA102	Negative (exploratory study)
Ames assay (T1072921)	S. typhimurium ± S9 TA1535, TA100, TA1537, TA98, TA102	negative
Cytogenetic screening (T407190)	Chinese hamster V79 cells	No increase in clastogenicity (exploratory study)
In vitro Chromosome aberration (T0072920)	Chinese hamster V79	Slight increase in aberrations that is within historical ranges
Micronucleus assay (T8072919) (T1073218)	Male mice given intraperitoneal injections	Exploratory and definitive studies. Increase in micronuclei that was within the historical control range.
Cytogenetic assay (T9073225)	Male NMRI mice given intraperitoneal injections	No apparent increase in aberrations

Reviewer's Summary of Genetic Toxicity Testing for BAY60-4552

Assay (Study number)	Test System	Results
Ames assay (T4076100)	S. typhimurium±S9 TA1535, TA100, TA1537, TA98, TA102	Negative
In vitro chromosome aberration (T5076101)	Chinese hamster V79 cells	Negative
Mouse micronucleus test (T6076102)	Male mice given intraperitoneal injections	Negative

Carcinogenicity

Two year carcinogenicity studies were conducted in rats and mice. The protocols for both studies were submitted to the Executive Carcinogenicity Assessment Committee (Exec CAC) for comments prior to commencement.

The Exec CAC concurred with the proposed doses of 50, 100 and 200 ppm dietary administration in mice, based on a maximally tolerated dose (mortality occurred 400 ppm with only mild effects at 200 ppm). The final study was adequate as there was neither increased mortality nor evidence of new toxicities. The Exec CAC determined that there was no signal for carcinogenic potential in the mouse study. In mice, all doses of BAY63-2521 produced measurable plasma levels of parent drug. The highest dose tested in mice produced plasma levels of BAY63-2521 from 1-1.6X the mean AUC reported for pulmonary hypertension patients receiving the maximally recommended dose of 2.5 mg t.i.d.

The Exec CAC also concurred with the proposed doses of 5, 10 and 20 mg/kg for the rat study. These doses were also based on maximally tolerated dose as demonstrated by decreased body weight gain. The final study was adequate as there was neither increased mortality nor evidence of new toxicities. The Exec CAC determined that there was no signal for carcinogenic potential in the rat study.

In rats, all doses of BAY63-2521 produced measurable plasma levels of parent drug. The highest dose tested in rats produced plasma levels of BAY63-2521 from 1.4-2.2X the mean AUC reported for pulmonary hypertension patients receiving the maximally recommended dose of 2.5 mg t.i.d.

Reproductive and Developmental Toxicity

BAY63-2521

Fertility and Early Embryonic Development in Rats: A dose-related prolongation of time to insemination was reported, possibly due to muscle relaxation and decreased blood pressure. No other effect on fertility in either sex was apparent in the reported data.

		MEAN VALUES FOR TIME TO INSEMINATION			
		0 MG/KG	3 MG/KG	10 MG/KG	30 MG/KG
Mating Days until Day 0 pc	MEAN	1.7 d	2.3	2.5	3.9
	S.D.	1.43	2.74	3.43	3.90
	N	24	24	24	24
	p-value	0.082			

Statistical key: d=Dunnett's-test

Embryofetal Development in Rats: Riociguat was administered at doses of 0, 1, 5, and 25 mg/kg/day, achieving overt toxicity. The high dose dams showed decreased food consumption throughout treatment, polyuria/polydipsia, and decreased weight gain. The mid-dose group showed a transient decrease in food consumption at the start of treatment.

Uncorrected body weight was decreased 12% ($p < 0.05$) at the mid-dose, and 41% ($p < 0.01$) at the high dose. When the body weight was corrected for weight of the uterus, only the high dose group showed reduced weight compared to the control animals (37%, $p < 0.01$). The weight of live fetuses was reduced in the high dose group by 10% ($p < 0.01$) compared to the control pups.

Postimplantation loss was significantly increased at doses ≥ 5 mg/kg (2.4x the control value, $p < 0.05$ to 3.1 X the control value, $p < 0.01$). Early resorptions were also increased at 25 mg/kg (7.3% of implantations vs 0 in the control group, $p < 0.01$). The incidence of ventricular septal defect was significantly increased at 25 mg/kg (HD). The drug treated litters showed increases in incomplete ossification of several bones. The fourth sacral vertebral arches were the only bones showing a statistically significant dose response. The incidence of wavy ribs was increased in all drug-treated groups without showing a dose-response.

Reviewer's Summary of malformations and plasma drug values

	Dose mg/kg			
	0	1	5	25
Ventricular septal defect	5(5)	4(2)	5(4)	22**(10)
Sum of malformations of heart +/- great arteries in %	8(7) 3.5(37)	8(5) 3.6(26)	7(4) 3.3(19)	23(10) 10.7(59)
Sum of wavy ribs	6(5)	16(14)	18*(17)	14(13)
Sacral vertebral arches incompletely ossified 4 th right	3(3)	13*(11)	14*(13)	21**(19)
Sacral vertebral arches incompletely ossified 4 th left	7(6)	12(10)	20*(19)	25**(23)
AUC ₀₋₂₄ $\mu\text{g hr/l}$		627	2197	10626
AUC ₍₀₋₂₄₎ $\mu\text{g hr/l}$ 4 week toxicology study for comparison		1.5 mg/kg 370	5 mg/kg 1244	30 mg/kg 6786

* $p < 0.05$, ** $p < 0.01$ Numbers in parentheses () indicate litter incidence.

Embryofetal Development in Rabbits: The rabbits were very sensitive to the pharmacology and extended pharmacology of riociguat. While all females mated, incidence of abortions increased with increased dose. Postimplantation loss and late resorptions were significantly ($p < 0.01$) increased in the HD group. There was no clear signal for any kind of increase in variations or terata.

Summary of gestation and intrauterine development parameters in rabbits treated with riociguat

	0 mg/kg	0.5 mg/kg	1.5 mg/kg	5 mg/kg
Fertility index(females w/ implantations)	95% 19	100 19	95 19	19 95
Gestation index (females w/ viable fetuses)	100 19	100 19	84 16	32 6**
Implantations: % corpora lutea	97.0	90.5	95.3	87.9
Postimplantation loss: % of implantations	5±0	4.6±1.0	2.5±1.00	31.4±0.00**
Late resorptions: % of implantations	5±0	4.6±1.0	2.5±1.00	31.4±0.00**
Preimplantation loss:% of corpora lutea	3.0	9.5	4.7	12.1
Placental weight in g	4.16	4.28	4.79*	5.09*
BAY63-2521 AUC ₀₋₂₄ µg.hr/l		5064	18898	62467
BAY60-4552 AUC ₀₋₂₄ µg.hr/l		1327	6014	17210

*p<0.05 , **p<0.01 compared to control

The Late Gestation and Post-Natal Development in Rats (Segment III) showed few clear effects. The F₀ dams in the Segment III study received 0, 1.5, 5 and 15 mg/kg/day of BAY 63-2521 from gestation day (GD) 6 to post-partum (PP) day 21. The duration of gestation showed statistically significant increases in the low dose (0.35 day, p<0.05) and in the high dose (0.62 day, p<0.01). The toxicological significance, if any, is not clear as this is an expected effect of the pharmacology of a drug that causes smooth muscle relaxation and lowers blood pressure.

The pup data from the Segment III study showed no mean difference in time to achievement of balanopreputial separation and vaginal opening across the groups. However, the mean weight of the drug-treated groups was less than that of the control groups when developmental landmarks were achieved. The lack of dose dependence suggests this is normal variability rather than slightly precocious development.

Mean age in days of pups reaching the criterion

	0mg/kg	1.5 mg/kg	5 mg/kg	15mg/kg
Number of pups	21	22	22	21
Pinnae detachment	2.60d	2.18d*	2.31d	2.02d**
Development of fur	9.58d	9.30d	9.37d	9.45d
Incisor eruption	10.44d	10.06	10.06	9.88
Number of pups	24	24	24	24
Balanopreputial separation	43.6	43.2	43.8	43.8
Body weight on completion	206.7	203.8	200.2	202.1
Number of pups	24	24	24	24
Vaginal opening	32.2	32.3	33.0	32.8
Body weight on completion	102.0	100.2	105.6	100.9

There were no obvious trends in the motor activity data. The F1 generation necropsy was restricted, as is usual, to gross observations. There were no long bone measurements which might have been informative given the histologic findings from the neonatal rat study. As is usual for a Segment III study, there was no histological analysis.

The F1 generation used for the fertility and mating evaluation showed few significant findings. The MD and HD groups had a higher number of stillborn pups. There were 5 stillbirths out of 258 pups (1.9%) in the MD and 9 out of 251 HD pups (3.6%) compared to 2 pups out of 311 total control pups (0.6%). It is not clear how many litters were affected.

Mean age in days of pups reaching the criterion

	0mg/kg	1.5 mg/kg	5 mg/kg	15mg/kg
Number of pups	21	22	22	21
Pinnae detachment	2.60d	2.18d*	2.31d	2.02d**
Development of fur	9.58d	9.30d	9.37d	9.45d
Incisor eruption	10.44d	10.06	10.06	9.88
Number of pups	24	24	24	24
Balanopreputial separation	43.6	43.2	43.8	43.8
Body weight on completion	206.7	203.8	200.2	202.1
Number of pups	24	24	24	24
Vaginal opening	32.2	32.3	33.0	32.8
Body weight on completion	102.0	100.2	105.6	100.9

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The F1 generation used for the fertility and mating evaluation showed few significant findings. The MD and HD groups had a higher number of stillborn pups. There were 5 stillbirths out of 258 pups (1.9%) in the MD and 9 out of 251 HD pups (3.6%) compared to 2 pups out of 311 total control pups (0.6%). It is not clear how many litters were affected. Toxicokinetics were not available for this study.

Pilot Juvenile Animal Study: The juvenile animal pilot study consisted of Wistar rat pups given once daily oral doses of 0, 3, 10 or 30 mg/kg/day of BAY63-2521 starting from post-natal day (PND) 6 for 14 consecutive days. The pups were then euthanized. Satellite animals were used for toxicokinetics. High dose animals of both sexes showed increased normalized weight of brain, heart, kidneys and spleen and decreased weight of liver. Histopathological effects in the femur and tibia were reported for doses of BAY63-2521 greater than or equal to 3 mg/kg in males and greater than or equal to 10 mg/kg in females.

Juvenile Animal Study: This was actually 3 separate studies using the same doses (0, 0.3, 1 and 3 mg/kg/day) for different periods of time.

Study number	Dosing period	Recovery
T6081458	14 weeks Satellite animals for tk	No recovery
T4082428	4 weeks	4 weeks
T4082428	4 weeks	No recovery
T8082404	13 weeks	8 weeks

The results were merged into one report. The high dose in this definitive study, 3 mg/kg/day, the low dose from the pilot study, did not achieve the same AUC as the pilot study.

	Dose of Riociguat (mg/kg/day)				
	0.3	1	3	10	30
Juvenile pilot study AUC _{0-last} µg hr/l			1660	5025	10681
C _{max} µg/l (pilot study)			371	915	2944
Definitive juvenile study AUC _{0-last} µg hr/l	70.4	206	573		
C _{max} µg/l (definitive study)	16.7	42.0	129		

All three of the studies showed varying degrees of electrolyte effects, in particular Ca, P, K, and Mg. Some of the changes persisted into the drug-free recovery periods. Possible causes include pharmacologic effects on the bone (although no histopathologic changes were reported), effects on renal function, parathyroids or muscle, or the variability of growing animals. A specific cause cannot be determined from the available data.

The urinalysis indicated decreased excretion of protein and urea when normalized for volume. There were no apparent effects reported in the hematology or functional observational battery.

There were few findings in the developmental assessments. There was a 1.5 day delay in balanopreputial separation at the high dose of 3 mg/kg/day.

A consistent finding across all three studies was statistically significant increases in testicular weight at doses greater than or equal to 1 mg/kg/day. The weight of epididymides was increased in two studies and decreased in the 4 week dosing study. There is no explanation offered for the weight changes.

The histopathology report was concise. Bones were not mentioned for study T8082404. Study T6081458 noted atrophy of testes and epididymides with aspermia for one high dose male. Both high dose and mid-dose males showed grossly enlarged livers with no corresponding histopathology. No bone effects were reported. The report for T4082428 stated that one high dose male showed erythroid hypoplasia of the bone marrow but “no treatment-related findings in the region of the femur and sternum.”

BAY60-4552

Developmental and reproductive toxicity studies were conducted for direct administration of BAY60-4552, the major metabolite of riociguat.

The fertility and early embryonic development study in rats had few findings of toxicological significance. There was no apparent effect of drug treatment on the number of estrus cycles recorded per observation period. However, we do not know duration or regularity of the cycles. Prolonged diestrus was recorded for the MD and HD groups, but without apparent dose-dependence. Overall, there was no obvious effect of drug treatment on either male or female fertility.

Fetal development (Segment II) was studied in both rats and rabbits. BAY60-4552 tested up to doses causing maternal toxicity in rabbits did not cause adverse fetal effects. A rat dose-ranging study and the definitive study showed the thyroid (missing or decreased size), skeletal system (delayed or incomplete ossification in numerous bones, wavy ribs) and heart (ventricular septal defect) at doses of 50 mg/kg/day of BAY60-4552. Post-implantation loss was doubled in this dose group (7.4% of implantations versus 3.9% of the control animals, $p < 0.086$). Only those bones also affected by the parent drug are summarized in the reviewer's table below.

	Dose of BAY60-4552 mg/kg			
	0	2	10	50
Ventricular septal defect	0	0	0	1(5)
Thyroid gland missing	0	1(5)	0	5(25)
Sacral vertebral arches 4 th left incomplete ossification	9(8)	15(10.8)	7(5)	34**(25.2)
Sacral vertebral arches 4 th right incomplete ossification	10(8.8)	13(9.4)	7(5)	30*(22.2)
Wavy ribs	2 (1.8)	4 (2.9)	1(0.7)	17**(12.6)

Numbers in parentheses are the percent litters affected.

The rat Segment II study included severe maternal toxicity that included sudden death of 2 high dose females. The clinical signs reported were piloerection, gasping breathing, sunken flanks and staggering gait. In light of this, the bone effects reported in this study are most likely a generalized delay due to the maternal effects (Carney and Kimmel, 2007; Augustine-Rauch, 2007).

Toxicokinetics were determined from satellite rats. Comparing these values to those from a 13 week repeat dose rat study (PH35976, reviewed in this amendment), it appears that pregnant rats show similar plasma values compared to non-gravid rats given the same doses.

Reviewer's comparison of BAY60-4552 plasma levels in gravid versus non-gravid female rats

	Non-Gravid Female Rats (PH35976)			Gravid Rats (PH35958)		
	Repeat dose			Repeat dose		
	3mg/kg	10 mg/kg	30 mg/kg	2 mg/kg	10 mg/kg	50 mg/kg
AUC ₀₋₂₄ µg.hr/l	4083	12244	41509	2545	12947	54548
C _{max} µg/l	638	1714	5950	299	1415	5606

Bone Effects

The bone effects were identified by histopathological analysis and reported in mice and rats only. There were no clear effects on bone length or quality of movement. There were variable reports of changes in the quantity of movement. The full reviews of the studies where these effects are reported are filed in DARRTS as listed in the table of contents of this review.

The different study reports provided different degrees of detail for the histologic lesions. The report for the 4 week oral gavage study in rats provided one of the most detailed descriptions. In males given riociguat at 15 mg/kg/day or more, the articular cartilage showed a distinct thickening of the hypertrophic zone of the femoral/tibial growth plates (physis) with irregular resorption zone and an increased thickness of the trabeculae of the primary spongiosa. In two 15 mg/kg and 1 recovery male, a minimal and focal thickening of the chondral part of the growth plate without distal involvement of the bone trabeculae was reported. The pathologist's report notes that :

Based on the literature, the lesions are considered to be related to the mode of action of the compound class. Increased intracellular cGMP levels are known as a strong stimulus for osteoblast activation.

The sponsor's cited reference: Mancini L, Moradi-Bidhendi, Brandi ML, MacIntyre I. Nitric oxide superoxide and peroxynitrite modulate osteoclast activity. *Biochem Biophys Res Commun.* 1998 Feb 24;243(3):785-90.

Longer duration studies, ending when the rats were older, did not report effects on the articular cartilage. The 13-week oral gavage study in rats showed slight increases in serum calcium with a corresponding increase in phosphorus (P) levels (a change suggestive of osteolysis). The histopathology showed an increased ratio of hematopoietic bone marrow to fat in femoral epiphysis in both sexes ≥ 3 mg/kg. This was also present in sternal marrow. No other changes in bone morphology were noted. However, the 26 week oral gavage study in rats reported hyperostosis of the femur in males at doses ≥ 10 mg/kg (AUC 2283 $\mu\text{g}\cdot\text{hr/l}$) and in females at the high dose of 40 mg/kg (AUC 13927 $\mu\text{g}\cdot\text{hr/l}$).

To examine the effects of riociguat on post-natal bone development, the sponsor conducted the standard late-gestation through weaning study (Segment III), a pilot juvenile animal study and a definitive juvenile animal study.

The Segment III, post-natal development study, using the standard indirect drug administration, did not show any bone effects. No histopathology was done. Toxicokinetic analysis for the pups was not conducted.

Rat pups in the pilot juvenile animal study were given oral doses of riociguat starting on post-natal day 6, administered daily for 14 days. The animals were euthanized on post-natal day 19. Histology of the femur and tibia showed disorganization of epiphyseal bone and marrow cavity with thickening of the trabecular bone and resultant decrease in the marrow cavity and marrow

cells. “Activated osteoblasts” and multinucleated osteoclasts were detectable. Hyperostosis and remodeling were reported for metaphyseal and diaphyseal bone in both sexes. These effects were reported for doses of BAY63-2521 greater than or equal to 3 mg/kg in males and ≥ 10 mg/kg in females.

The definitive juvenile animal study used doses of 0, 0.3, 1 and 3 mg/kg/day and was conducted in several sub-studies. The results of the sub-studies were combined into one report. Dosing was initiated at post-natal day 6 and continued for either

- 4 weeks with a 4 week drug-free recovery period,
- 14 weeks with no drug-free recovery, or
- 13 weeks with an 8 week drug-free recovery phase

The highest dose used in this study was at or below the LOAEL from the pilot study. While no histomorphological effects were reported for bone, there were increases in circulating K^+ and Ca^{2+} and a slight decrease in P during the dosing phases in both sexes of animals. Serum albumin concentrations did not change appreciably. This study is also noteworthy for the marked decrease in riociguat AUC from the beginning to the end of the dosing phase.

To characterize the bone effects in mature rats, the sponsor conducted a toxicology study with 40 male rats per dose (0, 10, and 50 mg/kg subsequently reduced to 25 mg/kg), aged 17 weeks at the start of dosing. Subgroups consisting of 10 males per group were euthanized after 4, 8, 13 or 26 weeks to provide time course information. The sponsor examined sternum, femur, humerus, vertebrae, and costae. Female rats can naturally develop osteopenia and might have been useful to see if there was an exacerbation of a naturally occurring phenomenon. Bone mineral density (BMD) was measured at several different locations on the femur using peripheral quantitative computed tomography (pQCT). The BMD cortical (diaphysis) showed a dose-related increase at 4 weeks ($p < 0.0006$), 8, 13, and 26 weeks ($p < 0.0001$ at each). At study day 183, alkaline phosphate was increased 21% relative to control (n.s.) and serum phosphorus was decreased 17% (n.s.). The isoform of alkaline phosphatase measured was not clarified.

Histopathology indicated slight ($< 5\%$, n.s.) increases in cortical bone mineral density in the diaphysis of the femur in the high dose group from week 4 onwards. The sponsor states that the bone effects were probably not related to treatment and that one high dose animal with profound effects most likely was affected by underlying metabolic disease. The sponsor did not elaborate on the reasoning for this point.

The pathology report for this study contained very minimal, one word descriptions of findings, reported in the summary tables for sternum and femoro-tibial bones. These findings include: osteoclasts increased and bone resorption increased. Findings for the humerus, vertebrae and costae include: fibrosis, necrosis, osteoclasts increased, and bone resorption increased. The one word descriptions found in the summary tables are not sufficient information for risk assessment purposes. An integrated assessment or discussion of the findings was not apparent. The sponsor has been asked to provide photomicrographs to illustrate the range of effects and to provide a detailed description and assessment of the findings in the vertebrae, costae, and humeri by a veterinary pathologist with expertise in bone pathology. The information was provided in SDN 019 (CDER stamp date May 30, 2013).

Of interest in the above report, there were 3 unscheduled euthanasias of note from the high dose group. These three animals showed bone pathology. In the sponsor's words:

In addition, the following histopathological changes were present in all bones (femur, patella, tibia, sternum, humerus, vertebrae and costae) examined: slight to marked bone resorption with a slight to marked increase in the number of large osteoclasts, and slight to moderate fibrosis (osteosclerosis). Intra-articular hemorrhages, fracture of the patella, sub-patellar hemorrhages and a moderate mixed cell infiltration in adjacent soft tissue were present in the femoral tibial joint. In the epiphysis of the humerus minimal necrosis was present.

The findings were attributed to metabolic disease.

Histomorphological effects were also noted in several studies of BAY60-4552. The high dose (100 mg/kg) animals of both sexes given BAY60-4552 orally for 4 weeks showed histomorphological changes to the femur. These changes were reported as thickening of the growth plate and disorganized trabecular bone. A 13-week study also showed changes primarily in females (and 1 male) of the high dose group (50 mg/kg). The changes were listed as being in the femur and included thickened growth plate, disorganized trabeculae, and diffuse hyperostosis. The 1 affected male was reported to have focal hyperostosis of the femur.

Histomorphological effects on bone were not reported for the dog studies. Because the rate of bone turnover in dogs is faster than humans but slower than rats, it is not likely that there would be effects detectable by light microscopy. Also, the studies were conducted as routine toxicology studies. While the sternum, tibia and femur were sampled in the dog studies, there was no description of how samples were chosen for histopathology and no indication that other bones were examined. A publication by Chennekatu et al. (1996) describes a three-year study of alendronate administration to dogs. The investigators examined the costochondral junction of the ribs, femur, tibia, and vertebral bone. They reported a dose-dependent delay in bone remodeling in the ribs of dogs treated with alendronate, but no similar changes in the tibia and no changes in the structural properties of femoral or vertebral bone.

Some effects on serum electrolyte levels may indicate modulations in bone metabolism. In the 4 week dog study, the high dose males showed decreased serum calcium relative to controls at both 2 weeks (-9%, ns) and 4 weeks (-5%, ns). This decrease was not apparent in the recovery period. The serum P levels also decreased; therefore the ratio of Ca:P changed slightly, but probably not to a biologically significant degree. Other studies in rats with administration of parent drug or BAY60-4552 also showed changes in serum Ca and or serum P levels. This may suggest a number of possible explanations: normal variability, renal alterations, bone alterations, and parathyroid changes. The small size of the parathyroid and the proximity to the thyroid gland make it difficult to study histopathologically. The usual diet of laboratory animals is rich in calcium and other minerals and in conjunction with the body's mechanisms for maintaining the physiologic ratio of calcium to phosphorous, could make it difficult to see clinical chemistry evidence of a metabolic derangement.

Reviewer's Summary of Bone Effects and Associated Plasma BAY63-2521

species	Duration Report #	comment	AUC µg.hr/l
BAY63-2521			
mouse	2 week PH 34519	thickening of growth plates in femur at 800 ppm (both sexes)	10767
rat	4 week PH 33408	Serum calcium increased, thickening of femur growth plates in males ≥ 15 mg/kg females: Serum calcium increased ≥ 5 mg/kg	3795 1244
rat	13-week PH 34877	increased diaphyseal bone remodeling and hyperostosis in both sexes. Males females increased ratio of hematopoietic to adipose tissue in the marrow was reported in both sexes at ≥ 3 mg(LD) slight change in Ca:P ratio in MD+HD females at end of study	34058 25786
rat	26 week study PH 35002	Diaphyseal remodeling and hyperostosis Males ≥ 10 mg/kg Females 40 mg/kg	2283 13927
rat	Pilot neonatal rat study, 14 days PH36257	disorganization of femur and tibia epiphyseal bone and marrow cavity with thickening of the trabecular bone and decrease in the marrow cavity and marrow cells. Hyperostosis and remodeling of metaphyseal and diaphyseal bone. Males ≥ 3 mg/kg Females ≥ 10 mg/kg	1660 5025
rat	Definitive neonatal rat study:	No bone findings Day 1 AUC _{0-t} for the 3 mg/kg (high dose) group Day 69 AUC _{0-t} for the 3 mg/kg (high dose) group	Males females 3470 4400 573 794
rat	26 week mechanistic study A43289	The only study to examine humerus, vertebrae, costae along with femur and tibia. Doses of 0, 10 and 50/25 mg/kg. For high dose animals: Increased osteoclasts, increased bone resorption, fibrosis, necrosis reported for sternum, femur/tibia, humerus, vertebrae. No necrosis reported for costae.	No tk provided for this study

Reviewer's Summary of Bone Effects and Associated Plasma BAY 60-4552 Levels

BAY60-4552			
rat	2 week PH36027	Hyperostosis of femur, males 140 mg/kg	44694
rat	4 week PH34599	Thickening of the femoral and tibial growth plates and disorganized trabecular bone in high dose males and females 100 mg/kg	64206
rat	13 week PH35365	thickened growth plates, diffuse hyperostosis: 1-7/10 HD females focal hyperostosis 1/10 HD male Day 1 Day 72	Males females 37503 54000 51428 82697
human	2.5 mg t.i.d. sponsor estimated based on multiple dose study #12166 in pulmonary hypertension patients		4161

The carcinogenicity studies did not report changes similar to those found in the shorter studies. It is not clear if the early changes are completely remodeled and therefore no longer apparent.

Summary of reported bone effects in the 2-year rat carcinogenicity study

	Dose mg/kg			
	0	5	10	20
Males				
Femur: osteodystrophy	9/48 (19)	15/49 (31)	12/47 (26)	11/42 (26)
Sternum: cartilage degeneration	22/49 (45)	19/49 (39)	22/47(47)	16/42(38)
Bone:				
Odontoma	0/48	1/49	1/47	0/42
Osteosarcoma	0/48	0/49	0/47	1/42
osteoma	0/48	0/49	0/47	0/42
Females				
Femur:osteodystrophy	0/49	1/49	2/50	1/50
Sternum: cartilage degeneration	18/49	15/48	20/47	15/50
Ovaries				
Tubulostromal adenoma	0/26	0/35	1/35	1/36
Benign luteoma	0	0	0	1/36
Benign thecoma	0	0	0	1/36
Granulosa CB tumor	0	0	2/35	1/36
Sex cord stromal MB tumor	0	0	1/35	0

Numbers in parentheses () are percentage incidence.

Information Provided in SDN 019

The sponsor divided the response into 4 sections. The sponsor's categories are shown next page.

1. Morphological changes seen in the skeleton of an isolated animal in the 26-week mechanistic study and their interpretation in the context of riociguat-related bone lesions.
2. Compilation of photomicrographs from the pilot study in juvenile animals illustrating the lesions observed with regard to their morphological features as well as with regard to their severity spectrum.
3. Compilation of photomicrographs from the repeat-dose studies in adolescent animals illustrating the lesions observed with regard to their morphological features as well as with regard to their severity spectrum.
4. Considerations on human relevance of bone findings in adolescent and juvenile rats.

Section 1.

The sponsor provided this restatement of the mechanistic study and a correction to the stated methodology:

As riociguat induces treatment-related findings in the long bones of adolescent, growing rats, a chronic study in full-grown rats with treatment duration of up to 26 weeks was performed. The animals were treated at 0, 15 and 25 mg/kg riociguat. The high dose group was initially started at 50 mg/kg however, due to clinical symptoms and especially body weight gain effects which could interfere with bone metabolism, the dose was reduced to 25 mg/kg after 9 days of treatment. 10 animals each were sacrificed at day 1 (to obtain base line values for bone marrow density measurement) as well as after 4, 8, 13 and 26 weeks of treatment (named interim sacrifices K1 to K5 in the Pathology Report).

The following bones were fixed for histopathological examination:

- Femur (incl. proximal tibia and knee joint)
- Humerus
- Sternum
- Vertebrae
- Costae

Of note, erroneously, in the original report, a restricted panel of bones examined histopathologically was mentioned to be examined. However, all bone samples of all animals were investigated.

Histopathology was performed on the bone specimen according to the following schedule

Interim Sacrifice	0 mg/kg					15 mg/kg					25 mg/kg				
	1	2	3	4	5	2	3	4	5	2	3	4	5		
Sternum	X	X	X	X	X	X	X	X	X	X	X	X	X		
Femur	X	X	X	X	X	X	X	X	X	X	X	X	X		
Humerus	X	X	X	X	X	X	X	X	X	X	X	X	X		
Vertebra	X	X	X	X	X	X	X	X	X	X	X	X	X		
Costa	X	X	X	X	X	X	X	X	X	X	X	X	X		

Primary histopathological examination was performed by an ESVP board-certified veterinary pathologist with long-standing history (> 10 years) in toxicological pathology.

The Division had requested that the slides be examined by a pathologist with expertise in bone. The slides from one animal were re-examined by a bone expert.

In consequence of the information request by the Agency, animal 221 showing morphological bone changes, was reviewed by Dr. Matthias Rinke, Head of Pathology, Bayer HealthCare AG and member of the INHAND (INternational **H**ARmonization of Nomenclature and **D**iagnostic criteria) Skeletal (bone, cartilage, tooth) working group. The INHAND task force formed by members of international STPs is currently establishing controlled terminology for neoplastic and non-neoplastic histopathological findings to support the FDA SEND initiative.

Thus, both the study pathologist and the reviewing pathologist have deep knowledge on bone morphology and bone-associated pathology.

As outlined in the original report, none of the animals surviving until the end of their scheduled treatment duration showed any bone-related findings.

Three high dose animals died or were sacrificed prematurely during the course of the study. Animal 225 showed a purulent pleuritis, indicating a gavage error as cause of death on day 9 of the study.

Animal 203 was found dead on day 21. This animal was emaciated but no obvious cause of death was discernible.

Whereas animal 225 (Figure 10) and 203 showed no bone lesions at all, animal 221 revealed alterations of the bone in samples all investigated which were described as bone resorption, increased osteoclasts, fibrosis/osteosclerosis and inflammation in the narrative of the pathology report.

The sponsor then described the lesions for animal 221. The main lesion was a fracture or rupture of the patella from the patellar tendons with intra-articular hemorrhage and thrombosis/necrosis of the patellar bone marrow. The sponsor described a moderate mixed cellular infiltration of the tendons and surrounding musculature as evidence that this happened in life and not post-mortem.

The sponsor then described bone resorption characterized by numerous osteoclasts and reduced bone matrix occurred especially in the subcartilage zone of the epiphysis of the femur but way less in the tibia where they were restricted to the region of the activated synovialis. In contrast to the lesions seen after treatment with riociguat, no hyperostosis of cancellous or diaphyseal bone was observed. Furthermore, growth plates were normal.

Another thrombus was noted, in the humerus:

In the epiphysis of the humerus, a wide zone of bone marrow necrosis was observed, most likely due to the presence of a thrombotic event while the bone marrow of the shaft was completely normal. The reported moderately increased number of osteoclasts, bone resorption and fibrosis could not be verified and were restricted to the area of necrosis in the epiphysis (Figure 7). Also the findings reported for sternum, costae (Figure 8), and vertebrae (Figure 9) appear to be less pronounced when compared to the massive local lesions in the injured knee joint.

In conclusion, the lesions described for animal 221 differ distinctly from the treatment-related lesions described in adolescent riociguat-treated animals. In contrast to animal 221, in riociguat-treated, adolescent animals, an increase of bone mass with hyperostosis in cancellous as well as in diaphyseal bone is seen.

In addition to the fact that the findings are morphologically different to those described as riociguat-related, that they were isolated, restricted to a single animal and not seen in animals sacrificed in close temporal relationship to the respective animal they are considered not to be treatment-related.

Overall, the primary origin of the lesions seen exclusively in the deceased animal 221 has to remain open; a treatment-relationship can be excluded since the findings are clearly different in morphology from those described as riociguat-related bone effect.

Reviewer's response:

Animal 221 was on a high dose treatment of 50 mg/kg/day for 8 days before unscheduled mortality occurred. There is a greater level of detail in this Information Response than the original description. From the report A43289, here is the sponsor's discussion of animal 221:

Histopathological examination of the high dose animal no. 221 which was sacrificed in moribund condition on day 8, revealed bone resorption, increased numbers of large osteoclasts, and fibrosis (osteosclerosis) in various bones. Biochemical examinations supported the mentioned bone findings by increases in ALP, inorganic phosphate, calcium, magnesium, as well as decreases in chloride and iron.

The pronounced histopathological bone lesions (resorption, fibrosis/osteosclerosis) noted in one high dose animal sacrificed for animal welfare reasons on day 8 were not considered to be toxicologically relevant since no comparable lesions were observed in further animals of this study or in previous studies with BAY 63-2521 at comparable dose levels (PH-33454).

In animal no. 221 all lobes of the lung had focal reddish discoloration and from the cut surface foamy outflow from the bronchioles were present. Histologically, a slight alveolar edema and minimal emphysema was present.

In addition, the following histopathological changes were present in all bones (femur, patella, tibia, sternum, humerus, vertebrae and costae) examined: slight to marked bone resorption with a slight to marked increase in the number of large osteoclasts, and slight to moderate fibrosis (osteosclerosis). Intra-articular hemorrhages, fracture of the patella, sub-patellar hemorrhages and a moderate mixed cell infiltration in adjacent soft tissue were present in the femoral tibial joint. In the epiphysis of the humerus minimal necrosis was present.

The pronounced histopathological lesions (bone resorption, increased osteoclasts and fibrosis/osteosclerosis) in femur, tibia, patella, humerus, costae and vertebrae, noted in one high dose animal (no.221) which was sacrificed for animal welfare reasons on day 8 were probably of low or no toxicological relevance since no comparable lesions were observed in any further animal. In addition, since the known hyperostosis observed in adult rats is based on a reverse effect (increased osteoblasts and/or reduced osteoclasts), a relationship of the mentioned findings to treatment is unlikely. Rather, it is likely that in that animal a metabolic disease was present.

The patella is noted as having a lesion but “fracture” was not elaborated upon.

The sponsor does not dismiss the possibility of a treatment related effect but notes that the lesions in animal 221 are somewhat different than those reported in other studies. However, thrombi (cardiac) were reported for high dose treated males in the two-year carcinogenicity study. Also, because so few bones were examined in the other toxicology studies, it cannot be definitively stated that these lesions are in fact unique to this study and this animal. It seems a unique coincidence to have bone lesions in an animal treated with the high dose of drug known to affect bone metabolism and to have those lesions be unrelated to the drug. It is also of some concern that the report has several inconsistencies in methodology. For example, the text on page 16 states that

5.1.3.3.3 Histological examination

In Table 5-2 organs to be weighted and organ/tissue sampling, fixation and processing are listed. All tissue samples were fixed in neutral buffered formalin (Fo), except femur, right and humerus right which were fixed in alcohol fixative.

On page 17, several more bones are listed:

Table 5-2: Organs selected for weighing and histological examination

Organ	Histol. examination				Organ	Histol. examination		
	K5	K4	K1/K2/ K3	Group		K4/K5	K1/K2/ K3	Group
	W	1,3	1,3			1,2,3	W	
Kidneys	yes	P	Fo	Fo	Humerus		P	P
Thyroid glands with parathyroid glands	yes	P	Fo	Fo	Vertebrae		P	P
Adrenal glands		P	Fo	Fo	Costae		P	P
Sternum (bone marrow)	yes	P	Fo	Fo	Femur right		E	E
Femur with Tibia (incl. joint bone marrow)		P	P	P	Humerus, right		E	E
		P	P	P	Macroscopic findings (if necessary for diagnostic evaluation)		P	P

K1 = scheduled sacrificed animals on day 1
 K2 = scheduled sacrificed animals at the end of the 4 week treatment period
 K3 = scheduled sacrificed animals at the end of the 8 week treatment period
 K4 = scheduled sacrificed animals at the end of the 13 week treatment period
 K5 = scheduled sacrificed animals at the end of the 26 week treatment period
 W = weight determination
 P = processed and examined histologically
 E = fixed for bone densitometry measurement
 Fo = fixation of organ/tissue samples, not processed for histological examination

In the current information amendment, the sponsor states that *all* bones were examined, not just the ones listed.

The methodology of the original report states that the pathology process was subject to a formal cross-check with a second pathologist. In the current Information Response description of the lesions, the sponsor states:

“the reported moderately increased number of osteoclasts, bone resorption and fibrosis could not be verified and were restricted to the area of necrosis in the epiphysis.”

The sponsor does not clarify the statement “could not be verified.” There was no statement of how disagreements or unverifiable findings were to be resolved.

5.1.3.3.4 Pathology cross check
 This study was subjected to a formal cross-check (cross-checking pathologist: DR. RAINER ERNST). The cross-check comprised the femur from all animal at the high-dose after 26 weeks and the femur from two animals in the high-dose group at 4, 8 and 13 weeks. Appendix 3 reflects the consensus diagnosis of the pathologists.

A final question is how a patellar fracture happened. A traumatic cause (e.g. rough handling) would be likely to have a tibial avulsion. In this case, the photomicrograph indicates that the tendon lesion is on the femoral side.

Section 2: Range of severity of lesions

The sponsor provided photographic examples of the range of severity of the lesions. In some cases, not all grades of lesions were represented. For example, the scales apparently ran from grade 1 (minimal) to grade 5(extensive/massive). The pictures for lesions of the tibial

metaphysis showed grade 2(slight) and grade 4 (severe) only. The pictures for lesions of the femur diaphysis showed grades 2(slight), 3(moderate) and 4(severe).

Section 3: Range of severity of lesions in adolescent animals

The sponsor provided photographic examples of the range of severity of the lesions noted in the adolescent rats.

Section 4: Sponsor's considerations of human relevance

The sponsor begins with a recapitulation of the species and studies in which changes were seen and the various exposure margins based on ratios of unbound drug.

The sponsor proposes that the absence of riociguat-associated bone findings in dogs, mice and adult rats, suggests that the drug induces morphological changes only during the phase of bone growth and rapid remodeling. In other words, the metabolic pathways are activated to beyond physiologic levels only during rapid growth. It was also noted that these changes occur at exposure levels close to therapeutic exposure.

Thus, the Sponsor concludes that one cannot exclude a risk for children treated chronically with riociguat. Although the clinical relevance of the findings in the bones of rapidly growing young rats is not fully understood, the age-dependency as well as the small therapeutic index shown in preclinical studies along with the physiological mechanism of the NO-sGC-CMP-PKG pathway suggests that children undergoing rapid and pronounced skeletal development may be susceptible for riociguat-related bone effects.

For adult patients the situation is different: data from the chronic rat study showing more mature bone structure and less active remodeling at about 2-fold human exposure and no morphological changes after 2-year treatment at up to 9-fold human exposure suggest that after full cessation of bone growth, remodeling stops, and bone homeostasis normalizes. In this context, it is of particular note that also in the spinal column, bone known to be of major importance in patients suffering from osteoporosis, no morphological were seen.

The sponsor's interpretation is that the bone findings are to be expected as related to mode of action. This is reasonable s based on the increase in cGMP produced by riociguat and the subsequent activation of Protein Kinase G (PKG). The sponsor cites five publications to support the involvement of the eNOS-NO-sGC-cGMP-PGK pathway in the regulation of bone homeostasis.

Sponsor's References

1. Wimalawansa S. Nitric oxide and bone. *Ann NY Acad Sci.* 2010;1192:391-403.
2. Rangaswami H, Schwappacher R, Tran T, Chan GC, Zhuang S, Boss GR, et al. Protein kinase G and focal adhesion kinase converge on Src/Akt/beta-catenin signaling module in osteoblast mechanotransduction. *J Biol Chem.* 2012 Jun 15;287(25):21509-19.
3. Rangaswami H, Schwappacher R, Marathe N, Zhuang S, Casteel DE, Haas B, et al. Cyclic GMP and protein kinase G control a Src-containing mechanosome in osteoblasts. *Sci Signal.* 2010;3(153):ra91.
4. Rangaswami H, Marathe N, Zhuang S, Chen Y, Yeh JC, Frangos JA, et al. Type II cGMP-dependent protein kinase mediates osteoblast mechanotransduction. *J Biol Chem.* 2009 May 29;284(22):14796-808.
5. Marathe N, Rangaswami H, Zhuang S, Boss GR, Pilz RB. Pro-survival effects of 17beta-estradiol on osteocytes are mediated by nitric oxide/cGMP via differential actions of cGMP-dependent protein kinases I and II. *J Biol Chem.* 2012 Jan 6;287(2):978-88.

I concur with the recapitulation of effects reported in the different species. However, the sponsor states that no effects were found in dogs. The studies were conducted as standard toxicology studies, examining the femoro-tibial joint and sternum, and not expanded to address a specific question of whether or not the bone effect occurred in dogs. The size of the femoro-tibial joint in dogs is much greater than the corresponding joint in rats, but we do not know how the samples for sectioning were collected, processed and selected. There was no indication of additional bones, e.g vertebrae, nasal turbinates, humerus, ribs, calvarium, examined in the dog studies.

Additionally, bone remodeling in dogs is slower than in the rat. A treatment period of 9-12 months is equivalent to 2-3 remodeling periods in the dog (Forwood et al 1995) where the bone remodeling period in the rat is proposed to be approximately 6 days (Vignery and Baron, 1980). Studies examining bisphosphonate adverse bone effects in dogs used periods of up to 2 years (Forwood et al 1995; Grynepas et al, 1994), 3 years (Chennekatu et al 1996) or more (Allen et al. 2008).

The sponsor's assertion that the 2-year rat study showed no changes in the spinal column is puzzling. The 2-year rat study methodology section does not list vertebral bodies or vertebrae as having been collected and/or examined. The methodology in the Pathology Report does not list the vertebrae as having been examined. The summary of neoplastic and non-neoplastic results for the nervous system (sections 3.1.1. and 4.1.1. in Pathology Report) do not list vertebrae. The

summaries of neoplastic and non-neoplastic results for the musculoskeletal system (sections 3.1.11 and 4.1.11 in the Pathology Report) do not discuss vertebrae. The summary incidence tables do not list vertebrae, only femur and sternum. “Knee joint” is listed under femur. This reviewer re-examined SDN 0140 (filed in DARRTS, October 17, 2011). This was a special examination of bones from the 2-year carcinogenicity. In this special analysis, the sponsor stated an examination of sternum, femur and tibia. Therefore, it is unclear when the sponsor examined vertebrae.

Summary

I concur with the sponsor that there is published data to support a direct effect modulated via sGC and eNOS-NO-sGC-cGMP-PGK pathway. Inhibiting NO synthase (NOS) can potentiate ovariectomized bone resorption in rats which suggests a link to the parathyroid axis (Kasten et al 1994).

Studies in vitro indicate that NO modulates both osteoblasts and osteoclasts. Osteoclastic resorption is potentiated during NOS inhibition (Kasten et al 1994). Decreased NO levels increase osteoclast recruitment and bone resorption (Collin-Osodby et al, 2000). Receptor activator of nuclear factor- $\kappa\beta$ (NF $\kappa\beta$) ligand (RANKL) is up-regulated in response to hormones and factors that are known to promote bone resorption, such as PTH and vitamin D. RANKL binds to RANK receptors on osteoclast precursors and stimulates differentiation toward the osteoclast lineage.

Multiple physiologic mechanisms exist to regulate extracellular calcium. The processes include intestinal Ca^{2+} absorption, mobilization of bone Ca^{2+} , and excretion of calcium in urine, feces and sweat. The overall balance involves interactions between the parathyroids, kidneys, bone, blood, and gastrointestinal tract. Understanding the effects of riociguat is clouded by the overall decreased blood pressure and the reduced gastrointestinal motility. The altered organ perfusion may affect oxygenation and metabolism of a given organ and the decreased efficiency of the gastrointestinal tract may affect the absorption of nutrients such as calcium. Measurement of parameters such as vitamin D levels, vitamin D metabolites, parathormone levels, and urinary calcium would allow for some assessment of parathyroid involvement. Examination of bones in addition to the standard femorotibial joint would permit a more complete picture of the skeletal effects of riociguat. Also, no evaluations were made of the drug's effect on bone strength, compressability or healing ability. Riociguat therefore is associated with an incompletely described effect upon bone metabolism and thus overall calcium metabolism.

Recommendations for bone questions

A well designed animal study that includes examination of bones such as mandible, nasal turbinates, calvarium, vertebrae, humerus, femur (including the neck) and tibia and clinical chemistry parameters such as levels of vitamin D and metabolites, parathyroid hormones, and urinary calcium excretion may help to address the issue of exacerbation of osteoporosis. Assessment of the mechanical properties of bone may be prudent. A clinical trial with appropriate monitoring and imaging (e.g. high resolution MRI) may also be of value.

Examples of Approved Drugs with Apparent Musculoskeletal Effects

Reviewer's Summary of Some Drugs with Skeletal System Effects

Drug or drug class	Effect	Estimated frequency
Corticosteroids	Osteoporosis, vertebral compression fractures	30-50% of those on chronic therapy (G&G)
	Osteonecrosis	
	Growth retardation	
fluoroquinolones	tendinopathy	≤2% (Melhus, 2005)
Antiepileptics Carbamazepine, gabapentin, phenobarbital, phenytoin, primidone	Osteomalacia/fracture	3.1% (Watson et al, 2006)
Heparin (unfractionated)	Osteoporosis, vertebral fractures	2-5% (Lefkou et al 2010)
GnRH analogues	osteoporosis	~5% (Bannwarth 2007)
bisphosphonates	osteonecrosis	Predominantly in cancer patients, 1-12%. In osteoporosis patients, 1 case per 100,000 person-years. (Khan 2008)
Aromatase inhibitors e.g. anastrozole	Musculoskeletal symptoms and fractures	11% (Muslimani et al 2009) Small sample 7% greater than compared to tamoxifen (Bannwarth, 2007)
Methotrexate	Bone pain, osteoporosis, stress fractures (methotrexate osteopathy)	Incidence unclear

Note: this table is not meant to be a comprehensive summary of drugs affecting bone metabolism. Rather, it is a meant to show examples of drugs with similar issues to riociguat.

Cardiac Damage

Rats showed little cardiac damage after administration of riociguat. The rat carcinogenicity study reported thrombosis in the heart and other organs of males but not cardiac morphologic changes.

Cardiac morphologic damage was noted in dogs after administration of either BAY63-2521 or BAY60-4552. The sponsor identified a NOAEL for riociguat of 1 mg/kg in a 4-week oral dosing study. In the same study, doses of 3 mg/kg and 2 mg/kg given twice a day caused deterioration of body condition in the dogs with signs of trembling, ptyalism, vomiting, tenesmus, and unsteady gait. Mean blood pressure decreased profoundly in these animals (a change of 60 mm Hg relative to the control group), suggesting that the signs were due to hypotension, poor organ perfusion, and possibly cerebral ischemia. The next tolerable dose was 2 mg/kg given once a day. The 52 week dog study with riociguat reported that cardiac troponins I and T were

measured days 9, 40, 89, 180, 271 and 362 and were “normal.” The sponsor argued that cardiac changes happened acutely during the period of adaptation to the altered hemodynamics. It might have been more informative to have measured cardiac troponins within the first 2-4 days of dosing, during the cardiovascular adaptation.

Direct administration of the active metabolite, BAY60-4552 also had data indicating cardiac morphologic damage. The 4 week toxicology study for BAY60-4552 included unscheduled mortality in 1 high dose (5 mg/kg) male dog in the second week. The necropsy revealed necrosis of the papillary muscles of the heart, myocarditis, and myocardial degeneration.

Following administration of the metabolite to dogs for 39 weeks, hypertrophy of the ventricular septum was reported for the high dose males of 3 mg/kg and the females beginning at the mid-dose of 1 mg/kg. The study pathologist interpreted this as a non-adverse adaptation.

As documented by a number of investigators and chronicled by Greaves (2007), vasodilating, antihypertensive drugs have been demonstrated to cause myocyte necrosis in the papillary muscles and subendocardial zones of the left ventricle. Such necrosis usually resolves into fibrosis. The hypotension and resultant reflex tachycardia caused by vasodilation is proposed to increase gradients of blood flow and tissue oxygenation, creating relative ischemia. Tachycardia also decreases the interval for cardiac filling, further aggravating the inadequate blood/oxygen supply. Response to exaggerated pharmacology is a reasonable explanation for the cardiac damage reported. One can also distinguish between direct and indirect cardiotoxic effects by co-administering a pressor or beta adrenergic receptor antagonist with the drug to see if hypotension or tachycardia is the cause. However, there is an inconsistency of reported effects. There are few if any cardiac findings mentioned in the short term toxicology studies where it is reasonable to expect a sudden drop in blood pressure.

Reviewer’s Summary of Cardiac Morphologic Changes for BAY63-2521

Duration of study	Dose mg/kg	findings	AUC $\mu\text{g hr/l}$
			BAY63-2521
13 weeks dog PH34778	≥ 0.3	Males: Endocarditis, perivascular edema of the myocardial arteries	483 (week 12)
	≥ 1	Males: medial hypertrophy of the myocardial arteries Females: perivascular edema + medial hypertrophy of the myocardial arteries	1721 (week 12)
	3	Females: endocarditis	3702 (week 12)
52 week dog A45725	2 (males)	1 HD (2 mg/kg) male unscheduled mortality day 279. Necropsy showed acute myocardial degeneration that was considered final circulatory decompensation.	2980 (week 50)
	≥ 0.3 (females)	Cardiac vascular hypertrophy was seen in $\frac{1}{4}$ females in each of the drug-treated groups.	828 (week 50)
2 year rat carcinogenicity PH36817	5, 10, 20 mg/kg	Thrombosis in heart, kidney, and other organs in males in all drug-treated groups. Not in females	≥ 1526

Reviewer's Summary of Cardiac Morphologic Changes for BAY 60-4552

BAY60-4552			AUC $\mu\text{g hr/l}$
2 weeks dog PH34800	≥ 3	Coronary arteritis, necrosis, perivascular edema, Epicarditis, endocarditis	7351
	10	M +F: fibrosis of papillary muscle	13198
4 weeks dog PH34862	1.5	Females: papillary fibrosis	4771 (Day 25)
	≥ 1.5	Cardiac vascular inflammatory changes (both sexes)	4771
	5	Males: papillary fibrosis	9609 (Day 25)
13 weeks dog PH36085	≥ 1	Medial hypertrophy of the papillary muscle: females	3633
	3	Medial hypertrophy of the papillary muscle:males	9678
13 weeks rats PH35365	50 mg/kg	Males 8/10 medial hypertrophy cardiac blood vessels	51428
	100 mg/kg	Females: 4/10 medial hypertrophy cardiac blood vessels	82697
39 week dog PH35948	≥ 1	LOAEL for cardiac effects: hypertrophy of the arterial media of the left heart papillary muscles	
		Hypertrophy of the ventricular septum(Females: ≥ 1 mg/kg ; Males: 3 mg/kg)	2222 (week 39) 3755 (week 39)

Reviewer's comparison summary of the 26 and 52 week studies of BAY63-2521 in dogs

	26 week dog study PH35050 0, 0.3, 1, 3 mg/kg/day	52 week dog study A45725 0, 0.3, 1, 2 mg/kg	AUC ₀₋₂₄ µg hr/l 26 week	AUC ₀₋₂₄ µg.hr/l 52 week
mortality	1 HD male, week 6. Necropsy showed a generalized lymphoid atrophy.	1HD male, day 279 after showing signs of anorexia, weight loss, diarrhea, vomiting, general debilitation. Necropsy showed acute myocardial degeneration	3954	2980
≥0.3 mg/kg	Diarrhea and vomiting, adrenal hypertrophy/hyperplasia in males Blood pressure lowering apparent at 13 weeks to the end of the study. Increased weight of adrenals (31%, n.s.) and thyroids+parathyroids (32% n.s.) normalized to body weight in males.	Decrease in systolic and diastolic blood pressure Adrenal hypertrophy/hyperplasia Vascular hypertrophy in cardiac arteries Blood pressure lowering was apparent in both sexes from day 36 to the end of the study. Inconsistent changes in normalized thyroid weight in males. Increased weight of ovaries (31%, n.s.) and uterus (129%, n.s.) normalized to body weight.	1123	828
≥1 mg/kg	Diarrhea and vomiting in both sexes. Adrenal hypertrophy/hyperplasia in females also Decreased weight of uterus/cervix/oviduct (71% n.s.) and ovaries (61% n.s.) normalized to body weight	Sialorrhea, vomiting, diarrhea	1683	1205
2.0 mg/kg	Not applicable	↓motor activity, sedation, emaciation, slight ↓ in T3	33954	2980
3.0 mg/kg	↓EROD activity in females	Significant decrease in food consumption, weight loss, muscle tremor within the first 9 days		

Metabolic Effects

Several studies suggested drug effects on serum glucose, food consumption, food efficiency, hepatic glycogen contents, serum triglyceride and cholesterol, thyroid weights, thyroid hormone levels and weights of reproductive organs. It is not clear if these effects should be viewed as separate toxicities or should be considered together. The sponsor did not integrate the various findings in the written reports. In this review, discrete phenomena from different toxicology studies are organized into separate tables.

The serum glucose/feed efficiency effects for riociguat are variable across studies. No pancreatic histopathology was reported to correlate with these findings. In general, animals receiving riociguat consumed more food than control animals, but either did not gain additional weight relative to the control group, or gained at a lower rate, thus gaining less weight over the duration of the study.

Liver-related effects for both riociguat and BAY60-4552 pertained to function with few morphologic changes. Decreases were reported for serum cholesterol, triglycerides, and total protein. Urea was reported to increase in some studies and decrease in others. The sponsor measured hepatic activity of certain enzymes at the end of both rat and dog toxicology studies. These enzymes included 7-ethoxycoumarin deethylase (ECOD), 7-ethoxyresorufin deethylase (EROD), aldrin epoxidase (ALD), carnitine acetyl transferase (CAT), epoxide hydrolase (EH), glutathione-S-transferase (GS-T), UDP-glucuronyltransferase (GLU-T). In both rat and dog studies, various enzymes were reported to have some degree of decreased activity relative to control. There was no consistent pattern of a specific enzyme showing decreased activity across studies.

Smooth muscle relaxation of the gastrointestinal tract and decreased or variable efficiency of nutrient absorption may cause the variable serum glucose, liver and thyroid effects. The safety pharmacology study indicated slowing of gastrointestinal transport. The majority of toxicology studies reported gastrointestinal effects including abdominal distention and/ or diarrhea. Decreased perfusion of the liver may also contribute to increased urea, decreased cholesterol or triglycerides, through a mechanism of reduced synthesis. It is possible that the alterations in gastrointestinal motility and the distention of the gut, may have caused alterations in various gastrointestinal peptides, or activated feedback mechanisms with the thyroid and the liver. These possibilities were not explored.

Nutritional deficits can also be marked by changes in circulating thyroid hormones, typically with low T3 levels (Mebis et al, 2012). Reduced T3 relative to the control group was reported in the rat 26 week study and the dog 52 week study. There were no apparent thyroid effects in shorter term dog studies.

Changes in the reproductive or endocrine organs were apparent in several studies. Again, it is unclear if this is a primary effect or secondary to decreased perfusion of the listed organs. There is little, if any, other suggestion of a primary endocrine effect.

The clinical implications to altered gastrointestinal transit and efficiency of absorption include the possibility of altered absorption of concomitant medications and the potential for destabilizing glycemic control in those with diabetes mellitus. Whether or not this occurs should be discernible from clinical data.

Reviewer's Summary of Possible Glucose Metabolism Effects for Riociguat(all changes relative to control group)

Species	Duration of study	BAY63-2521	AUC ₀₋₂₄ μg.hr/l
Rat	2 week PH34791	17% increase in food consumption with 11% decrease in body weight gain relative to the control group.	18675
Mouse PH34865	13 week 0,42	Males: plasma glucose increased	4942
Mouse PH34865	13 week 0,67	females :plasma glucose mildly increased	4305
mouse	13 week PH 34866	35% (m) and 27%(f) increase in food consumption with minimal increase in rate of body weight gain. Hypertrophy of liver in both sexes	2211 m 2848 f
rat	4 week PH33408	≥5mg/kg/day Males gained a slower rate (-4 to -11%) than controls despite slightly increased food consumption Females gained at a higher rate than the controls (+5%) with 13% increase in food consumption. Increased normalized liver weight	5 mg/kg: 1140 m 30 mg/kg 6766 f
rat	13 week PH34674	Drug treated males gained less weight than controls (11%), no effect on female body weight. Both sexes of drug-treated animals ate more than the control groups in apparent dose-response. In g/kg of bodyweight/day : Males +11%, females +5% (neither was statistically significant)	Combined sexes 956
rat	26 week bone study A43289	In the initial week of the study, HD males ate 11% less than did the control group with a significant body weight loss. After the dosage for the high dose group was decreased, the HD group then consumed a statistically significant amount more than the control group to day 54 and to the end of the study. The LD group consumed approximately the same amount as the control group although in the last 3 weeks of the study they consumed slightly more than the control group, sometimes reaching statistical significance. From day 89 to 180, a dose response of increasing food consumption with increasing dose was apparent.	AUC not done

Reviewer's Summary of Possible Glucose Metabolism Effects for BAY60-4552

Species	Duration of study	BAY60-4552	AUC ₀₋₂₄ μg.hr/l
Rat PH34791	2 week	Food consumption increased in the HD groups with decreased body weight	≥4010 (day 12)
Rat PH34599	4 week	100 mg/kg M ↓serum glucose 5%(p<0.01) Food consumption ↑ in MD and HD groups but body weight ↓ in both groups.	≥16290 (day 22)
Rat PH 33408	4 week	Increased food consumption and decreased weight at 5, 15 and 30 mg/kg in males	≥ 1140 day 29
Rat PH35365	13 week	Food consumption ↑ in HD group with decreased body weight Decreased hepatic glycogen: males 1/10 @50 mg/kg Females: 1/10 @ 30 mg/kg ; 2/10 @100 mg/kg	51428 47423
Rat PH35987	13 week	Increased food consumption in the HD group with decreased body weight	32894
Rat PH34674	13 week	Increased food consumption and decreased body weight in both sexes, all drug-treated groups	≥956 Day 91
Rat PH34877	13 week	↑food intake in male md and hd with decreased body weight. ↑food intake in md and hd females with no difference in body weight from controls	≥5141 males ≥4127 females Day 88/89
Mouse PH36240	2 week	↑food intake in LD and HD groups with decreased body weight ↓ serum glucose in LD group	≥6028 (day 12)
Mouse PH35973	14 week	↑food intake in LD and HD groups with no increased gain over controls.	≥2987 (day 88/89)
All changes relative to the control group			

Endocrine organs

There is no clear signal for a hormonal (e.g. endocrine disruption) effect. It is unclear how significantly differences in perfusion of the endocrine/reproductive organs contributes to the variable effects noted below.

Reviewer's Summary of Possible Endocrine Organ Effects

Species	Duration of study	BAY63-2521	AUC ₀₋₂₄ µg.hr/l
Rat PH34877	13 week dietary	T3 decreased at 20 and 100 mg/kg	≥5141
Rat PH35002	26 weeks	T3 ↓15%(p<0.05) in hdf (40 mg/kg)and md f (10mg/kg) T4 ↑51% (p<0.05) in hdf day 89 Normalized weights of epididymides (↓10%, p<0.05), prostate (↓15%, p<0.05), and seminal vesicles(↓16%, p<0.05) were changed in the hdm.	Md 2283 Hd 13927
Rat A 43289	6-month bone study	Thyroid weights normalized to body:% difference from control 4 weeks: -5%(10 mg/kg), -10% (25 mg/kg) 8 weeks: +4%(10 mg/kg), +3%(25 mg/kg) 13 weeks: -4% -15 26 weeks: -18% -23%	Not found
Rat PH 36817	2 year carcinogenic- ity	Diffuse hyperplasia of C-cells of thyroid Males : 1/48, 1/49, 0/47,5/42 (p<0.05) Females : 8/49, 15/49, 11/50, 16/50 (n.s.)	M : 5923 F : 9099
Dog PH 35050	26 weeks	Dose related increase in absolute and normalized thyroid weight (+32%,n.s.) in males (hd 3 mg/kg) Females: Non-dose related increase in normalized thyroid weight (25%, n.s.) dose related ↓normalized weight of uterus/cervix/oviduct (71% ns) ↓normalized weight of ovaries (61% ns) at hd compared to the control group	LD 1103 HD 4654
Dog A 45725	52 weeks	Males: +35% (p<0.05) increase in normalized thyroid weight rel to control at 1 mg/kg(md), +14% (ns) at 0.3 mg/kg (LD). No histologic correlate Thyroid T3 values were significantly decreased in the HD males up to 20%(p<0.01). T4 was increased by up 52% (p<0.01) in HD females with a corresponding increase in TSH. There were no histologic correlates for these findings. "sick euthyroid syndrome" in dogs typically manifests with low T4 values Females: increase in normalized ovarian weight +16% increase at 0.3 mg/kg +97% 1 mg/kg (p<0.05) +31% increase at 2 mg/kg Increase in normalized uterine weight +68% increase at 0.3 mg/kg +365% increase at 1 mg/kg (p<0.05) +129% increase at 2 mg/kg	HD 2980 LD 828 AUC values are for combined sexes
All changes reported relative to the control group.			

Reviewer's Summary of Possible Endocrine Organ Effects for BAY60-4552

			AUC ₀₋₂₄ μg.hr/l
dog	2 week PH34800	activation of the uterine glandular epithelium, mucous secretion and luminal dilation occurred in 2 sexually mature animals each at ≥3 mg/kg. Luminal dilation or mucous secretion occurred in 1 female control and at 1 mg/kg. Lactation was present in one control, 2 @ 3 mg/kg and 1 @ 10 mg/kg. The mammary tissue of the other HD female was not available for evaluation. This may be degrees of pseudopregnancy	≥7351
dog	13 week PH36085	Decreased absolute and normalized weight of the female repro tract (70% of the control group, n.s) ≥0.3 mg/kg. This was not described as a gross lesion.	1603 9678
rat	4 week PH34599	Testes weight showed a dose related increase (4- 7%) while prostate and seminal vesicle weight were decreased at the HD (100 mg/kg) by 26% and 32% respectively. Histologically, the prostate and seminal vesicles were atrophic in several HD males, consistent with the decreases in organ weight. There was also a decrease in uterine weight (20%) in the HD females. In 3 out of 10 HD females, the uterus was reported to be juvenile, characterized by small lumina and an endometrium composed of small cells with spindle shaped nuclei. The superficial epithelium consisted of densely packed cuboidal epithelial cells. Corresponding to the uterine findings, 2 of the affected females contained abundant mucous and desquamated epithelial cells.	64206
rat	13 week PH35365	Males T3 @50 mg/kg ↓20% from control (p<0.01) on day 30, ↓12% from control (p<0.05) day 91 Females changes only on day 87 @100 mg/kg T4↑52% over control (p<0.01); TSH dose related increase of 28%over control (p<0.05) No histologic correlate to these changes.	Males: 51428 Females: 47423
All changes reported relative to control group.			

Liver*BAY63-2521*

Changes noted pertained primarily to function in the shorter term studies and both function and morphology in the longer term studies.

Reviewer's Summary of Possible Liver Effects of Riociguat: Mouse Studies

study	Doses mg/kg	effects
2 week mouse:males PH34519	0,16, 56	56 mg/kg: Decreased cholesterol (-22%), urea(-21%) and triglycerides(-22%) Normalized liver weight +8
2 week mouse: females PH34519	0,27,96	Decreased cholesterol (-14%), urea(-15%) and triglycerides(-28%) Normalized liver weight +6
13 week mice dietary males PH34866	0,3,16,86	At HD: Decreased protein (-7%)and albumin (-8) Histo: hypertrophy at HD 8/10 AUC not calculated due to lethality
13 week mice dietary females PH34866	0,6,24,124	At HD: Decreased protein (-13%, p<0.01)and albumin (-16, p<0.01) Histo: hypertrophy 2/10 MD, 6/10 HD AUC not calculated due to lethality
13 week mice dietary: males PH34865	0,42	Normalized liver weight:+4 ns, histo: hypertrophy 7/10 Serum cholesterol -16% ns
13 week mice dietary females PH34865	0,67	Normalized liver weight:+18%, p<0.01 histo: hypertrophy 4/10 Serum cholesterol -13, p<0.05
Carcinogenicity: mice PH36818	Females: 0,8,16,32	Hepatic inflammation: 0/3/3/5 p<0.05 Foamy/enlarged macrophages: 7/11/15/21 p<0.01
Carcinogenicity: mice PH36818	Males: 0, 6,12,25	Hepatic inflammation: 1/2/2/4 Foamy/enlarged macrophages: 8/9/10/23 p<0.01
All effects reported relative to control groups.		

The rat studies included both functional and morphologic liver changes.

Reviewer's Summary of Possible Liver Effects with Riociguat: Rat and Dog Studies

study	Doses mg/kg	effects
2 week rat: males PH34791	0, 5,23,99	Cytoplasmic change/hypertrophy: 0/5, 0/5, 2/5, 4/5 Increased total bilirubin at HD
2 week rat: females PH34792	0, 7, 30, 101	Cytoplasmic change/hypertrophy: 0/5, 0/5, 0/5, 3/5 Increased total bilirubin at HD
4 week rats PH33408	0, 1.5, 5, 15, 30	Increased serum urea and protein, both sexes Liver histo: cytoplasmic change. Males: 0/10, 0/10, 4/10, 10/10, 10/10 Females: 0/10, 0/10, 0/10, 6/10, 7/10 MD AUC: 3795 μ g hr/l for males
13 week rats gavage PH34674	0, 3,10,30	Males: \uparrow ALT 32%, p<0.05; \downarrow total protein -6 p<0.01; \downarrow triglycerides -26, \uparrow total bilirubin, slight increase in liver weight at HD Females : \uparrow ALT 8% , n.s.; \uparrow total protein 4; \downarrow triglycerides -45, p<0.05 \uparrow total bilirubin,slight increase in liver weight \geq 10 mg/kg, condensed hepatocellular cytoplasm
13 week rats Dietary PH34877	Males: 0, 4, 20, 101	\downarrow triglycerides -48%, p<0.01, \downarrow total protein -8, p<0.01 Histo: cytoplasmic condensation 0/10,0/10, 4/10, 10/10 Bile duct activation/hyperplasia: 0/10, 0/10, 0/10, 9/10
13 week rats Dietary PH34877	Females: 0,4,21,105	\downarrow triglycerides -5%, \downarrow total protein -3 Histo: cytoplasmic condensation 2/10,2/10, 3/10, 8/10 Bile duct activation/hyperplasia: 0/10, 0/10, 0/10, 8/10
26 week rats gavage PH35002	0,2.5, 10, 40	<u>Males:</u> \downarrow triglycerides -46%, p<0.05; \downarrow total protein -5%; \uparrow urea +14 p<0.05 \downarrow periportal fat in HD m <u>females:</u> \downarrow triglycerides -27%; \downarrow total protein -10, p<0.01; \uparrow urea +11 ; \downarrow albumin -8%, p<0.01 Hypertrophy/cytoplasmic change of centrilobular hepatocytes in HD \downarrow periportal fat in MD and HD AUC ₀₋₂₄ for MD : 2283 μ g hr/l AUC ₀₋₂₄ for HD: 13927 μ g.hr/l
Carcinogenicity: rats PH36817	females	Liver: biliary cysts 9/14/10/16
Carcinogenicity: rats PH36817	males	Liver: biliary cysts 5/7/10/12 p \leq 0.05
4 week dog study PH33454	0.3, 1, 3/2	Increased hepatic glycogen content in the HD animals. Increased serum urea in both sexes, all doses.

Direct administration of the metabolite produced primarily clinical chemistry changes suggesting functional changes in the liver.

Reviewer's Summary of Possible Liver Effects: BAY60-4552

study	Doses mg/kg	effects
14 week mice PH35973	0,100, 300, 1000 ppm	Males decreased triglycerides -38%, p<0.05 Increased creatinine +12%, p<0.05
4 week rat PH34599	0, 4, 20, 100	Males: Increased urea +34% p<0.01 (>20 mg/kg)
13 week rat PH35978	Males: 0,50 Females:0,100	Males: increased urea +53%, p<0.01 Females: increased urea +33, p<0.01, female liver: relative weight +25% p<0.01
13 week rat PH35987	0, 10, 30, 100	Increased serum urea males: +20%, p<0.05 Increased serum urea females: +10% ns
13 week rat PH35365	M:0,5,15,50 F:0,10, 30, 100	Increased serum urea 35-60% (p<0.01) in HD males Increased serum urea 35% (p<0.01) in HD females; increased normalized liver weight 21% (p<0.01)
All effects reported relative to control.		

Renal

The indications of renal effects are summarized in the reviewer's tables below. Several factors make it difficult to discern whether there is a primary toxicity or not. These confounders include 1) the bone effects with the attendant modulation of serum phosphorous and calcium possibly altering renal function and 2) the decreased blood pressure overcoming renal vascular autoregulation. It is noted in Greaves (2007) that high doses of angiotensin converting enzyme inhibitors have been associated with renal tubular dilation, something noted in the histopathology for riociguat and M1. However, dogs are usually sensitive to such effects and in this case do not show evidence to suggest a discernible renal effect.

The sponsor felt that the renal effects were attributable to the M1 metabolite. A 13-week time course study in female rats (PH35975) with direct administration of BAY60-4552 indicated the following changes:

PH35975

The most prominent histopathological findings in the kidneys are the following (the findings are given with their first day of treatment-related occurrence; already existing changes persisted during the further course of treatment):

First observed at necropsy on day 3:

- Epithelial degeneration/cellular debris in the papilla
- Focal tubular degeneration (one rat)
- Ductular dilation in the papilla
- Diffuse dilation of cortical tubules

First observed at necropsy on day 15:

- Hypertrophy/hyperplasia of the distal cortical tubules

First observed at necropsy on day 29:

- Hyperplasia of the ductular epithelium papilla
- Prominent epithelium of the collecting ducts
- Mononuclear cell infiltration

First observed at necropsy on day 48:

- Interstitial swelling at the tip of the papilla
- Mitosis of the ductular epithelium

First observed at necropsy on day 57:

- Interstitial fibrosis

First observed at necropsy on day 71:

- Degenerative lesions in the cortical tubules of the P3-segment
- Inflammation of the pelvis (one rat)

At final necropsy on day 92, no additional histopathological changes were observed.

The sponsor's summary of plasma level exposure for the time course study is shown below:

Dose	[mg/kg]	100
		Mean geom.
AUC(0-24)	[µg·h/L]	97017
AUC(0-24) _{norm}	[kg·h/L]	0.970
C _{max}	[µg/L]	10958
C _{max, norm}	[kg/L]	0.110
C(24)/C _{max}	[%]	2.10
t _{max}	[h]	1.00

Two other studies with direct administration of BAY60-4552 showed marked renal effects. The pathologist's comments for these studies are shown below:

PH35365

The most prominent microscopic lesions were noticed in the kidneys with the distal tubule and the collecting ducts as specific target of BAY 60-4552. A hypertrophy/hyperplasia of the epithelial cells of the distal tubule occurred in the medulla and cortex of high dose rats of both sexes. The collecting ducts of the papilla showed an epithelial hyperplasia in almost all high dose females and two high dose males. In some of the affected females, degenerative changes in hyperplastic collecting ducts were also present as well as an increased mononuclear cell infiltration and a tubular dilation in the cortex and/or papilla. The latter was also seen in a few high dose males.

PH35976

In the kidneys, epithelial hyperplasia of the papillary collecting ducts was observed at the renal papilla of three males of the high dose level (left kidney specimen, Davidson's fixative) and could be seen in one of these animals (No. 69) also at the specimen of the right kidney. In females, the incidence of basophilic tubules was reduced at the high dose level. The incidence of corticomedullary mineralization was lower in the left kidney of females at the high concentration level. This could not be seen in the kidney fixed in formalin where the incidence was similar throughout the groups including control (incidence 16/13/9/9).

From the viewpoint of pathology, the no effect level is 3 mg/kg in males. For females, a no-effect level could not be established.

The toxicokinetics determined on day 164 are shown below:

	Gender Dose [mg/kg]	Male			Female		
		3	10	30	3	10	30
AUC(0-24)	[$\mu\text{g}\cdot\text{h/L}$]	2909	11420	33002	4085	12244	41509
AUC(0-24) _{norm}	[$\text{kg}\cdot\text{h/L}$]	0.970	1.14	1.10	1.36	1.22	1.38
C _{max}	[$\mu\text{g/L}$]	435	1170	4315	638	1714	5950

Plasma levels of BAY60-4552 when administered alone or after riociguat administration

species	dose	Riociguat administered			BAY60-4552 administered		
		AUC ₀₋₂₄ $\mu\text{g h/l}$ total exposure	C _{max}	AUC ₀₋₂₄ $\mu\text{g.h/l}$ Unbound exposure	AUC ₀₋₂₄ $\mu\text{g.h/l}$ total exposure	C _{max} $\mu\text{g/l}$	AUC ₀₋₂₄ $\mu\text{g.h/l}$ Unbound exposure
mouse	100ppm	331	23	57	2987	165	511
rat	30 mg/kg	1125	208	120	33002	4315	3531
Human AUC for BAY60-4552: 2604 $\mu\text{g}\cdot\text{hr/l}$ after 2.5 mg t.i.d. (Study 12166)							
Human C _{max} for BAY60-4552: 111ng/ml (range: 58-202 ng/ml)							

The rat AUC at 30 mg/kg is 13X the AUC reported for pulmonary hypertension patients taking 2.5 mg of riociguat three times a day. There is no margin of safety between the mouse AUC value and the human AUC value.

Reviewer's Summary of Possible Renal Effects of Riociguat

Study	Doses mg/kg	Effects
13 week rat PH34674	0,3,10,30	Serum P: males +9 %, females +17% (both $p \leq 0.05$) Serum Ca increased males +5%, females +5% ($p \leq 0.01$)
13 week rat PH 34877	0, 4, 20, 100	Serum calcium decreased in both sexes of HD animals 4% $p \leq 0.01$ Histo: lipofuscin in proximal tubules HD m, 3/10 MDF, 9/10 HDF
26 week rat PH35002	0, 2.5, 10, 40	Histo: lipofuscin in proximal tubules HDm, 1/20LDF, 6/20 MDF, 17/20 HDF
4 week dog PH33454	0, 0.3, 1, 3/2	Urinalysis: HD males and females only showed + protein, bilirubin, and triple phosphate crystals. Histopath showed increased basophilia in both these groups.

Reviewer's Summary of Potential Renal Effects from BAY60-4552

study	Doses mg/kg	Effects
3 day time course in rats PH36334	M: 15, 50 mg/kg F: 30, 100 mg/kg	Increased excretion of NAG, LDH, Increased NAG/creatinine, LDH/creatinine Histopathology primarily in HD females. Degenerative and regenerative changes. \uparrow mitosis in tubular epithelium in both sexes. Intraluminal debris in collecting ducts, epithelial hyperplasia/degeneration, ductular dilation in females.
14 week mice PH35973 dietary	0, 100, 300, 1000 ppm in diet M: 17, 50, 166 mg/kg F: 28, 84, 226 mg/kg	Creatinine: dose related increase up to +22% $p < 0.05$ in males.
2 week rat PH36027 dietary	Males: 13, 43, 140 Females: 17, 52, 134	Histo: hypertrophy of epithelium distal cortical tubules 5/5 HDm, 5/5 HDF Diffuse +/- or segmental dilation of renal tubules 4/5HDm, 3/5 HDF Intraluminal accumulation of degenerated cells 4/5HDm, 1/5HDF
4 week rat PH34599	0, 4,20, 100	Normalized renal weight: +17% ($p < 0.01$) HDm, +3% (ns) HDF Histo: hypertrophy of epithelial cells in distal tubules 10/10 HDm, 6/10 HDF Epithelial hypertrophy of papillary collecting ducts 7/10 HDm, 3/10 HDF
13 week rat PH35987	0, 10, 30, 100	Histo: hyperplasia/hypertrophy distal cortical tubules 10/10HDm, 10/10 HDF, signs of continuous tubular degeneration/regeneration (HD rats and MD m) Epithelial hyperplasia/papilla 2/10 HDm, 9/10 HDF Ductular dilation/papilla 3/10 HDm, 10/10HDF « Adaptation to vasodilation » Increased excretion of LDH
13 week rat PH35975	Females only 0 100	Sequential euthanasias to assess renal findings. Urinary LDH activity increased from day 57. Normalized excreted creatinine, protein, urea, NAG and LDH were increased starting day 29. Time dependent increase in kidney weight beginning with Day 29 euthanasia From day 48: enlargement, discoloration and surface changes. Histopathologic changes seen beginning day 3: cellulat debris, epithelial degeneration in renal papilla, Signs of tubular and ductular activation/regeneration seen over the whole investigation period.
28 week rat PH35976	0,3,10,30	Urinalysis: dose-related increase in NAG excretion +55% $p < 0.01$ m; +21%ns f Histo: epithelial hyperplasia papillary collecting ducts 3/20HD m

In summary, riociguat shows profound hypotensive effects that the sponsor uses as a surrogate for efficacy in pulmonary hypertension. The hazard characterization shows direct effects of teratogenicity and adverse effects on bone. Adverse effects on kidney, liver, serum glucose and endocrine function are difficult to classify as either direct or indirect effects. The approvability depends upon the clinical benefit offered by riociguat versus the potential liabilities.

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