CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

203085Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Stivarga (regorafenib)

Date: September 10, 2012
To: File for NDA 203085
From: John K. Leighton, PhD, DABT
Acting Director, Division of Hematology Oncology Toxicology
Office of Hematology and Oncology Products

I have examined pharmacology/toxicology supporting review of Drs. Goheer and McDougal and secondary memorandum and labeling provided by Dr. Helm. I concur with Dr. Helm's conclusion that Stivarga may be approved and that no additional nonclinical studies are needed for the proposed indication.

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/s/

JOHN K LEIGHTON
09/10/2012

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:	203-085
Supporting document/s:	IND 75,642, EDR Location:
	\\CDSESUB1/EVSPROD\NDA203085\203085.enx
Applicant's letter date:	April 30, 2012
CDER stamp date:	April 30, 2012
Product:	Regorafenib (Stivarga®)
Indication:	Metastatic colorectal cancer
Applicant:	Bayer Healthcare Pharmaceuticals, Inc.
	340 Changebridge Road
	Pine Brook, NJ 07058
Review Division:	Division of Hematology Oncology Toxicology
	(Division of Oncology Products 2)
Reviewer:	M. Anwar Goheer, Ph.D.
	Andrew McDougal, Ph.D., D.A.B.T.
Supervisor/Team Leader:	Whitney S. Helms, Ph.D.
Division Director:	John Leighton, Ph.D.
	(Patricia Keegan, M.D.)
Project Manager:	Monica L. Hughes

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1 Executive Summary

1.1 Introduction

New Drug Application (NDA) 203-085 was submitted to the U.S. Food and Drug Administration for evaluation for full approval of regorafenib (Stivarga®) for the treatment of patients with metastatic colorectal cancer. Regorafenib is a new molecular entity kinase inhibitor, which inhibits multiple membrane bound and intracellular kinases involved in a wide range of normal cellular functions and in pathologic processes such as oncogenesis, tumor angiogenesis, and maintenance of the tumor microenvironment. The recommended dose of regorafenib for the treatment of metastatic colorectal cancer (CRC) is 160 mg (4 tablets) daily for 3 weeks followed by 1 week off therapy to comprise a cycle of 4 weeks. Nonclinical pharmacology, pharmacokinetic and toxicology studies have been submitted to support the approval of regorafenib for the proposed indication.

1.2 Brief Discussion of Nonclinical Findings

Nonclinical primary pharmacology studies were designed to evaluate the mechanism of action and activity of regorafenib. Regorafenib and two metabolites of the drug present at high levels in human serum, M-2 and M-5, were tested in either biochemical assays or in cellular assays examining the phosphorylation of downstream targets. Kinases inhibited at the lowest concentrations of regorafenib included RET and several RET variants, PTK5, VEGFR-1,-2, and -3, FGFR-1 and -2, DDR2, SAPK2, Lyn, Tie2, Abl, TrkA, EphA2, KIT and several Kit variants, c-RAF, BRAF, and $BRAF^{V600E}$. With each of these kinases, both the M-2 and M-5 metabolites showed inhibitory activity that was similar to and occasionally higher than the activity of the regorafenib parent compound.

Regorafenib also exhibited activity in *in vivo* studies performed in mice and rats. An *in vivo* experiment specifically examining potential anti-VEGF activity was conducted with both regorafenib and the M-2 and M-5 metabolites. At a dose level of 1 mg/kg, regorafenib and each of its metabolites were able to prevent VEGF-induced reduction of blood pressure in cathetized Wister rats. Non-VEGF specific effects of regorafenib on angiogenesis were also analyzed using magnetic resonance imaging (MRI) to determine the amount of tumor blood vessel development in regorafenib-treated rats implanted with exogenous tumors. Decreased MRI signals in tumors from rats treated with either regorafenib or the M-2 metabolite suggest that the drug has anti-angiogenic activity. Regorafenib also showed anti-tumor activity, primarily inhibition of tumor growth, in several mouse tumor implant models including some investigating the drug's activity against CRC cell lines.

Target organs for regorafenib-mediated toxicity identified in toxicology studies conducted using rats and dogs included the liver, kidney, adrenal gland, thyroid, pancreas, gastrointestinal tract, hematopoietic/lymphoid system, reproductive system, and skeletal system. Findings of changes in dentin and epiphyseal growth plates were present in both species. These changes have been associated with many VEGF inhibitors and may be relevant to a pediatric population. Evidence of gastrointestinal

toxicity included findings of liquid feces/blood in the feces, vomiting of whitish/yellowish mucus/foam/watery liquid/food mash in the 13-week dog study at doses resulting in regorafenib exposures approximately 50% of the exposure in humans. A single dose study in rats also demonstrated decreases in gastric motility following administration of regorafenib. In the hematopoietic system there were findings of bone marrow hypocellularity, atrophy of the spleen, lymph nodes, and thymus in rats at doses resulting in exposures similar to the exposure in humans at the recommended daily dose. In dogs, thymic atrophy was observed at the high dose levels in all studies; atrophy was also observed in lymph nodes. Both rats and dogs had histopathological findings in the liver along with elevations in liver enzymes noted in short and long term repeat dose toxicology studies. Hematological changes including neutropenia, thrombocytopenia, and lymphopenia as well as elevations in liver enzymes were seen clinically and hepatotoxicity is included in a black box warning for Stivarga.

Skin toxicity was observed in dogs at all dose levels of regorafenib administration in a 13-week study. Toxicity was evidenced by histopathological findings of dyskeratosis, hyperkeratosis, acanthosis, and dermatitis, along with hair growth arrest. Similarly, in a 52-week study, dogs displayed dose-dependent increases in findings of fur and skin/mucosa alteration (hair loss, abscess like lesions). Administration of regorafenib to both rats and dogs also resulted in increases in thyroid stimulating hormone (TSH). In rats this change was accompanied by an increase in thyroxine (T4) levels at doses approximately 40% higher than those observed in humans. In dogs the change in TSH (up to 7 fold higher than levels in control animals) was not accompanied by increases triiodothyronine (T3) and (T4) but was observed in high dose animals from Week 6 onwards in a 52-week study; at this dose level regorafenib exposure in dogs was approximately 53% of the human exposure at the recommended daily dose. Skin toxicity and rising TSH levels have been reported clinically as well.

Renal toxicity was observed in all repeat-dose toxicology studies conducted with regorafenib. Renal findings in rats and dogs included glomerulpathy, tubular degeneration/regeneration, tubular dilation, and interstitial fibrosis. No renal toxicity was noted in 1-month studies with either the M-2 or the M-5 metabolite which suggests that differences in metabolism between humans, rats, and dogs leading to significantly higher human exposures to M-2 and M-5 compared to the species used for toxicological assessment may account for higher levels of renal toxicity seen in animals compared to humans in trials conducted to support marketing.

Cardiovascular safety was examined in both single and repeat-dose toxicology studies in dogs. In repeat-dose studies conducted in rats there histopathological findings in the heart including perivascular/interstial edema and pericarditis in the 4-week rat study and thickening of the atrioventricular valve in the 26-week rat study. In dogs none of the studies revealed significant changes in ECG parameters. In *in vitro* experiments regorafenib itself showed low potential for QTc prolongation with no prolongation of action potential duration in a Purkinje fiber assay and an IC_{50} of 27μM in the hERG assay; however, the M-2 and M-5 metabolites had IC_{50}s of 1.1 and 1.8 μM, respectively, in the hERG assay suggesting a considerably higher potential for QTc prolongation. The M-2 and M-5 metabolites were not present in rats or dogs at significant levels, thus the animal studies may have underpredicted the potential for regorafenib induced QTc prolongation in humans. To address this issue, single dose

cardiovascular safety studies in dogs were conducted using each of the metabolites; however, there were no clearly adverse effects noted for either metabolite in these studies and in 1-month repeat-dose toxicology studies conducted in mice using each of the metabolites, no unique toxicities compared to those observed in animals administered regorafenib were identified.

Dedicated studies examining fertility and pre- and post-natal development were not conducted to support the treatment of patients with advanced cancer. In general toxicology studies, female rats administered regorafenib at dose levels resulting in exposures similar to those observed in humans at the clinically recommended dose had histopathological findings of increased necrotic corpus lutea and atrophy in the ovaries and uterus. Males in the same dose group had increases in histopathological findings of mononuclear infiltration and cellular debris as well as decreased weight of the testes, prostate, and seminal vesicles compared to control animals. Findings in the epididymides had not resolved by the end of the recovery period; there were also findings of tubular atrophy and degeneration in the testes and atrophy of the seminal vesicles noted at the end of the 4 week recovery period in these animals. Similarly, in 13-week studies conducted in male dogs at dose levels ≥ 400 mg/m^2 (approximately half of the human exposure at the clinically recommended dose of 160 mg/day by AUC) had histopathological findings of retarded maturation of the testes along with aspermia/oligospermia in the epididymides. In females, findings of reduced follicular development and increased follicular degeneration were noted at the same dose levels. These findings suggest that regorafenib could affect fertility in humans.

Embryofetal studies were conducted in Wistar rats and Himalayan rabbits. In both species, at doses resulting in exposures significantly lower than the human exposure at the recommended daily dose, there were increases in post-implantation loss and teratogenic effects including skeletal and cardiovascular malformations and renal findings of dilation of the renal pelvis or hydronephrosis. Total resorption of litters in rats was sometimes observed at dose levels resulting in exposures as low as approximately 10% of the exposure in humans at the recommended dose. Pregnancy category D is recommended.

In a distribution study in pregnant rats that were administered radiolabelled regorafenib there was clear exposure to the fetus. Exposure in the fetal adrenal glands exceeded the maternal blood concentration. Fetal brain concentration exceeded the maternal brain concentration by 2-fold indicating significantly higher penetration of the blood/brain barrier in the fetus compared to the dam. In the mammary gland exposure was approximately 2-fold higher than that in maternal blood correlating with a finding of high levels of radiolabelled regorafenib or its metabolites secreted in milk observed in a dedicated excretion study performed in rats. These studies suggest a high risk for neonatal exposure to regorafenib in breast milk from women taking Stivarga.

Regorafenib was not mutagenic in *in vitro* or *in vivo* assessments of genotoxicity; however, the M-2 metabolite was clastogenic in an *in vitro* assay suggesting that the drug may have mutagenic potential in humans. No carcinogenicity studies were conducted to support the marketing application for regorafenib. These studies are not required for the evaluation of drug safety in products intended for the treatment of patients with advanced cancer. The nonclinical data from the studies submitted to this NDA are adequate to support an assessment of the safety of regorafenib for the

treatment of patients with metastatic colorectal cancer; there are no nonclinical findings or outstanding issues that would prevent the approval of regorafenib for the treatment of this patient population.

1.3 Recommendations

1.3.1 Approvability

Approvable
There are no non-clinical findings or outstanding issues that would preclude the approval of regorafenib in the proposed indication.

1.3.2 Additional Non Clinical Recommendations None

1.3.3 Labeling

Labeling will be addressed in a separate review.

2 Drug Information

2.1 Drug

Trade Name:	Stivarga®
Generic Name:	Regorafenib
Code Name:	BAY 73-4506, (b) (4)
CAS Registry Number:	(b) (4) Monohydrate: 1019206-88-2
Chemical Name	(b) (4)
	(b) (4)
Molecular Formula/Molecular Weight:	(b) (4)
Structure:	

Pharmacologic class: Kinase Inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

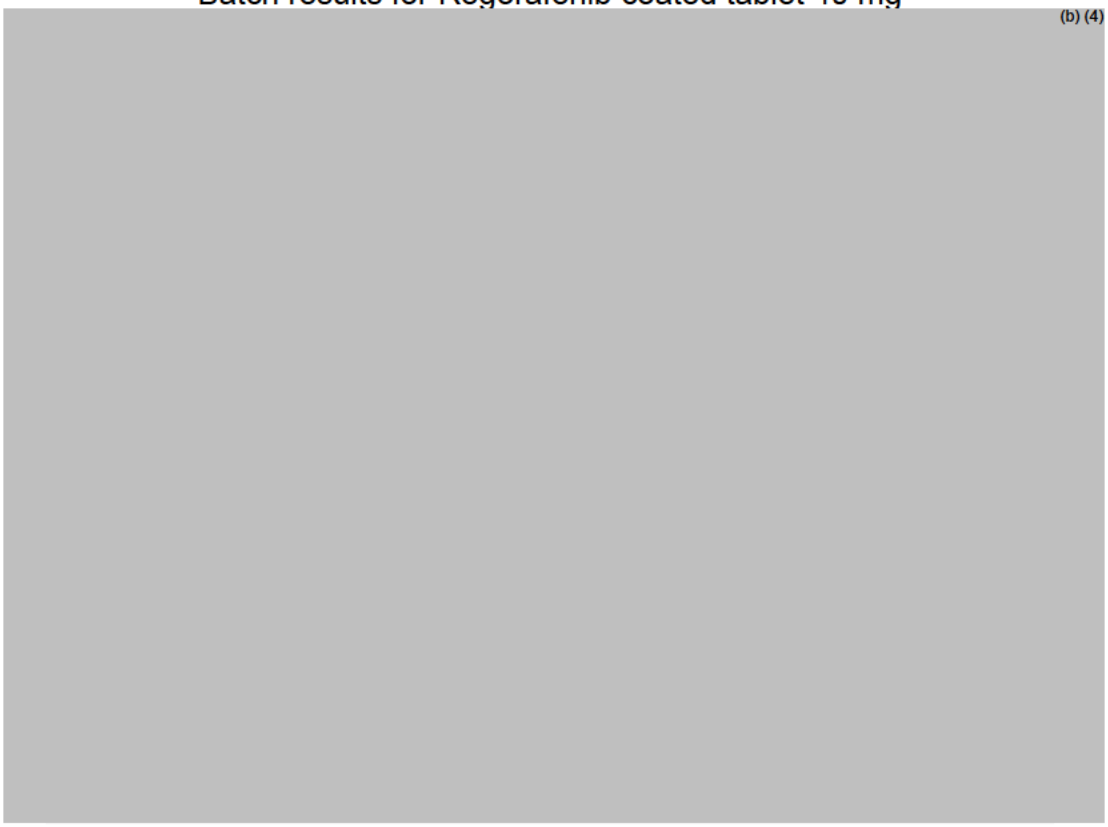
INDs 75,642 & (b) (4)

2.3 Drug Formulation

Composition	Function	Amount (mg)
Drug Substance		
Regorafenib	Active drug substance	40
Excipients	-	
Cellulose microcrystalline Croscarmellose sodium Magnesium stearate Povidone Silica colloidal (b) (4) (b) (4) (b) (4)	(b) (4)	
Weight (coated tablet)	-	472.00

Regorafenib monohydrate (reference to standard: specification) is used as drug substance for the manufacture of Regorafenib tablets. 40.00 mg Regorafenib is equivalent to 41.49 mg Regorafenib monohydrate. (b) (4)

Batch results for Regorafenib coated tablet 40 mg



Source: *P.5.4.01 Batch Analyses / Regorafenib coated tablet 40 mg*

(excerpted from the Applicant's submission)

2.4 Comments on Novel Excipients None

2.5 Comments on Impurities/Degradants of Concern

Regorafenib drug substance contains the impurity (b) (4) which was demonstrated to be mutagenic in an *in vitro* Ames test. The proposed specification for controlling (b) (4) in the regorafenib drug substance is (b) (4) In the 13-week dog study animals were exposed to batch of regorafenib with a level of (b) (4) (b) (4) (Batch no. BX01U82). In this study, dogs were able to tolerate a daily dose of 400 mg/m^2/day regorafenib which would result in a daily dose of (b) (4) of approximately (b) (4) The proposed specification of (b) (4) would result in a daily dose of approximately (b) (4) of (b) (4) - (b) (4) at the recommended daily dose of 160 mg regorafenib/day. The theoretical threshold of toxicological concern for genotoxic impurities has traditionally been 1.5 µg/day; however, this limit is based on a lifetime risk of developing cancer due to chronic exposure to a genotoxic agent. Regorafenib has been developed for the treatment of patients with advanced cancer, and, as has been discussed in ICH S9, does not adequately capture the risk-benefit ratio for the patient population. Considering all these factors, the proposed specification for (b) (4) is acceptable.

(b) (4) is a non-genotoxic impurity in regorafenib. In the above mentioned study, dogs were able to tolerate a dose of regorafenib resulting in an (b) (4) dose of (b) (4). At a specification of (b) (4) humans would receive a daily dose of approximately (b) (4) of (b) (4) at the recommended daily dose of 160 mg regorafenib/day. Thus, the impurity is qualified by nonclinical data.

2.6 Proposed Clinical Population and Dosing Regimen

Regorafenib (Stivarga®) is indicated for patients with metastatic colorectal cancer (CRC) who have been previously treated with , (b) (4), fluoropyrimidine-based chemotherapy, an anti-VEGF therapy, and , if KRAS wild type, an anti-EGFR therapy. The recommended dose is 160 mg (4 tablets) daily for 3 weeks followed by 1 week off to comprise a four week cycle. The tablets should be swallowed whole after a light meal. Treatment should be continued as long as benefit is observed or until unacceptable toxicity occurs. The dose can be reduced to 120 mg or 80 mg due to toxicity.

Route of Administration: Oral

2.7 Regulatory Background:

Original IND (75,642) was submitted to the FDA on July 24, 2006

3 STUDIES SUBMITTED

3.1 Studies Reviewed

Report #	Report Title
Primary Pharmacodynamics (NDA module 4.2.1.1)	
A57121	Kinase profiler
A58227	Kinase profiling
A58230	• Inhibitory potential of 6 compounds using a cellular TIE2 phosphorylation assay • Inhibitory potential of 7 compounds using a cellular TIE2 phosphorylation assay • Inhibitory potential of 12 compounds using a cellular TIE2, KIT, and B-RAF-VE phosphorylation assay
A58229	Multiplexed cytotoxicity assay
A58234	*In vivo* pharmacodynamics of BAY 73-4506 and its metabolites by Magnetic Resonance Imaging (MRI) after various administrations in rats

A57101	Investigation of 4 development compounds (regorafenib, its metabolites M-2 and M-5 and sorafenib) for tumor growth inhibition in the HT-29 CRC xenograft model on NMRI nu/nu mice
A57105	Investigation of 4 development compounds (regorafenib, its metabolites M-2 and M-5 and sorafenib) for tumor growth inhibition in the MDA MB-231 breast xenograft model on NMRI nu/nu mice
A58231	Efficacy of regorafenib on the survival of syngeneic mice with orthotopically transplanted H129 hepatoma
A57118	• Evaluation of BAY 734506 in combination with Irinotecan in the Oxaliplatin resistant human colorectal tumor model Co8183 • Evaluation of BAY 734506 in combination with Irinotecan in the Oxaliplatin resistant human colorectal tumor model Co8434 • Evaluation of BAY 734506 in combination with Irinotecan in the Oxaliplatin resistant human colorectal tumor model Co8435 • Evaluation of BAY 734506 in combination with Irinotecan in the Oxaliplatin resistant human colorectal tumor model Co5896
A58233	Efficacy of regorafenib and sorafenib on primary tumor and metastases formation in the syngeneic orthotopic 4T1 breast cancer model in Balb/C mice
Secondary Pharmacodynamics (NDA Module 4.2.1.2)	
PH-36660	Effects of regorafenib and its metabolites M-2 and M-5 in an *in vivo* assay for the systemic effects of exogenous VEGF in rats
Safety Pharmacology (NDA Module 4.2.1.3)	
PH-33109	Effects of BAY 73-4506 on the HERG K^+ current in stably transfected HEK293 cells
PH-33827	Effects of BAY 73-4506 on the action potential of isolated rabbit cardiac Purkinje fibers
PH-33840	Effect of a single oral administration of BAY 73-4506 on the behavioral and physiological state, open-field behavior, and body temperature of rats
PH-33856	BAY 73-4506: Effects of a single oral administration on the convulsive threshold dose of pentylenetetrazole, on the nocifensive responsiveness to heat, and on the duration of

	hexobarbital-induced anesthesia in rats
PH-34006	BAY 73-4506: Effect of a single oral administration on renal function, blood pharmacology and lipid metabolism of rats
PH-33925	BAY 73-4506: Effect of a single oral administration on blood glucose of fasted and fed rats
PH-33841	BAY 73-4506: Effects of a single oral administration on gastrointestinal motility in rats
PH-34043	BAY 73-4506: Effects on the contractility of the isolated guinea pig ileum
PH-33963	BAY 73-4506: Influence on haemodynamics, ECG and respiration in anaesthetized dogs after single intraduodenal administration
PH-35619	BAY 73-4506: Influence on cardio-hemodynamics, ECG and respiration in anesthetized dogs after cumulative intravenous infusions
PH35502	BAY 75-7495: Effects on the hERG K+ current in stably transfected HEK293 cells
PH-35438	BAY 75-7495: Effect of a single oral administration on the behavioral and physiological state, open-field behavior, and body temperature of rats
PH-35628	BAY 75-7495 (metabolite M-2 of BAY 73-4506): Influence on cardio-hemodynamics, ECG and respiration in anesthetized dogs after cumulative intravenous infusions
PH-33519	BAY 81-8752: Effects on the hERG K+ current in stably transfected HEK293 cells
PH-35409	BAY 81-8752: Effect of a single oral administration on the behavioral and physiological state, open-field behavior, and body temperature of rats
PH-35620	BAY 81-8752 (Metabolite M-5 of BAY 73-4506): Influence on cardio-hemodynamics, ECG and respiration in anesthetized dogs after cumulative intravenous infusions

Pharmacokinetics

Report #	Report Title
Distribution	
PH-34096.	Investigation of the Stability in Plasma, Binding to Plasma Proteins, Reversibility of Binding, and Erythrocyte/Plasma Partitioning of [^{14}C]BAY 73-4506 *In Vitro*
PH-33804	Whole-body Autoradiography in Rats after Single Intravenous and Oral Administration of [^{14}C]BAY 73-4506. Study no. I 5001743.
A53543	Quantitative Whole-body Autoradiography. Distribution of Radioactivity and Elimination from Blood, Organs, Tissues, and Fetuses after Single Oral Administration of [^{14}C]BAY 73-4506 to Pregnant Rats. Study no. I

Report #	Report Title
	5145-5
Metabolism	
PH-33760	[^{14}C]BAY 73-4506: Species Comparison Based on Phase I Metabolism in Liver Microsomes of Different Species Including Man
Excretion	
A53331	Secretion of Radioactivity into Milk of Lactating Rats after Single Oral Administration of [^{14}C]BAY 73-4506
PH-33884	Absorption and Excretion of the Radioactivity in Male Wistar Rats after Single Administration of [^{14}C]BAY 73-4506. Study No. I 5001752, I 4558-2

GENERAL TOXICOLOGY

Report #	Report Title
Repeat-Dose Toxicity	
PH-35874	Chronic Oral Toxicity Study in Rats (6-Months Administration by Gavage). Study No. T2078241
PH-73-4506	Subchronic Oral Toxicity Study in Beagle Dogs (13 Week Administration by Gavage). Study No. T3076046
A45739	Systemic toxicity study in dogs (M+F) with daily intragastric administration over a period of approx. 52 weeks. Study No: TXST20070037 (T6077787)
Special Toxicology Studies	
PH-35885	BAY 75-7495 (metabolite M-2): Repeated Dose Systemic Toxicity Study in CD-1 Mice (4-Weeks Administration by Gavage). Study No T0077259
PH-35852	BAY81-8752 (Metabolite M-5): Subacute Oral Toxicity Study in CD-I Mice (4 Weeks Administration by Gavage). Study No. T4079477

GENETIC TOXICOLOGY

Report #	Report Title
PH-33605	Salmonella/Microsome Test. Plate Incorporation and Preincubation Method. Study No. T 1074307
PH-33732	*In Vitro* Chromosome Aberration Test with Chinese Hamster V79 Cells. Study No. T 2074308
PH-33682	Micronucleus Test on the Male Mouse. Study No. T 3074309
Other Genetic Toxicity Studies	
PH-35197	BAY 75-7495: Salmonella/Microsome Test Plate Incorporation and Preincubation Method. Study No. T 0077772
PH-35596	BAY 81-8752: Salmonella/Microsome Test Plate Incorporation and Preincubation Method. Study No.: T 1079285
A57981	Evaluation of (b) (4) in a bacterial reverse mutation study using Salmonella typhimurium (Ames-Test). Study No. TOXT7082944
PH-35108	(b) (4) Salmonella/Microsome Test Plate Incorporation Method. Study No.: T 8077761

Report #	Report Title
PH-35194	(b) (4) Salmonella/Microsome Test Plate Incorporation and Preincubation Method. Study No. T 3077775,
PH-33994	(b) (4) BAY 73-4506. Salmonella/Microsome Test Plate incorporation Method. Study No. T 3074778
PH-33995	(b) (4) l. *In Vitro* Chromosome Aberration Test With Chinese Hamster V79 Cells. Study No.: T 4074779
PH-35637	BAY 81-8752: *In Vitro* Chromosome Aberration Test with Chinese Hamster V79 Cells. Study No. T 0079284
PH-35189	BAY 75-7495: *In Vitro* Chromosome Aberration Test With Chinese Hamster V79 Cells. Study No. T 9077771
Report No. A48891	(b) (4) - Bone Marrow Micronucleus Test and Liver Comet Assay In Male and Female Rats after Oral Administration over 3 Days. Study No. TOXT8081081

REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

Embryo-Fetal Development (NDA Module 4.2.3.5.2)	
PH-36547	Pilot prenatal developmental toxicity study in rats after oral administration
PH-36036	Developmental toxicity study in rabbits after oral administration

3.2 Studies Not Reviewed

PHARMACOKINETICS/TOXICOKINETICS*

Report #	Report Title
Analytical Methods and Validation	
PH-33332.	(b) (4)
PH-33342	
PH-35490	
PH-35693	
A59116	Bioanalytical Methods and Validation for Preclinical and Toxicokinetic Studies
A59117	Bioanalytical Methods and Validation for Clinical Studies
PH-34021	(b) (4)

Report #	Report Title
PH-34893	(b) (4)
Absorption	
A55575	Plasma Pharmacokinetics of BAY 73-4506 and BAY 75-7495 (M 2) and BAY 81-8752 (M-5) in Female Nude Mice after Single Peroral and Multiple Administration of BAY 73-4506, BAY 75-7495 and BAY 81-8752. Study Nos. I 4940-7, I 5006-1
PH-35862	Pharmacokinetics of BAY 75-7495 (M-2), BAY 73-4506, and BAY 81-8752 (M-5) in Male Wistar Rats after Single Oral Administration of BAY 75-7495. Study No. I 4830-5
PH-36084	Pharmacokinetics of BAY 81-8752 (M-5) and BAY 75-1098 (M-4) in Male Wistar Rats after Single Oral Administration of BAY 81-8752. Study No.: I 4920-5
PH-34034	Pharmacokinetics of Unchanged Compound and [^{14}C]BAY 73-4506 Radioactivity in Male Wistar Rats after Single Intravenous and Oral Administration. Study Nos. I 5001761, I 4609-9, I 4624-6
A49045	Pharmacokinetics of BAY 73-4506 and Total Radioactivity in Female Beagle Dogs after Single Administration of [^{14}C]BAY 73-4506. Study Nos. I 4715-7, I 4975-5
PH-34014.	Pharmacokinetics of Unchanged BAY 73-4506 in Female Beagle Dogs after Single Intravenous and Oral Administration. Study No. I 5001770
I 4687-5	Pharmacokinetics of BAY 73-4506 and its Metabolites BAY 75-7495 (M-2), M-3, BAY 75-1098 (M-4) and BAY 81-8752 (M-5) in Female Rhesus Monkeys after Single Oral Administration of BAY 73-4506.
Distribution	
A44224	*In vitro* investigations for [^{3}H] BAY 75-1098 and [^{3}H] BAY 81-8752 Binding to plasma proteins in different species *in vitro*. Study No(s). KINE 080154, I 5113-0
PH-34277	Investigation of the Stability in Plasma and the Binding to Plasma Proteins of [^{14}C]BAY 75-7495, the M-2 Metabolite of BAY 73-4506. Study Nos. B4-757495-51, B8-734506-59
A47928	Extended Investigations of the Binding to Plasma Proteins and Interaction Studies of [^{14}C]BAY 73-4506 *In Vitro* in Human Plasma. Study Nos. I 4871-0, I 4874-3, I 4876-5,
PH-35209	Quantitative Whole-body Autoradiography. Distribution of Radioactivity and Elimination from Blood, Organs, and Tissues after Single Oral Administration to Male Wistar and Long Evans Rats of [^{14}C]BAY 73-4506. Study No. I 4859-6,

Report #	Report Title
Metabolism	
A57473	[^{14C}]BAY 73-4506: *In Vitro* Metabolic Profiling and Species Comparison in Hepatocytes
A51918	[^{14}C]BAY 73-4506: Biotransformation in Mice. Study No(s). I 4688-6/A
A49684	[^{14}C]BAY 73-4506: Biotransformation in Rats. Study No(s). I 4609-9/D, I 4558-2/A, I 5001752/01 + /02 + /03
A59256	[^{14}C]BAY 73-4506: Biotransformation in Beagle Dogs. Study No(s). I 4715-7/A + /B + /C
A50624	Inhibitory Potency of BAY 73-4506 and Its Metabolites towards Human Dihydropyrimidine Dehydrogenase *in Vitro*
A58506	Identification of Enzymes Involved in the Oxidative Metabolism in Human *In vitro*
A57553	Determination of the Inhibitory Potency of Metabolites of BAY 73-4506, M-2 (BAY 75-7495) and M-5 (BAY 81-8752) Towards Human CYP Isoforms *In Vitro.* Study No(s). KINM 100100-ELB, KINM 100101-ELB
PH-34703	Evaluation of the CYP Induction Potential of BAY 73-4506 in Cultured Human Hepatocytes
A59022	[^{14}C]BAY 73-4506: Biotransformation in Man. Study No(s). KINM 110009-ELB
A59099	Identification of Human UDP-glucuronosyl Transferase (UGT) Isoforms Involved in the *In Vitro* Metabolism of BAY 73-4506
PH-34036	Determination of the Inhibitory Potency of BAY 73-4506 Towards Human UDP-glucuronosyltransferases (UGTs)
PH-34364	Determination of the Inhibitory Potency of BAY 73-4506 towards Human CYP Isoforms
PH-35818	Determination of the Inhibitory Potential of Metabolites M-2 (BAY 75-7495) and M 5 (BAY 81-8752) Towards Human UDP-glucuronosyltransferases (UGTs)
Pharmacokinetic drug interactions	
A59108	Effects of Drugs on the Glucuronidation of BAY 73-4506 *in Vitro.* Study No(s). KINM 110098-ELB
A59249	Effects of Drugs on the *N*-Oxidation of BAY 73-4506 *in Vitro.* Study No(s). KINM 110098-ELB
Other Pharmacokinetic Studies	
A58768	*In Vitro* Studies in MDCKII-BCRP Cells to Evaluate the BCRP Substrate

Report #	Report Title
	Characteristics. Study No(s). GH110318, GH110319, B115150, B115151
A58796	*In Vitro* Studies in L-MDR1 Cells to Evaluate the P-gp Substrate Characteristics. Study No(s). : IC110106, IC110108, B115147
PH-36201	In Vitro Studies in L-MDR1 Cells to Evaluate the P-gp Inhibition Potential
PH-36293	Determination of the Inhibitory Potential towards Human BCRP using MDCKII-BCRP Cells
PH-36645	Investigation on cell permeability of BAY 73-4506 in Caco-2 cells with regard to BCS classification
PH-36646	*In Vitro* Studies in HEK-OATP1B1 and HEK-OATP1B3 Cells to Evaluate the Substrate Characteristics and the Inhibitory Potential
R-8644	*In vitro* studies to determine the inhibitory potential of BAY 73-4506 towards OAT1, OAT3 and OCT2

*some *in vitro* PK studies were evaluated in the review of clinical pharmacology

GENERAL TOXICOLOGY

Report #	Report Title
Single-Dose Toxicity	
PH-33610	Acute toxicity in the mouse and rat after oral administration. Study Nos. T 8074656, T 9074657
PH-33610A	Acute toxicity in the rat and mouse after oral administration. Study Nos. T 8074656, T 9074657
Repeat-Dose Toxicity	
PH-34500	Subacute Oral Toxicity Study in CD-1 Mice (4 Weeks Administration by Gavage). Study No. T 3076262
PH-35918	Subacute Oral Toxicity Study in CD-I Mice, 5 Weeks Administration by Gavage. Study No. T5079478
PH-33468	Subacute Oral Toxicity Study in Rats (Two Weeks Administration by Gavage). Study No. T6073132
PH-34484	Subchronic Oral Toxicity Study in Rats, 13 Weeks Administration by Gavage with a Subsequent Recovery Period of 4 Weeks. Study No. T 4075651

The applicant provided nonclinical references (papers, abstracts and posters) which are summarized by the applicant (in NDA Module 2.6.2 Pharmacology Written Summary). These papers were not fully reviewed:

- Chapman MS Expert Opin Investig Drugs 2011:20:209-220
- Fichtner I Eur J Cancer 2004:40:298-307
- Huynh H EORTC AACR NCI 2011:B2
- Linardou H Cancer Treat Rev 2011:37:221-233
- Pratilas CA Clin Cancer Res 2010:16:3329-3334
- Su M-Y J Magn Reson Imaging 1999:9:128-137
- Villanueva A Gastroenterology 2011:140:1410-1426
- Wilhelm S EORTC AACR NCI 2007:B260
- Wilhelm S EORTC AACR NCI 2009:B4
- Wilhelm S IJC 2011:129:245-255
- Zopf D AACR 2010:1666
- Zopf D AACR 2011:4262

3.3 Previous Reviews Referenced

Pharmacology/Toxicology review of IND 75,642 by Dr. Haleh Saber

TOXICOLOGY

Report #	Report Title
Repeat-Dose Toxicity	
PH-34206	Subacute Oral Toxicity Study in Rats, 4 Weeks Administration by Gavage with a Subsequent Recovery Period of 4 Weeks. Study No. TI074622
PH-34182	Subacute Oral Toxicity Study in Beagle Dogs (4 Week Gavage Study with 4 Week Recovery Period). Study No. T2074704

4 PHARMACOLOGY

4.1 Primary Pharmacology

The applicant claims (NDA Module 2.6.2 Pharmacology Written Summary) that:

- "Regorafenib (BAY 73-4506) is a novel, oral, multi-kinase inhibitor of angiogenic, stromal and oncogenic (receptor tyrosine) kinases (TK)"
- Regorafenib has a distinct kinase inhibition profile from sunitinib and sorafenib.

The applicant provided three study reports (reviewed below) showing that regorafenib binds (report # A58227) and inhibits (reports # A57121 and # A58230) multiple tyrosine kinases under the *in vitro* conditions tested. Additionally, the applicant provided a paper, Zopf et al. 2010, which reported that regorafenib inhibited VEFGR2 (IC_{50} = 40 nM) and KIT(K642E; IC_{50} = 12 nM)

Study title: **Kinase profiler**	
Study no.:	A57121 Other study #s listed: • BAY051 • SAG103 • SAG161 • SAG175 • SAG199
Study report location:	NDA module 4.2.1 Primary Pharmacodynamics
Report date:	Not dated
Conducting laboratory and location:	(b) (4)
GLP compliance:	No
Drugs:	Regorafenib (BAY051), M-2 and M-5

Key Study Findings:

- Of the kinases screened, regorafenib was most potent against Ret > PTK5 ≈ PDGFRα(V561D), Flt1, KDR> FGFR1, DDR2 under the *in vitro* conditions tested
- Regorafenib, and the metabolites M-2 and M-5, inhibited multiple tyrosine kinases under the *in vitro* conditions tested

Method notes:

- Regorafenib, M-2 and M-5 were screened at 1 µM against a panel of 175 kinases (experiment 1; data not presented in this review)
- For specific kinases (experiments 2-5), additional doses of regorafenib, M-2 and M-5 were screened to identify the 50% inhibitory concentration (IC_{50})
- In a follow-up experiment, IC_{50} values were determined for regorafenib, M-2 and M-5 against mutant forms of KIT, TIE2 and PDGFR alfa
- Data for other compounds were also reported (but were not reviewed for this NDA)
- **Note**: Flt1 is VEGFR1; KDR is VEGFR2

Table 1: Kinase IC_{50} values for regorafenib, M-2 and M-5 (report # A57121)

Kinase	Regorafenib (BAY40, BAY 73-4506): IC_{50} (nM)	M-2 (BAY41, BAY 75-7495) : IC_{50} (nM)	M-5 (BAY42 (BAY 81-8752) : IC_{50} (nM)
2nd experiment			
Ret(h)	1	1	1
PTK5(h)	8	6	5
FGFR1(h)	26	46	84

Kinase	Regorafenib (BAY40, BAY 73-4506): IC_{50} (nM)	M-2 (BAY41, BAY 75-7495) : IC_{50} (nM)	M-5 (BAY42 (BAY 81-8752) : IC_{50} (nM)
DDR2(h)	29	36	35
SAPK2a(h)	44	41	56
Lyn(h)	44	46	79
FGFR2(h)	50	78	152
SAPK2b(h)	56	51	54
Abl(h)	56	63	39
TrkA	74	16	16
EphA2(h)	85	40	44
cKit(h)	807	715	684
PDGFRβ(h)	>1000	134	221
3rd experiment			
Flt1(h)	16	9	11
EGFR	ND	> 1000	ND
4th experiment			
Ret(h)	2	ND	
PDGFRα(V561D)(h)	6		
Flt1(h)	10		
KDR(h)	10		
Tie2(R849W)(h)	43		
PDGFRα(D842V)(h)	50		
Tie2(Y897S)(h)	70		
Flt3(h)	162		
Tie2(h)	471		
PDGFRα(H)	886		
5th experiment (selected data)			
cKit(V560G)(h)	13	ND	
c-RAF(h)	67		
cKit(V654A)(h)	79		
Hck(h)	85		
Abl(T315I)(h)	149		
Mer(h)	172		
cKit(D816H)(h)	239		
Yes(h)	531		
Rse(h)	663		
Axl(h)	895		

ND: not determined

Study title: **Kinase profiling**	
Study no.:	A58227 Other study #s listed: • BSP001-01-p-00001 • BSP002-01-s-00001 • BSP003-01-p-00001
Study report location:	NDA module 4.2.1 Primary Pharmacodynamics
Report date:	Not dated
Conducting laboratory and location:	(b) (4)
GLP compliance:	No
Drugs:	Regorafenib, M-2 and M-5 (no lot #s or purity information provided)

Key Study Findings

- Under the conditions tested, regorafenib bound most strongly to Kit and Kit variants, PDGFR, PDGFRβ, and RET. Strong binding was also observed to RET variants, VEGFR, and 12 other kinases.
- M-2 and M-5 had similar, but not identical, binding profiles compared to regorafenib.

Method notes

- Competitive binding of regorafenib, M-2 and M-5 to a panel of kinases was assessed in several *in vitro* screening assays, and reported as K_d values.
  - 68 Kd values were reported for regorafenib
  - 8 Kd values were reported for M-2
  - The M-5 metabolite of regorafenib was also assessed (in experiment #4, results not summarized in this review)

Results notes

- In experiment 1, kinases were screened against a single dose (1 μM):
  - strongest binding of regorafenib was observed to Kit [as well as Kit(L576P), Kit(V559D), and Kit(V559D,T670I)], PDGFRβ, and RET
  - Very strong binding of regorafenib was observed to RET(M918T), RET(V804L), RET(V804M), CDK11, CDKL2, CSF1R, DDR1, ERK8, FLT3, FLT3 (K663Q), FLT4, LOK, p38-beta, PDGFRA, and VEGFR2
  - M-2 and M-5 had binding profiles similar to, but not identical to, those of regorafenib.
    - M-2 bound more strongly than regorafenib to: JNK2, MAPK4K, MKNK1, p38-alpha, p38-beta, PDGFRA especially; and also CDK11, CSF1R, EPHA8, ERK8, FLT1, FLT3, FLT4, LCK, LOK
    - M-5 bound more strongly than regorafenib to: MAP4K4, MKNK1, MKNK2 especially; and also CDK11, CDK8, CSF1R, DDR1, EPHA8, LOK

- In experiment 2, the only remarkable data are for regorafenib, as shown in Table 2 of this review (M-2 was screened for a few kinases, and M-5 was not screened):

Table 2: Selected K_D values for regorafenib (report # A58227)

Kinase	Regorafenib (BSP-1, BAY 73-4506): K_D (nM)
DDR1	0.77
ZAK	2
HIPK4	4.5
FLT3	4.8
RET	5.2
KIT	6.9
PDGFRB	8.3
DDR2	9.7
CSF1R	10
LOK	10
FLT4	15
RET(V804L)	15
ABL1-nonphosphorylated	16
ERK8	17
PDGFRA	19
EPHA6	23
FLT1	27
TIE1	27
VEGFR2	28
P38-beta	28
FRK	42
BRAF(V600E)	42
P38-alpha	48
CDK11	43
BRAF	52
CDKL2	58
RAF1	59
MKN2	64
MUSK	67
CDK8	73
YSK4	94
SLK	98

Study title: **Inhibitory potential of 6 compounds using a cellular TIE2 phosphorylation assay. Inhibitory potential of 7 compounds using a cellular TIE2 phosphorylation assay. Inhibitory potential of 12 compounds using a cellular TIE2, KIT, and B-RAF-VE phosphorylation assay.**	
Study no.:	A58230 Original study report numbers: • 3911 • 3949 • 4125
Study report location:	NDA module 4.2.1 Primary Pharmacodynamics
Report date:	March 20, 2009
Conducting laboratory and location:	(b) (4)
GLP compliance:	no
Drug:	Regorafenib, M-2 and M-5 (no lot #s or purity information provided)

Key Study Findings

Under the conditions tested *in vitro*, the following IC_{50} values were determined:

- Regorafenib:
  - TIE2 autophosporylation, IC_{50} range of 24 to 41 nM
  - KIT IC_{50} = 23 nM (2.3E-08)
  - B-RAF-VE IC_{50} = 69 nM (6.9E-08)
- M-2:
  - TIE2: IC_{50} = 90 nM (9.0E-08)
  - KIT IC_{50} = 13 nM (1.3E-08)
  - B-RAF-VE IC_{50} = 21 nM (2.1E-08)
- M-5:
  - TIE2: IC_{50} = 180 nM (1.8E-07)
  - KIT IC_{50} = 110 nM (1.1E-07)
  - B-RAF-VE IC_{50} = 27 nM (2.7E-08)

Method notes:

- CHO cells were transfected to overexpress one of three human kinases, then treated with ligand to stimulate phosphorylation
  - For TIE2: 10 mM of sodium orthovandadate for 15 minutes
  - For KIT: 100 ng/ml of SCF for 3 minutes
  - For B-RAF-V600E: 1 µM of tamoxifen for 1 hour
- Prior to ligand stimulation, cells were treated with regorafenib, M-2, M-5, or other compounds for 90 minutes.
- Quantitation of phosphorylation was assessed using an ELISA with a anti-phosphotyrosine detection antibody

Study title: **Multiplexed cytotoxicity assay**	
Study no.:	A58229 Original study # AA96857
Study report location:	NDA module 4.2.1 Primary Pharmacodynamics
Report date:	October 29, 2010
Conducting laboratory and location:	(b) (4)
GLP compliance:	no
Drug:	Regorafenib (BSP001-0734; lot # and purity information not provided)

Key Study Finding

- The activity of regorafenib to inhibit proliferation, inhibit mitosis, and induce apoptosis was evaluated i*n vitro* in a panel of 25 human colorectal cancer cell lines and 7 pancreatic cancer cell lines
- Regorafenib inhibited cell proliferation, with IC_{50} values ranging from 40 nM to 10 µM
- Regorafenib clearly induced apoptosis in only 4 cell lines

Method notes

- Each cell line was grown in the same media; cells were plated for 24 hours and incubated with regorafenib for 72 hours, then fixed and evaluated by immunohistochemistry for:
  - Cell proliferation (by measuring incorporation of a nuclear dye relative to cell count)
  - Late stage apoptosis (using an antibody against activated caspase-3)
  - Mitosis (using an antibody against phospho-histone-3)
- The report provides results for other compounds (data not reviewed for this NDA)

Results notes

- The study report provided raw data, but no interpretation or conclusion. Therefore, the applicant's interpretation is useful in reviewing this report (see Table 3 below, from NDA Module 2.6.2 (Pharmacology Written Summary).
- For this review, the data superscripted with "c" refers to data from this report. Data superscripted with "b" are from a published paper.

Table 3: Regorafenib inhibited cell proliferation *in vitro* (report # A58229)

Cell proliferation activity[a]	IC_{50} (nM) ± SD (n)	genotyping[d]		
		KRAS	BRAF	other
VEGF/HUVEC[b]	2.6 ± 0.8 (2)	n.d.	n.d.	n.d.
FGF2/HUVEC[b]	127 ± 13 (2)	n.d.	n.d.	n.d.
PDGF-BB/HAoSMC[b]	146 ± 114 (6)	n.d.	n.d.	n.d.
GIST 882[b]	45 ± 20 (2)	n.d.	n.d.	KIT^{K642E}
TT, thyroid[b]	34 ± 8 (2)	n.d.	n.d.	RET^{C634W}
MDA-MB-231s, breast[b]	401 ± 88 (2)	G13D	G464V	CDKN2A,NF2,TP53
HepG2, liver[b]	560 ± 200 (3)	$NRAS^{Q61L}$	wt	CTNNB1
A375, melanoma[b]	900 (1)	wt	V600E	CDKN2A
SW620, colon[b,c]	967 ± 287 (2)	G12V	wt	APC,SMAD4,TP53
Colo-205, colon[b,c]	3269 (1)/4590	wt	V600E	APC,SMAD4,TP53, MAP2K4
HT-29, colon[c]	3890	wt	V600E	APC,PI3KCA, SMAD4,TP53
Mia PaCa-2, pancreas[c]	5310	G12C	wt	Notch 1
HCT-15, colon[c]	7610	G13D	wt	APC,BRCA1,MSH6, PI3KCA,TP53
BxPC3, pancreas[c]	>10000	wt	wt	CDKN2A,MAP2K4, SMAD4,TP53

[a] tumor cells were maintained and propogated using standard procedures; [b] Wilhelm et al 2011; [c] report A58299;
[d] data from COSMIC data base

Study title: ***In vivo* pharmacodynamics of BAY 73-4506 and its metabolites by magnetic resonance imaging (MRI) after various administrations in rats**	
Study no.:	A584234 Other study #s: • KM08132 • KM09228
Study report location:	NDA Module 4.2.1.1 Primary Pharmacodynamics
Report date:	January 20, 2012
Conducting laboratory and location:	(b) (4) Bayer Pharma AG Berlin, Germany
Date of study initiation:	June 2008
GLP compliance:	No
Drug, lot #, and % purity:	Regorafenib (purity not reported). Batch #s: • IV solution: H01152A01 (02.12.2009) for KM09228 • Oral solution: 5785A01 (23.06.2008) for KM08132 and H01131A01 (25.11.2009) for KM09228

Key Study Finding

- Under the conditions tested, regorafenib and M-2 transiently reduced the permeability of tumor blood vessels in tumor-bearing rats

Method notes:

- Female Fischer 344 rats were implanted with a rat brain glioblastoma cell line (GS9L cells) intramuscularly, into the left hind limb.
- Nine or ten days after inoculation, when tumors had reached a size of ~ 300 to 700 mm^3, and the magnetic resonance imaging (MRI) study was initiated
- MRIs were performed under isoflurane anesthesia using Gadomer-17 as the contrast agent; imaging was measured pre-dose, and post-dose at various time points (up to 8 days post-dose)
- Groups of 8 or 9 rats received regorafenib or M-2: 10 mg/kg once orally, 10 mg/kg/day for 4 consecutive days orally, 7.5 mg/kg once orally, or 7.5 mg/kg once iv.
- The authors state that IAUC360 (initial area under the MR signal versus time curve for 360 seconds of MR measurement) is a measure of blood vessel state that "depends on total tumor blood vessel volume and total permeability surface area of tumor vessels" (report page 13)

Results notes:

- After a single dose of regorafenib, a reduction in IAUC360 was observed from 8 hours to 2 days post-dose

- After multiple dosing with regorafenib, a reduction in IAUC30 was observed that persisted until 2 days after the last dose
- The authors concluded, and this reviewer concurs, that no differences were apparent between regorafenib and M-2, or between oral versus iv dosing.

The applicant submitted 5 studies demonstrating the anti-tumor activity of regorafenib in tumor-bearing mice. These studies have been reviewed. Briefly:

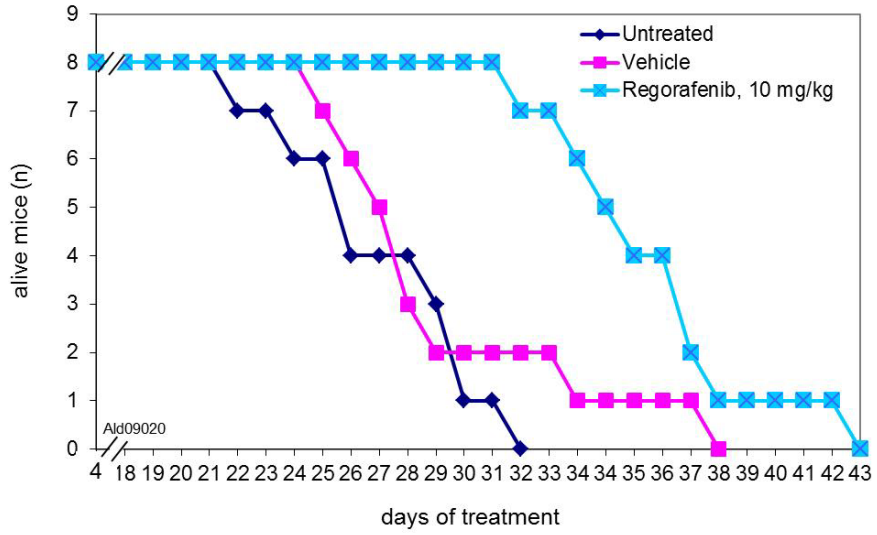
- Title: "**Investigation of 4 development compounds (regorafenib, its metabolites M-2 and M-5 and sorafenib) for tumor growth inhibition in the HT-29 CRC xenograft model on NMRI nu/nu mice**"
  - Study # A57101. Not GLP
  - Female athymic nude mice (NMRI: nu/nu) were implanted subcutaneously with a human colorectal cancer cell line (HT-29 cells).
  - Eleven days after inoculation, groups of 9 mice were dosed orally by gavage daily for 27 days
  - Doses:
    - Regorafenib: 0, 3, or 10 mg/kg of regorafenib
    - M-2: 3 or 10 mg/kg
    - M-5: 3 or 10 mg/kg
    - An additional compound (sorafenib) was tested as a positive control.
  - Regorafenib, M-2, and M-5 treatment slowed tumor growth, but did not cause shrinkage.
  - The 10 mg/kg doses were more active than the 3 mg/kg doses, and no clear differences were apparent among regorafenib, M-2 and M-5
  - The doses appeared tolerated; diarrhea and body weight loss (-2 to -3%) were observed in all dose groups (including vehicle controls; no treatment-relationship apparent)
  - No clear differences for anti-tumor activity apparent among regorafenib, M-2 and M-5.
  - **Note**: The report provides results for the positive control sorafenib; comparing the anti-tumor activities of regorafenib versus sorafenib is beyond the scope of this review.

- Title: "**Investigation of 4 development compounds (regorafenib, its metabolites M-2 and M-5 and sorafenib) for tumor growth inhibition in the MDA MB-231 breast xenograft model on NMRI nu/nu mice**"
  - Study # A57105. Not GLP.
  - Female athymic nude mice (NMRI: nu/nu) were implanted subcutaneously with a human breast cancer cell line (MDA MB-231)
  - Thirteen days after inoculation, groups of 9 mice were dosed orally by gavage daily for 27 days
  - Doses:
    - Regorafenib: 0, 3 or 10 mg/kg of regorafenib
    - M-2: 3 or 10 mg/kg

        - M-5: 3 or 10 mg/kg
        - An additional compound (sorafenib) was tested as a positive control.
    - Regorafenib, M-2 and M-5 treatment slowed tumor growth, but did not cause shrinkage.
        - The authors concluded, and this reviewer agrees, that the 10 mg/kg doses appeared to cause tumor stasis, but re-growth was observed at the 3 mg/kg doses
    - The doses appeared tolerated; diarrhea and body weight loss (-2 to -5%) were observed in all dose groups (including vehicle controls; no treatment-relationship apparent)
    - No clear differences in tumor activity apparent among regorafenib, M-2 and M-5.
    - **Note**: The report provides results for the positive control sorafenib; comparing the anti-tumor activities of regorafenib versus sorafenib is beyond the scope of this review.

- Title: "**Efficacy of regorafenib on the survival of syngeneic mice with orthotopically transplanted H129 hepatoma**"
    - Report # A58231
    - In C3H/HeN mice, H129 hepatoma cells (a syngenic tumor) were implanted into the liver (upper left lobe). [Sex not reported]
    - Four or five days after implant, groups of 8 mice were untreated, dosed with vehicle or dosed with 10 mg/kg/day of regorafenib orally until death
    - Regorafenib improved survival compared to vehicle:
        - Controls: 50% survived to 27 days, all died by day 31
        - 10 mg/kg regorafenib: 50% survived to day 35, all died by day 43.
    - Regorafenib deceased body weight (approximately -8% compared to base-line)
    - From the report (page 14):

Figure 1: Regorafenib (10 mg/kg/day orally) prolonged survival in mice bearing implanted hepatomas (report # A58231)



- Report # A57118
  - The report has four titles (for four sub-reports, none are GLP):
    - **"Evaluation of BAY 734506 in combination with Irinotecan in the oxaliplatin resistant human colorectal tumor model Co8183"**
    - **"Evaluation of BAY 734506 in combination with Irinotecan in the Oxaliplatin resistant human colorectal tumor model Co8434"**
    - **"Evaluation of BAY 734506 in combination with Irinotecan in the Oxaliplatin resistant human colorectal tumor model Co8435"**
    - **"Evaluation of BAY 734506 in combination with Irinotecan in the Oxaliplatin resistant human colorectal tumor model Co5896"**
  - Method notes:
    - Four human colorectal cancer cell lines (Co8183, Co8434, Co8435, and Co5896) were tested in male athymic nude mice (NMRI nu/nu)
    - Groups of 8 mice received vehicle, irinotecan alone (15 mg/kg ip, 5 days per week), regorafenib alone (10 mg/kg orally daily), oxaliplatin alone (5 mg/kg ip, 5 days per week), or the combination of irinotecan and regorafenib (at their respective stand-alone doses and dosing schedules)
    - End of dosing was tumor-specific
  - Results notes:
    - Oxaliplatin did not exhibit anti-tumor activity (as expected for these cell lines)
    - Regorafenib (alone or in combination with irinotecan) caused weight-loss (approximately -5 to -12% from baseline)

- In Co8183 cells: irinotecan alone, regorafenib alone, and the combination slowed tumor growth (but did not cause stasis or regression). The combination was more active.
- In Co8434 cells: irinotecan alone was highly active (alone or in combination), regorafenib was not active alone and did not appear to increase the activity of irinotecan
- In Co8435 and Co 5896 cells: regorafenib inhibited tumor growth. Irinotecan alone was highly active. No additive or synergistic effect was observed with the combination.

- Title: " **Efficacy of regorafenib and sorafenib on primary tumor and metastases formation in the syngeneic orthotopic 4T1 breast cancer model in Balb/C mice"**
  - Report # A58233 (not GLP)
  - Female Balb/C mice were implanted with a mouse breast cancer cell line (4T1 cells) in the inguinal mammary fat pad.
  - Three different experiments were conducted, with daily dosing beginning 8 to 10 days after inoculation, and continuing "for about" 20 days (not specified in more detail in the study report)
  - Groups of 10 mice received 0 or 10 mg/kg of regorafenib per day. A separate study investigated sorafenib as a positive control.
  - Regorafenib inhibited tumor growth (as determined by comparing tumor weights at end of treatment) and tumor metastasis to the lung (assessed histologically)
  - Regorafenib caused weight loss (up to -5%)

4.2 Secondary Pharmacology

The applicant listed one study (report # PH-3660, reviewed below) as relevant to secondary pharmacology. The applicant characterized the *in vitro* anti-tumor and anti-vascular effects of M-2 and M-5 (report #s A57101, A57105, A57121, A58227, A58230, A58234, PH-36660; reports reviewed above) as well as the general toxicology of M-2 and M-5 (reviewed below). The activity of the M-2 and M-5 metabolites may be considered secondary pharmacology.

Study title: **Effects of regorafenib and its metabolites M-2 and M-5 in an *in vivo* assay for the systemic effects of exogenous VEGF in rats**	
Study no.:	PH-36660
Study report location:	NDA module 4.2.1.2 Secondary Pharmacodynamics
Report date:	November 29, 2011
Conducting laboratory:	Bayer HealthCare (location not reported)
GLP compliance:	No
Drugs:	Regorafenib, M-2, M-5 (lot # and purity not reported)

Key Study Findings

- Under the conditions tested, 1 mg/kg of regorafenib, M-2, or M-5 administered intravenously inhibited the activity of vascular endothelial growth factor (VEGF); no clear differences apparent
- 0.1 mg/kg of regorafenib attenuated the VEGF-induced effect (less potent than 1 mg/kg of regorafenib

Method notes

- Blood pressure was measured in catheterized male Wistar rats
- Groups of 6 male rats were dosed iv with control vehicle, 0.1, or 1 mg/kg of regorafenib, 1 mg/kg of M-2, or 1 mg/kg of M-5, and then 9 µg/kg of recombinant human VEGF was administered IV 10 minutes later, and blood pressure (systolic and diastolic) was measured for 25 minutes.

Results notes

- VEGF induced an immediate reduction of blood pressure (25 to 40 mm Hg) in control rats; the response peaked at approximately 10 minutes post-dose
- 1 mg/kg of regorafenib, 1 mg/kg of M-2, or 1 mg/kg of M-5 prevented the decrease in blood pressure
    - The authors suggest M-2 was less active, but this reviewer disagrees
- 0.1 mg/kg of regorafenib attenuated the VEGF-induced decrease in blood pressure, but the effect was not complete (approximately 20 mm Hg drop in systolic blood pressure)

4.3 Safety Pharmacology

The applicant concludes (NDA Module 2.6.2 Pharmacology Written Summary, sub-section 1.3 Safety Pharmacology) that "regorafenib is devoid of substantial adverse effects on cardiovascular (incl. ECG), respiratory, and CNS function"; likewise no adverse effects were reported for either the M-2 or M-5 metabolites. Sixteen safety pharmacology studies were submitted to NDA 203085.

Regorafenib was weakly active in the hERG assay (report # PH-33109), and this activity was confirmed in an assay with rabbit Purkinje fibers (report # 33827); however, both the M-2 (report # PH-33502) and M-5 (report # PH-33519) metabolites exhibited potent hERG inhibition. Therefore, it is unclear whether the clinically-observed minimal "effects of regorafenib at t_{max} on the QT_C intervals" (NDA module 2.5 Clinical Overview, page 39) are due to the direct activity of regorafenib or are due to the formation of metabolites in patients.

The safety pharmacology study intended to evaluate the effects of regorafenib on behavior, physiological state and body temperature in rats (report # PH-33840) was inadequate by design – the duration of observations did not encompass the C_{max} following oral absorption. Notably, the rat behavior safety pharmacology study for M-2

(report # PH-35438) had a longer observation window and detected slight toxicity (increased body temperature, increased activity, lying prone, stereotypic paw licking). The rat behavior safety pharmacology study for the M-5 metabolite did not detect any treatment-related changes (report # PH-35409).

The applicant evaluated hemodynamics, ECG and respiration in dogs following a single dose of regorafenib administered 'intraduodenally' (report # PH-33963) and intravenously (report # 35619); no treatment-related toxicity was observed. These endpoints were evaluated in dogs for the M-2 (report # PH-35628) and M-5 (report # PH-35620) metabolites administered iv.

Study title: **Effects of BAY 73-4506 on the HERG K+ current in stably transfected HEK293 cells**	
Study no.:	PH-33109 Other study #: T 2072850
Report date:	May 5, 2006
Study report location:	NDA module 4.2.1.3 Safety Pharmacology
Conducting laboratory and location:	Bayer HealthCare AG Wuppertal, Germany
Date of study initiation:	September 9, 2003
GLP compliance:	No
Drug, lot #, and % purity:	Regorafenib (BAY 73-4506), batch # 58669-090-3, purity > 98%

Key Study Findings

- A dose-response was observed for regorafenib inhibition of the hERG channel *in vitro*. The authors concluded that regorafenib was a "low-potency blocker of the HERG K^+ current. … high concentrations of BAY 73-4506 [regorafenib] possess the potential to delay the repolarization of cardiac action potentials."
    - IC_{50} = 27 µM (55.9 ng/L)
    - IC_{20} = 12 µM (24.9 ng/L)

Method and Results Notes

- HEK293 (human embryonic kidney cells) were transfected to express the human ether-a-go-go-related gene (hERG) potassium (K^+) channel.
- The concentrations tested were 0, 0.1, 1, 10 and 20 µM
    - Inhibition was observed 10 µM
    - The solubility limit in the assay was 20 µM; maximal inhibition was not observed at this concentration
- Note: The conversion from µM to ng/L above was calculated using the molecular weight provided in the study report (page 5) of 482.8 (the free-base molecular weight, because this was the molecular weight reported; presumably the concentrations were calculated using this molecular weight)

- Note: Although regorafenib appears to be a low-potency hERG blocker, the M-2 and M-5 metabolites are high-potency inhibitors (see reviews of reports # PH-33502 and # PH-33519 below).

Study title: **BAY 75-7595. Effects on the hERG K+ current in stably transfected HEK293 cells.**	
Study no.:	PH-35502 Other study #: T7078390
Report date:	September 1, 2008
Study report location:	NDA module 4.2.1.3 Safety Pharmacology
Conducting laboratory and location:	Bayer HealthCare AG Wuppertal, Germany
Date of study initiation:	April 23, 2008, 2008
GLP compliance and QA statement:	Yes, signed
Drug, lot #, and % purity:	M-2 (BAY 75-7495), batch # BXR3BSX, purity 91.5% (concentrations corrected for purity)

Key Study Findings

- M-2 (BAY 75-7495, molecular weight (b) (4)) exhibited a concentration-dependent inhibition of the hERG-mediated tail current amplitude. The authors considered M-2 to be "a potent blocker of the hERG K^+ current" (report page 5)
  - IC_{50} = 1.1 µM (2.2 ng/L)
  - IC_{20} = 0.4 µM (0.81 ng/L)

Study title: **BAY 81-8752. Effects on the hERG K+ current in stably transfected HEK293 cells.**	
Study no.:	PH-35519 Other study #: T2078395
Report date:	September 17, 2008
Study report location:	NDA module 4.2.1.3 Safety Pharmacology
Conducting laboratory and location:	Bayer HealthCare AG Wuppertal, Germany
Date of study initiation:	May 14, 2008
GLP compliance and QA statement:	Yes, signed
Drug, lot #, and % purity:	M-5 (BAY 81-8752), batch # BXR3TVA, purity 98.4% (concentrations corrected for purity)

Key Study Findings

- M-5 (BAY 81-8752, molecular weight (b) (4)) exhibited a concentration-dependent inhibition of the hERG-mediated tail current amplitude. Like M-2, the authors considered M-5 to be "a potent blocker of the hERG K^+ current" (report page 5)
  - IC_{50} = 1.8 µM (3.7 ng/L)
  - IC_{20} = 0.4 µM (0.83 ng/L)

Study title: **Effects of BAY 73-4506 on the action potential of isolated rabbit cardiac Purkinje fibers**	
Study no.:	PH-33827 Other study #: T 1074848
Report date:	April 18, 2005
Study report location:	NDA module 4.2.1.3 Safety Pharmacology
Conducting laboratory and location:	Bayer HealthCare AG Wuppertal, Germany
Date of study initiation:	February 24, 2005
GLP compliance:	No
Drug, lot #, and % purity:	Regorafenib (BAY 73-4506), batch # BX01K3D, purity = 95.5% (doses corrected for purity)

Key Study Findings

- Regorafenib did not prolong the action potential duration at 20% depolarization (APD_{20}), APD_{50} or APD_{90} at 0.2 or 2 µmol/L
- At 20 µmol/L (0.04 µg/L) regorafenib inhibited the APD_{50} (statistically significant difference compared to vehicle control) by -23%; but did not inhibit APD_{20} or APD_{90}.
- The authors concluded, "The shift of the plateau potential to more negative values together with the more pronounced shortening of APD_{50} than of APD_{90}, however, are suggestive of an inhibition of Ca^{2+} inward currents by BAY 73-4506 at concentrations approaching the solubility limit." (report page III).

Methods and Results Notes

- Fresh Purkinje fibers were isolated from the cardiac ventricles of rabbits (age and strain not reported) and were mounted in a horizontal organ batch for these experiments. Each preparation was equilibrated for approximately 60 minutes, until "stable" pre-specified criteria were met for the resting membrane potential and action potential amplitude.
- Other endpoints measured (not affected by regorafenib) include: changes of resting membrane potential (RMP), action potential amplitude (APA), maximal

depolarization velocity (V_{max}), and plateau potential at 35 milliseconds after the V_{max}.

- The concentrations tested were 0, 0.2, 2 and 20 µM of regorafenib. Reportedly, higher concentrations could not be tested due to solubility limits under the conditions tested. This reviewer infers that nominally higher *in vivo* concentrations may be achievable at least in part because of the reported high binding of regorafenib to serum albumin (reference is made to report # PH-24096).
- Note: Although Figure 1 in the study report (report page 10) is not legible, the numerical data provided in Table 4 (report page 11) are adequate to review the results. Therefore, no revision of the report is required to fix the figure.

Study title: **Effect of a single oral administration of BAY 73-4506 on the behavioral and physiological state, open-field behavior, and body temperature of rats**

Study no.:	PH-33840 Other study #: T 1074550
Report date:	April 29, 2005
Study report location:	NDA module 4.2.1.3 Safety Pharmacology
Conducting laboratory and location:	Bayer HealthCare AG Wuppertal, Germany
Date of study initiation:	January 25, 2005
GLP compliance and QA statement:	Yes, signed
Drug, lot #, and % purity:	Regorafenib (BAY 73-4506), batch # 040607-010 (purity 9.9%, doses corrected for purity)

Key Study Findings

- **This study is of limited usefulness for regulatory purposes**. Endpoints were only measured for 2 hours post-dose, and the applicant reports a T_{max} of 4 to 6 hours in rats following oral dosing of regorafenib (NDA Module 2.6.4 Pharmacokinetics Written Summary)
- No treatment-related effects were observed up to 2 hours post-dose, after male rats were dosed once orally with regorafenib (up to 50 mg/kg)

Methods

Doses:	0, 2, 10 or 50 mg/kg
Frequency of dosing:	Once (single dose)
Route of administration:	Oral gavage
Dose volume:	10 ml/kg
Formulation/Vehicle:	Tap water; controls received the placebo powder (PLCA POWD 000, batch # 040624-000)
Species/Strain:	Male rats (HsdCpb strain)
Number/Sex/Group:	6 males/dose

Age: Approximately 8 weeks old at time of dosing
Weight: 211 to 250 g

Method notes

- Food and water were available *ad libitum* (no fasting prior to dosing)
- Pre-dose body temperature was measured
- At approximately 30, 60, 90 and 120 minutes post-dose, animals were taken from their cages and assess for symptoms/behavior for approximately 4 minutes (including piloerection, ptosis, exophthalmus, lacrimation, salivation, diarrhea, vocalization, difficulty in respiration, posture and gait, stereotypic behavior, involuntary movements, alterations in motor activity and vigilance) and physiological state), with specific attention given to response to touch.
- At approximately 30 minutes and 2 hours post-dose (after the evaluation of behavior and physiological state), animals were placed singly into open-field boxes. Endpoints automatically assessed over 5 minutes included traveled distance, resting time, and rearing.
- Body temperature was measured electronically using a stomach probe after each behavioral assessment.

Results notes

- No symptoms/behavior changes were noted for any animal
- No changes in open-field behavior or temperature were apparent
- The results of the rat behavior safety pharmacology study for M-2 (report # PH-35438) raise the theoretical concern that regorafenib-treated rats might have exhibited toxicity if observed longer post-dose; this study detected small treatment-related effects for M-2 at 4 hours (see review below).

Study title: **BAY 75-7495. Effect of a single oral administration on the behavioral and physiological state, open-field behavior, and body temperature of rats**

Study no.:	PH-35438 Other study #: T8078364
Report date	July 3, 2008
Study report location:	NDA module 4.2.1.3 Safety Pharmacology
Conducting laboratory and location:	Bayer HealthCare AG Wuppertal, Germany
Date of study initiation:	April 15, 2008
GLP compliance and QA statement:	Yes, signed
Drug, lot #, and % purity:	M-2 (BAY 75-7495), batch # BXR3BSX (purity 91.5%, doses corrected for purity)

Key Study Findings

- The authors considered the results as showing “no substantial effects on the behavioral and physiological state of rats” treated with M-2. This reviewer disagrees

- The mid-dose tested, 5 mg/kg of M-2, is a NOAEL. At the high-dose, 20 mg/kg of M-2, there was increased activity at 4 hours post-dose and slightly increased body temperature at 2 and 4 hours post-dose. The reversibility of these changes was not assessed.
- One high-dose animal (1/6 at 20 mg/kg of M-2) exhibited transient prone position and stereotypic licking, suggesting a possible effect of M-2 on behavior.

Methods

Doses:	0, 1, 5, 20 mg/kg of M-2
Frequency of dosing:	Once (single-dose study)
Route of administration:	Oral gavage
Dose volume:	6.25 or 5 ml/kg
Formulation/Vehicle:	42.% PEG400, 42.5% propylene glycol, 15% Pluronic F68
Species/Strain:	Male rats (HsdCpb strain)
Number/Sex/Group:	6 males/dose
Age:	Approximately 7 weeks old at time of dosing
Weight:	182 to 207 g

Method notes:

- Food and water were available *ad libitum* (no fasting prior to dosing)
- At approximately 30 minutes, 2 hours and 4 hours post-dose, rats were taken from their cages and assessed for symptoms/behavior and physiological state), with specific attention given to response to touch.
- At approximately 30 minutes and 4 hours post-dose (after the evaluation of behavior and physiological state), animals were placed singly into open-field boxes. Endpoints automatically assessed over 5 minutes included traveled distance, resting time, and rearing.
- Body temperature was measured electronically using a stomach probe pre-dose and after each behavioral assessment

Results notes:

- One high-dose rat (1/6) displayed prone position and stereotypic licking in the open-field box, 30 minutes after dosing. The authors considered this an incidental finding.
- Body temperature increased for the 20 mg/kg group at 2 and 4 hours.
    - The authors noted this effect, but considered it “without any biological relevance due to the small size of the increase and the small standard deviations”. This reviewer disagrees, and considers the effect treatment-related and pharmacologically-relevant
    - From the report (page 12):

Table 4: M-2 slightly increased body temperature in a rat behavioral safety pharmacology study (report # PH-35438)

Dose of M-2 (mg/kg)	Body temperature (^{o}C) at approximate time after treatment (hours)			
	0 (pre-dose)	0.5	2	4
0	36.9 ± 0.2	37.6 ± 0.5	37.7 ± 0.2	37.8 ± 0.2
1	36.7 ± 0.2	38.2 ± 0.3	37.9 ± 0.2*	37.9 ± 0.2
5	36.8 ± 0.2	38.0 ± 0.3	37.8 ± 0.1	38.0 ± 0.2
20	36.8 ± 0.2	38.0 ± 0.9	38.0 ± 0.1*	38.4 ± 0.2*

Data expressed as mean ± standard deviation
* statistically significantly different from controls, $p < 0.05$

- In the open-field test, control animals exhibited habituation to the test box (i.e. a decrease in activity at 4 hours compared to 30 minutes); habituation was also observed at 1 and 5 mg/kg. The 20 mg/kg group exhibited an attenuation of the habituation.
  - The authors noted the attenuation at 20 mg/kg and considered it treatment-related, but not a sign of toxicity.
  - From the report (pages 11-12):

Dose of M-2 (mg/kg)	Traveled distance (meters) at approximate time after treatment (hours)		Number of rearings at approximate time after treatment (hours)	
	0.5	4	0.5	4
0	31.5 ± 14.9	24.4 ± 13.4	8.5 ± 4.8	7.8 ± 6.1
1	32.9 ± 10.7	24.9 ± 14.9	11.7 ± 7.0	7.7 ± 5.7
5	30.8 ± 16.0	23.8 ± 8.0	12.0 ± 9.0	8.7 ± 5.5
20	35.2 ± 17.2	33.7 ± 20.0	9.5 ± 7.0	11.8 ± 6.8

Study title: **BAY 81-8752. Effect of a single oral administration on the behavioral and physiological state, open-field behavior, and body temperature of rats**	
Study no.:	PH-35409 Other study #: T7078363
Report date	June 16, 2008
Study report location:	NDA module 4.2.1.3 Safety Pharmacology
Conducting laboratory and location:	Bayer HealthCare AG Wuppertal, Germany
Date of study initiation:	April 17, 2008
GLP compliance and QA statement:	Yes, signed
Drug, lot #, and % purity:	M-5 (BAY 81-8752), batch # BXR3TVA (purity 97.5, doses corrected for purity)

Key Study Findings

- No treatment-related effects were observed for M-5 in this study
  - The methodology for this study was similar to the M-2 study (report # PH-35438) reviewed above.
  - Groups of 6 male rats received single oral doses of 0, 1, 5, or 20 mg/kg of M-5 by oral gavage (vehicle was 42.5% PEG400, 42.5% propylene glycol, 15% Pluronic F68).
  - Behavior/symptoms were assessed at 0.5, 2 and 5 hours post-dose.
  - The open-field test was conducted at 2 and 5 hours post-dose.
  - Body temperature was measured pre-dose and after assessment of behavior (i.e. at 0.5, 2 and 5 hours post-dose)

Study title: **BAY 73-4506: influence on haemodynamics, ECG and respiration in anaesthetized dogs after single intraduodenal administration**	
Study no.:	PH-33963 Other study #: T 9074549
Report date:	July 18, 2005
Study report location:	NDA module 4.2.1.3 Safety Pharmacology
Conducting laboratory and location:	Bayer HealthCare AG Wuppertal, Germany
Date of study initiation:	February 15, 2009 (i.e. after the iv study, report # PH-35619)
GLP compliance and QA statement:	Yes, signed
Drug, lot #, and % purity:	Regorafenib (BAY 73-4506), batch # BX01K3D, purity 95.2% (doses adjusted for purity)

Key Study Findings

- Single intraduodenal doses of 10, 30 or 100 mg/kg of regorafenib had no apparent effects on hemodynamics, ECG, or respiration endpoints in beagle dogs.
- Intraduodenal dosing was used because the dogs were anesthetized during dosing.

Methods

Doses:	0, 10, 30 or 100 mg/kg of regorafenib
Frequency of dosing:	Once (single dose study)
Route of administration:	Intraduodenal
Dose volume:	2 ml/kg
Formulation/Vehicle:	0.5% aqueous Tylose MH 300 suspension
Species/Strain:	Beagle dogs, not treatment-naive
Number/Sex/Group:	3 per dose (1 male and 2 females per dose)
Age:	9 months to 5 years
Weight:	10.9 to 14.1 kg

Method notes

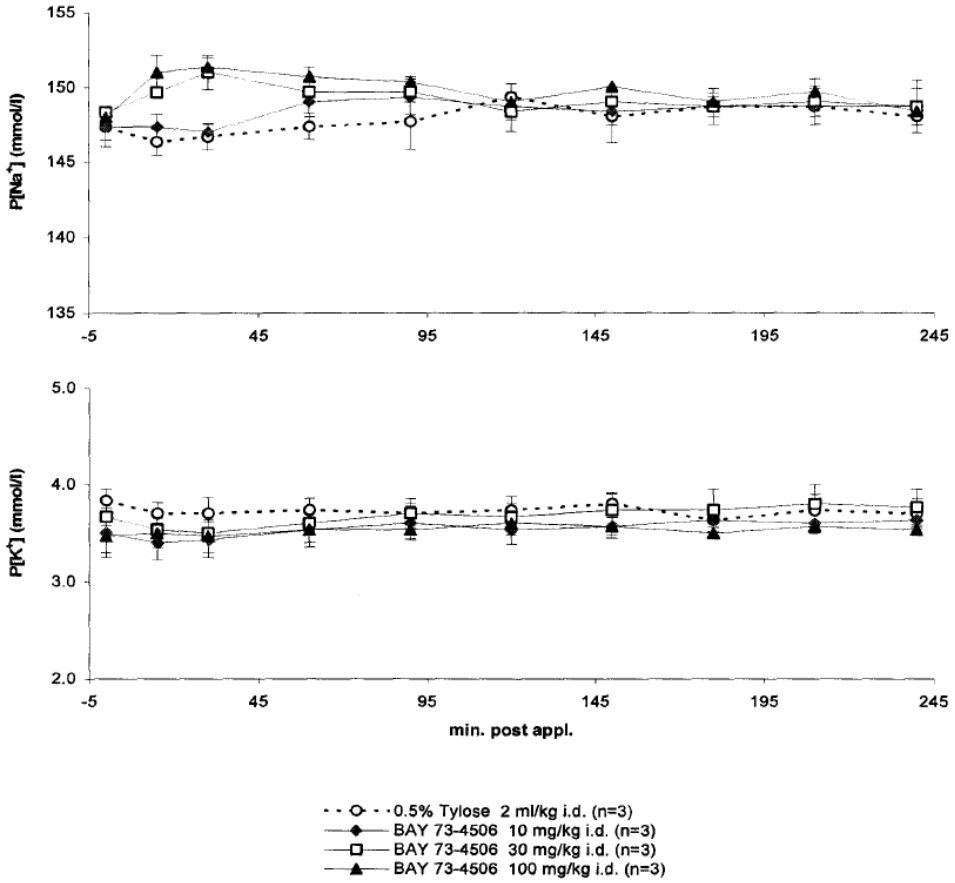
- Animals were caged individually and fasted on the day preceding the experiment. Water was available *ad libitum*.
- Multiple drugs were administered that might have confounded the results or obscured subtle changes, as these agents are recognized as affecting heart rate and breathing. The theoretical interactive effects of these agents with regorafenib are unclear:
    - Anaesthetized with droperidol/fantanyl/nitrous oxide
    - Aropine to reduce parasympathetic drive
    - Alcuronium chloride for skeletal muscle relation
    - Artificial respiration with enhanced oxygen
    - IV infusion of potassium and sodium
    - Body temperature kept at 37-38^{o}C by use of a heated pad
    - The site of catheter (for intraduodenum administration) was prepared with local anaesthetic (bupivacaine hydrochloride).
- Multiple catheters were placed to measure hemodynamic endpoints; their placement might theoretically have obscured subtle treatment-related changes
    - A catheter-tip manometer was placed through the femoral artery into the abdominal aorta, to measure blood pressure (systolic, diastolic, mean arterial blood pressure)
    - A catheter was placed via the left carotid artery, with an intraluminal velocity sensor placed into the ascending aorta and a manometer with bridge amplifier into the left ventricle, to measure cardiac output, cardiac left ventricular pressure, rate of rise of left ventricular pressure (dP/Dt), left ventricular end diastolic pressure

  - A catheter was placed via the left jugular vein into the cranial vena cava to measure central venous pressure
  - Stroke volume and total peripheral resistance were calculated
- A standard three lead limb ECG was recorded, for measurement of RR-interval, PQ-interval, QT-interval, and QRS complex. QT interval, QT corrected for heart rate (QTc) by Bazet's formula (QTcB) and Fridericia's formula (QTcF0 and T-wave height (T-H) were calculated.
- Endotracheal tubes were placed for artificial respiration; the tube had a pressure transducer and airflow sensor to measure: respiration rate, respiration pressure (peak of inspiratory pressure and expiratory pressure), respiration volumes (tidal and minute volume), oxygen concentrations in the inspiratory airflow (FiO_2), endexspiratory airflow (EtO_2), endespiratory carbon dioxide ($EtCO_2$) and nitrogen oxide in the inspiratory airflow (FiN_2O). Calculated parameters were dynamic compliance and resistance, and O2-difference.
- Blood samples were collected from the left femoral artery to measure arterial pH, partial O_2, CO_2 pressure, plasma sodium, plasma potassium, hematocrit, standard bicarbonate, and base escess (BE)
- Dogs were stabilized after anaesthesia for at least 30 minutes before test agent administration, and were then monitored for 240 minutes
- Blood was drawn from an arterial catheter at 0, 30, 60, 120 and 240 minutes for PK analysis

Results notes

- The authors concluded that no treatment-related changes were observed. This reviewer disagrees: a slight increase in plasma sodium was apparent for the 30 and 100 mg/kg groups at 15 and 30 minutes post-dose, returning to baseline by $\geq$ 60 minutes post-dose (report page 26). This change is not clearly adverse. Because it precedes the T_{max}, this effect may reflect the chemical/physical properties of regorafenib on electrolyte and water movement from the gut into systemic circulation.
- From the study report (page 26):

Figure 2: Slight transient plasma sodium elevation in regorafenib-treated dogs (report # PH-3393)



- C_{max} did not increase from 30 to 100 mg/kg; the authors speculate that absorption was limited under the conditions tested. From the report (page 75):

Table 5: Dog PK parameters following single intraduodenal doses of regorafenib (report # PH-33963)

Dose (mg/kg)→ Parameter↓	10	30	100
AUC (0-t_n)	0.189	0.425	0.361
AUC(0-t_n)$_{norm}$	0.0189	0.0142	0.00361
C_{max}	0.0639	0.127	0.110
$C_{max,norm}$	0.00639	0.00424	0.00110
t_{max}	1.59	1.26	2.52

Study title: **BAY 73-4506: influence on haemodynamics, ECG and respiration in anaesthetized dogs after single intravenous infusions administration**

Study no.:	PH-35619 Other study #: T 8078328
Report date:	November 24, 2008
Study report location:	NDA module 4.2.1.3 Safety Pharmacology
Conducting laboratory and location:	Bayer HealthCare AG Wuppertal, Germany
Date of study initiation:	January 23, 2008 (i.e. preceding the intraduodenal study, report # PH-33963)
GLP compliance and QA statement:	Yes, signed
Drug, lot #, and % purity:	Regorafenib (BAY 73-4506), batch # BXR3JXC, purity 96.4% (doses adjusted for purity)

Key Study Findings

- Intravenous infusion of increasing amounts of regorafenib (0.25 mg/kg, 0.75 mg/kg, or 2.25 mg/kg) over a total of 90 minutes had no apparent effect on safety pharmacology.
- The authors chose IV infusion, with the goal of achieving higher exposures than feasible from oral dosing

Methods

Dosing information:	• Two groups of 4 dogs were used: 2 male and 2 females for vehicle control; 2 males and 2 females for regorafenib treatment • The regorafenib-group received 0.25 mg/kg over 30 minutes, then 0.75 mg/kg over 30 minutes, then 2.25 mg/kg over 30 minutes (total infusion time of 90 minutes; no wash-out periods or breaks between doses)) • Dose volume for both groups was 0.17 ml/kg for the first 30 minutes, then 0.5 ml/kg for the second 30 minutes, then 1.5 ml/kg for the last 30 minutes
Formulation/Vehicle:	0.5% aqueous Tylose MH 300 suspension
Species/Strain:	Beagle dogs, not treatment-naive
Number/Sex/Group:	2/sex/dose
Age:	2 to 3 years
Weight:	10.1 to 12.8 kg

Method notes

- The procedures and endpoints are essentially similar as for the study reviewed above (report # PH-33963).

Results notes

- No treatment-related toxicity was observed. The following treatment-related effects were detected (but are not clearly adverse):
  - Slightly increased diastolic blood pressure
  - Slightly increased hemolysis
- All animals exhibited effects secondary to the infusion volume: increased total peripheral resistance, increased arterial blood pressure, decreased stroke volume, reduced dP/dt, increased left ventricular end diastolic pressure, increased central venous pressure. These changes might theoretically have masked subtle treatment-related changes.
- PK analysis did not detect M-2 or M-5. Plasma concentrations were dose-proportional. From the report (page 81):

Table 6: Dog PK parameters following intravenous infusion of regorafenib (report # PH-35619)

Dose (mg/kg)→ Parameter↓	3.25 (cumulative dose)
AUC (0-3.5) (µg*h/L)	6377
$AUC(0\text{-}3.5)_{norm}$ (µg*h/L)	1.96
C_{max} (µg/L)	4603
$C_{max,norm}$ (µg/L)	1.42
$C(3.5)/C_{max,norm}$ (µg/L)	32.7
t_{max} (h)	1.5

Study title: **BAY 75-7495 (metabolite M-2 of BAY 73-4506). Influence on cardio-hemodynamics, ECG and respiration in anesthetized dogs after cumulative intravenous infusions**	
Study no.:	PH-35628 Other study #: T 9078329
Report date:	November 27, 2008
Study report location:	NDA module 4.2.1.3 Safety Pharmacology
Conducting laboratory and location:	Bayer HealthCare AG Wuppertal, Germany
Date of study initiation:	February 1, 2008
GLP compliance and QA statement:	Yes, signed
Drug, lot #, and % purity:	M-2 (BAY 75-7495), batch # BXR3BSX, purity 91.5% (doses corrected for purity)

Key Study Findings

- No treatment-related toxicity was observed for M-2.
- The study design was essentially similar to the safety pharmacology study for regorafenib reviewed above (report # PH-35619)
- IV infusion of 0.25 mg/kg over 30 minutes, then 0.75 mg/kg over 30 minutes, then 2.25 mg/kg over 30 minutes (cumulative dose of 3.25 mg/kg)
- Formulated in 70% polyethylene 400, 20% water, 10% ethanol (96% ethanol)
- PK data were collected and reported (not tabulated for this review)

Study title: **BAY 81-8752 (metabolite M-5 of BAY 73-4506). Influence on cardio-hemodynamics, ECG and respiration in anesthetized dogs after cumulative intravenous infusions**

Study no.:	PH-35620 Other study #: T 1078330
Report date:	November 24, 2008
Study report location:	NDA module 4.2.1.3 Safety Pharmacology
Conducting laboratory and location:	Bayer HealthCare AG Wuppertal, Germany
Date of study initiation:	February 1, 2008
GLP compliance and QA statement:	Yes, signed
Drug, lot #, and % purity:	M-5 (BAY 81-8752), batch # BXR3TVA, purity 97.5% (doses corrected for purity)

Key Study Findings

- M-5 caused slight changes: reduced hematocrit, increased plasma potassium, and hemolysis.
- The study design was essentially similar to the safety pharmacology study for regorafenib reviewed above (report # PH-35619) and the study for M-2 reviewed above (report # 35628)
- IV infusion of 0.25 mg/kg over 30 minutes, then 0.75 mg/kg over 30 minutes, then 2.25 mg/kg over 30 minutes (cumulative dose of 3.25 mg/kg)
- Formulated in 70% polyethylene 400, 20% water, 10% ethanol (96% ethanol)
- PK data were collected and reported (not tabulated for this review)

Study title: **BAY 73-4506: Effects of a single oral administration on the convulsive threshold dose of pentylenetetrazole, on the nocifensive responsiveness to heat, and on the duration of hexobarbital-induced anesthesia in rats**

Study no.:	PH-33856 Other study #: T 2074551
Report date	May 11, 2005
Study report location:	NDA module 4.2.1.3 Safety Pharmacology
Conducting laboratory and location:	Bayer HealthCare AG Wuppertal, Germany
Date of study initiation:	January 20, 2005
GLP compliance and QA statement:	Yes, signed
Drug, lot #, and % purity:	Regorafenib (BAY 73-4506), batch # 040607-010, purity 9.9% (doses corrected for purity)

Key Study Findings

- Single oral doses of 2, 10 or 50 mg/kg of regorafenib had no apparent effect on the endpoints measured (convulsive threshold, nociferensive response to heat, duration of sleep time)
- This study is of very limited regulatory usefulness, because the endpoints were measured 30 and 45 minutes post-dose (i.e. before the T_{max})

Method and Results Notes

- Male rats (Hsd:CpB: WU) were approximately 8 weeks old and weighed 187 to 235 g at time of treatment.
    - Control animals received the PLAC POWD 000 placebo. Regorafenib and the placebo powder were suspended in tap water
    - The report states “oral” administration, but does not explicitly specify “gavage” administration.
- Pentylenetetrazole test:
    - Groups of 7 male rats were dosed with 0, 2, 10 or 50 mg/kg of regorafenib orally.
    - Thirty minutes later, pentylenetetrazole (9 mg/ml) was infused into the tail vein at a rate of 1.0 ml/minute, and the infusion was stopped when rats began to convulse.
    - The mount of pentylenetetrazole solution required to induce seizures was used to calculate the convulsive threshold
- Hot plate and hexobarbital test
    - These tests were done consecutively on the same groups of rats
    - Groups of 8 male rats were dosed with 0, 2, 10, or 50 mg/kg or regorafenib orally
    - Thirty minutes later, rats were placed singly on a hotplate (about 52^{o}C), and the latency to licking of hindpaws (as a distinct pain-induced reaction) was measured. The maximum time on the hotplate was 90 seconds.

  - Fifteen minutes after dosing, these same animals were injected intraperitoneally with hexobarbital (75 mg/kg), and then placed on a warm bench (30°C). Hexobarbital causes anaesthesia, and the time interval between loss and recovery of the righting reflex was recorded.
- No treatment-related effects were apparent for regorafenib.

Study title: **BAY 73-4506: Effect of a single oral administration on renal function, blood pharmacology and lipid metabolism of rats**	
Study no.:	PH-34006 Other study #: T 7075104
Report date	August 10, 2005
Study report location:	NDA module 4.2.1.3 Safety Pharmacology
Conducting laboratory and location:	Bayer HealthCare AG Wuppertal, Germany
Date of study initiation:	February 1, 2005
GLP compliance and QA statement:	Yes, signed
Drug, lot #, and % purity:	Regorafenib (BAY 73-4506), batch # 04607-010, purity 9.9% (doses corrected for purity)

Key Study Findings

- No treatment-related effects detected, for the limited endpoints measured at 2 hours post-dose, in rats after single oral dosing with 2, 10 or 50 mg/kg of regorafenib

Methods and Results Notes

- Groups of 10 male rats (Hsd:Cpb:WU), approximately 8 weeks old and 176 to 212 g at time of dosing, received 3 ml/rat of demineralized water by oral gavage (to facilitate diuresis) and then were dosed orally 15 minutes later with 0, 2, 10 or 50 mg/kg of regorafenib.
  - The report does not explicitly state that gavage was the route of administration (this reviewer presumes that oral gavage was used).
  - Control animals received PLAC POWD 000 (placebo powder); the placebo and regorafenib were suspended in tap water.
- After dosing, rats were placed singly into diuresis cages without water, for 2 hours, then euthanized with blood collection.
- The report does not clearly indicate whether the urine analyzed was from the cage floor, or from bladder puncture (this reviewer presumes that cage urine was collected and analyzed).
- Endpoints measured: urine volume, urine electrolytes (sodium, potassium chloride), blood parameters (leukocyte, erythrocyte, platelet, hematocrit) hemoglobin (total blood hemoglobin and free plasma hemoglobin), coagulation

(thrombin time and thrombolplastin time), and lipid parameters (triglyciderides, cholesterol).
- No treatment-related changes in the endpoints measured were observed.

Study title: **BAY 73-4506: Effect of a single oral administration on blood glucose of fasted and fed rats**	
Study no.:	PH-33925 Other study #: T 6075103
Report date	June 24, 2005
Study report location:	NDA module 4.2.1.3 Safety Pharmacology
Conducting laboratory and location:	Bayer HealthCare AG Wuppertal, Germany
Date of study initiation:	January 31, 2005
GLP compliance and QA statement:	Yes, signed
Drug, lot #, and % purity:	Regorafenib (BAY 73-4506), batch # 04607-010, purity 9.9% (doses corrected for purity)

Key Study Findings

- Single oral dosing with regorafenib decreased blood glucose concentration by up to -22% in fasted (but not fed) rats; the biological significance is not clear from this study report.

Methods and Results Notes

- Male rats (Hsd:Cpb:WU), approximately 8 weeks old and 183 to 228 g at time of dosing, were used
- Rats were either fed ad libitum or fasted overnight preceding the experiment (duration of fasting not otherwise specified).
- Groups of 10 male rats received 0, 2, 10 or 50 mg/kg of regorafenib, with or without fasting
    - The report does not explicitly state the gavage was the route of administration (this reviewer presumes that oral gavage was used).
    - Control animals received PLAC POWD 000 (placebo powder); the placebo and regorafenib were suspended in tap water.
- Blood samples were collected (from the sublingual vein plexus) at 30, 60, 120 and 180 minutes post-dose, to measure blood glucose.
- No treatment-related effects were noted in non-fasted rats. In fasted rats, control animals showed an increase in glucose levels over time, which appeared attenuated in the treated rats. As the authors note, “the mean decrease in blood glucose concentration relative to time-matched vehicle controls was in the order of 22%.” From the report (page 10):

Table 7: Blood glucose levels in rats after acute oral dosing with regorafenib (report # PH-33925)

Post-dose time point	Blood glucose concentration (mmol/L)			
	0	2 mg/kg	10 mg/kg	50 mg/kg
FASTED RATS (n=6)				
30 minutes	3.61	3.31	3.67	3.46
1 hour	3.79	3.40	3.69	3.39
2 hours	4.37	3.66 *	3.91	3.55 *
3 hours	4.52	3.53 *	4.12	3.65
NON-FASTED RATS (n=6)				
30 minutes	5.82	5.90	6.09	5.94
1 hour	5.88	5.77	5.80	5.69
2 hours	6.00	5.77	5.90	5.59
3 hours	6.07	6.55	5.92	5.76

* Statistically significantly different from controls, $p < 0.05$

Study title: **BAY 73-4506: Effects of a single oral administration on gastrointestinal motility in rats**

Study no.:	PH-33841 Other study #: T 3074552
Report date	April 29, 2005
Study report location:	NDA module 4.2.1.3 Safety Pharmacology
Conducting laboratory and location:	Bayer HealthCare AG Wuppertal, Germany
Date of study initiation:	January 26, 2005
GLP compliance and QA statement:	Yes, signed
Drug, lot #, and % purity:	Regorafenib (BAY 73-4506), batch # 04607-010, purity 9.9% (doses corrected for purity)

Key Study Findings

- Orally administered regorafenib inhibited gastrointestinal motility, with clear dose-dependence

Methods and Results Notes

- Male rats (Hsd:Cpb:WU), approximately 8 weeks old and 216 to 253 g at time of dosing, were used.
- The report (page 8) indicates that food and water were available *ad libitum*.

- Groups of 5 male rats received single oral doses of 0, 2, 10 or 50 mg/kg of regorafenib
  - Control animals received the PLAC POWD 000 placebo. Regorafenib and the placebo powder were suspended in tap water
  - The report states "oral" administration, but does not explicitly specify "gavage" administration.
- Thirty minutes after dosing, all animals received a suspension of 300 mg/rat of barium sulfate (in 3 ml of demineralized water with 5% w/v Tylose MH 300).
- Thirty minutes later, each animal was euthanized and the length of the small bowel containing barium sulfate was measured. From the report (page 10):

Table 8: Regorafenib slowed the intestinal transit of barium sulfate in rats (report # PH-33841)

Dose (mg/kg)	Length of intestine covered by barium sulfate (centimeters)
0	82.7 ± 2.7
2	71.8 ± 5.5 *
10	70.0 ± 7.0 *
50	62.0 ± 5.0 *

Data presented as means ± standard deviations
* Statistically significantly different from controls, $p < 0.05$

Study title: **BAY 73-4506: Effects on the contractility of the isolated guinea pig ileum**	
Study no.:	PH-34034 Other study #: T 4074553
Report date	September 1, 2005
Study report location:	NDA module 4.2.1.3 Safety Pharmacology
Conducting laboratory and location:	Bayer HealthCare AG Wuppertal, Germany
Date of study initiation:	February 4, 2005
GLP compliance and QA statement:	Yes, signed
Drug, lot #, and % purity:	Regorafenib (BAY 73-4506), batch # BX01K3D, purity 95.2% (doses corrected for purity)

Key Study Findings

- No treatment-related effect was apparent for regorafenib on the contractility of pig ileum under the conditions tested

Methods and Results Notes

- The ileum of a treatment-naïve male guinea pig was obtained; segments were suspended in an oxygenated Tyrode solution, and isotonic contractions under tension were measured by a force transducer.
- Sections were exposed to 0, 100 ng/ml or 1 µg/ml of regorafenib for 2 minutes, and then one of four inducing agents was added (acetylcholine, barium, serotonin, or histamine) for 30 seconds, followed by a washout. Each ileum segment was exposed four times (i.e. to regorafenib then an inducing agent, until each inducing agent had been tested).
- Regorafenib alone did not induce contractions or relaxation of the isolated pig ileum under the conditions tested, up to 1 µg/ml
- Regorafenib did not affect the ileal contractions induced by acetylcholine, barium chloride, serotonin or histamine.

5 PHARMACOKINETICS/ADME/TOXICOKINETIC

5.1 PK/ADME

Distribution

Study title: **Investigation of the Stability in Plasma, Binding to Plasma Proteins, Reversibility of Binding, and Erythrocyte/Plasma Partitioning of [^{14}C]BAY 73-4506 *In Vitro.***

Study nos.:	B5-734506-53, B6-734506-54, B8-734506-56, I 4929-4
Report no.:	PH-34096.
Study report location:	BSP GDD-GED-DMPK-DP-NPW, Building 468, Bayer HealthCare AG, 42096Wuppertal, Germany.
Conducting laboratory and location:	BSP GDD-GED-DMPK-DP-NPW, Building 468, Bayer HealthCare AG, 42096 Wuppertal, Germany
Date of study initiation:	April 01, 2004
GLP compliance:	No
QA statement:	Yes
Drug, lot #, and % purity:	BAY 73-4506, bath # BX01HPS 6282199 [^{14C}]BAY 73-4506, 98% purity
Labeling position	4-{4-[({[4-chloro-3-(trifluoromethyl) phenyl]amino}carbonyl)-amino]-3-fluorophenyl}-N-methylpyridine-2-[^{14}C]carboxamide
Species/strain	See table below

Key Study Findings

- BAY 73-4506 was stable in plasma of mouse, rat, rabbit, dog, Rhesus monkey and man for at least 2 h at 37^{o}C.

- Protein binding was high in all species investigated independent of the concentration range tested.
- The main binding protein in human plasma was serum albumin.
- BAY 73-4506 was moderately distributed into blood cells of rat, dog, and man.

Methods

The stability of BAY 73-4506 for 2 hours at 37 was evaluated by HPLC coupled to an MS/MS-detection method. Protein binding was determined by the distribution between Transil® and diluted plasma. Radioactivity was determined by liquid scintillation counting.

Table: 1 Protein binding was investigated in the plasma of the following strains:

Species		Strain / Supplier
Rat	(male/female)	Wistar (Hsd/Cpb:Wu), (b) (4) (b) (4)
Dog	(female)	Beagle, (b) (4)
Rabbit	(female)	CHBB,
Mouse	(male)	CD-1, (b) (4)
Monkey	(female)	Rhesus, (b) (4)
Man	(male/female)	Caucasian, healthy volunteer

Results

(Tables excerpted from Applicant's submission)

Table 9: Binding of BAY 73-4506 to plasma proteins of selected species

Species	Transil® volume [µL]	Dilution plasma	C_{total} Transil® dispersion [µg/L]	C_{plasma} diluted [µg/L]	C_{plasma} undiluted plasma [mg/L]	f_u [%]	Mean f_u [%]
Dog, female	120	0.200	204	89.4	0.508	0.966	0.971
Dog, female	120	0.200	1070	436	2.48	1.10	(CV=10.1)
Dog, female	120	0.200	1980	917	5.21	0.862	
Dog, female	120	0.200	5430	2370	13.5	0.962	
Human, male	120	0.133	140	65.1	0.556	0.575	0.488
Human, male	120	0.133	644	346	2.96	0.426	(CV=18.6)
Human, male	120	0.133	1370	648	5.53	0.555	
Human, male	120	0.133	3840	2130	18.2	0.394	
Human, male V 1	120	0.133	661	397	3.39	0.328	0.374
Human, male V 2	120	0.133	668	372	3.18	0.392	(CV=26.5)
Human, male V 3	120	0.133	671	404	3.45	0.326	
Human, male V 4	120	0.133	670	425	3.63	0.285	
Human, male V 5	120	0.133	668	321	2.74	0.537	
Monkey, Rhesus, female	120	0.667	635	339	0.578	2.12	2.16
Monkey, Rhesus, female	120	0.667	3420	1910	3.26	1.92	(CV=10.1)
Monkey, Rhesus, female	120	0.667	6120	3260	5.55	2.14	
Monkey, Rhesus, female	120	0.667	19800	9870	16.8	2.45	
Mouse, male	120	0.111	116	48.8	0.500	0.576	0.575
Mouse, male	120	0.111	538	231	2.37	0.555	(CV=6.80)
Mouse, male	120	0.111	1170	469	4.80	0.630	
Mouse, male	120	0.111	3260	1420	14.5	0.541	
Rabbit, female	120	0.333	346	147	0.502	1.68	1.68
Rabbit, female	120	0.333	1790	759	2.59	1.69	(CV=0.298)
Rabbit, female	120	0.333	3410	1440	4.93	1.69	
Rabbit, female	120	0.333	9950	4220	14.4	1.69	
Rat, male	120	0.143	152	56.8	0.452	0.906	0.723
Rat, male	120	0.143	701	302	2.40	0.710	(CV=18.0)
Rat, male	120	0.143	1460	685	5.44	0.602	
Rat, male	120	0.143	4110	1820	14.4	0.674	
AGP	120	1.00	4840	237	0.269	76.9	76.9
HSA	120	0.133	668	133	1.14	2.21	2.21
10% FCS	120	1.00	1060	99.3	0.113	35.8	41.2
10% FCS	120	1.00	4700	379	0.431	42.7	(CV=9.83)
10% FCS	120	1.00	10100	778	0.884	45.4	
10% FCS	120	1.00	28300	2380	2.70	40.8	

V = volunteer
CV = variation coefficient in %
AGP = α1-acidic glycoprotein
HSA = human serum albumin
FCS = fetal calf serum

Table 10: Stability in plasma of different species at 37 °C after 2 h of incubation.

Species	Type	Storage period [h]	Concentration spiked [µg/mL]	DEV [%]	n	Evaluation
Rat	Plasma 37 °C	2	500	-0.0134	5	Stable
Rat	Plasma 37 °C	2	5	-1.97	5	Stable
Mouse	Plasma 37 °C	2	500	-0.489	5	Stable
Mouse	Plasma 37 °C	2	5	1.49	5	Stable
Rabbit	Plasma 37 °C	2	500	-2.02	5	Stable
Rabbit	Plasma 37 °C	2	5	-3.15	5	Stable
Dog	Plasma 37 °C	2	500	0.132	5	Stable
Dog	Plasma 37 °C	2	5	0.0681	5	Stable
Monkey	Plasma 37 °C	2	500	0.296	5	Stable
Monkey	Plasma 37 °C	2	5	0.245	5	Stable
Human	Plasma 37 °C	2	500	1.45	5	Stable
Human	Plasma 37 °C	2	5	1.40	5	Stable

Table 11: Binding of BAY 73-4506 to human plasma proteins at different pH

Species	Transil® volume [µL]	Dilution plasma	C_{total} Transil® dispersion [µg/L]	C_{plasma} [µg/L]	C_{plasma} calculated in undiluted plasma [µg/L]	Mean f_u [%]
pH 7.20	60	0.250	244	130	592	0.705
pH 7.45	60	0.250	249	133	606	0.703
pH 7.59	60	0.250	246	140	635	0.615
pH 7.79	60	0.250	251	142	645	0.622
Control pH 7.79	60	0.250	232	134	609	0.591

Table 12: Reversibility of protein binding of BAY 73-4506 *in vitro*

	Recovery [%]	
	Rat	Human
1st Supernatant	92.8	95.2
2nd Supernatant	6.17	3.67
3rd Supernatant	1.02	0.473
4th Supernatant	0.361	0.227
Total	100	99.6

Table 13: *In vitro* partitioning of BAY 73-4506 between plasma and blood cells

Species	Hematocrit	Conc. in blood C_B [mg/L]	Conc. in plasma C_P [mg/L]	Plasma/ blood ratio C_P/C_B	Plasma/blood ratio mean C_P/C_B	Conc. in erythrocytes C_E [mg/L]	Partition coefficient $P_{E/P}$
Rat, male	0.420	0.983	1.43	1.46		0.360	0.251
Rat, male	0.420	5.09	7.43	1.46		1.87	0.252
Rat, male	0.420	9.49	14.6	1.54		2.39	0.163
Rat, male	0.420	25.1	38.4	1.53	1.50	6.78	0.177
Dog female	0.490	0.987	1.21	1.22		0.757	0.627
Dog female	0.490	5.13	6.87	1.34		3.31	0.482
Dog female	0.490	10.3	14.6	1.42		5.76	0.395
Dog female	0.490	29.9	46.0	1.54	1.38	13.1	0.285
Human, male	0.470	0.974	1.49	1.53		0.392	0.265
Human, male	0.470	5.04	7.70	1.53		2.03	0.265
Human, male	0.473	9.58	15.6	1.63		2.85	0.185
Human, male	0.473	24.4	40.7	1.67	1.59	6.39	0.157

Study title: **Whole-body Autoradiography in Rats after Single Intravenous and Oral Administration of [^{14}C]BAY 73-4506**

Study no.:	I 5001743
Report no.:	PH-33804
Study report location:	PH-R&D-PD-P Preclinical Pharmacokinetics Bayer HealthCare AG, D-42096 Wuppertal, Germany
Conducting laboratory and location:	PH-R&D-PD-P-PPK-EK, Building 468, Bayer HealthCare AG, D-42096 Wuppertal, Germany
Date of study initiation:	May 11, 2004
GLP compliance:	No
QA statement:	Yes
Drug, lot #, and % purity:	[^{14}C]BAY 73-4506, 99% purity
Labeling position:	4-{4-[({[4-chloro-3-(trifluoromethyl) phenyl]amino}carbonyl)amino]-3-fluorophenoxy}-N-methylpyridine-2-[^{14}C]carboxamide
Specific radioactivity:	2.30 MBq/mg
Species/strain:	See table below

Key Study Findings

- [^{14}C]BAY 73-4506 and/or its metabolites were rapidly distributed to all organs and tissues.
- Adrenal cortex, liver, gastrointestinal tract and bile-ducts showed, qualitatively, the highest exposures of [^{14}C]BAY 73-4506.
- After 7 days, the liver, kidney cortex and outer medulla still retained a moderate residue of radioactivity
- Low radioactivity was observed in the brain, spinal cord, compact bone, and eye lens.
- No significant evidence of specific affinity of substance-associated radioactivity for melanin-bearing tissues was detected in pigmented Long Evan rat.

Methods

(Tables excerpted from Applicant's submission)

Species, specification and suppliers

Study no./trial no.	I 5001743/1, 2, 5	I 5001743/3	I 5001743/4
Species	Wistar rat, male	Wistar rat, female	Long Evans rat, male, pigm.
Strain	Hsd Cpb: WU	Hsd Cpb: WU	LE/JANV.
Breeder	(b) (4)		
Body weight (range)	193 - 206 g	185 - 189 g	181.1 g
Age	approx. 8 weeks	approx. 8 weeks	approx. 8 weeks
Number of animals	9	2	1
Individual animal nos.	206 – 213, 217	214 - 215	216

Summary of experimental data

Trial no.	Animal no. /sex	Route	Target dose [mg/kg]	Body weight [kg]	Admin. volume [ml]	Admin. solution [MBq/ml]	Dilution labeled + non-labeled	Indiv. rad. dose [MBq/kg]	Individual dose [mg/kg]	Sacrifice after admin.
1	206/m	i.v.	3	0.2058	1.04	1.35	none	6.84	2.97	5 min
1	207/m	i.v.	3	0.2048	1.04	1.35	none	6.87	2.99	2 h
2	208/m	p.o.	3	0.1959	0.99	1.35	none	6.84	2.97	2 h
2	209/m	p.o.	3	0.1939	0.98	1.35	none	6.84	2.97	4 h
2	210/m	p.o.	3	0.1981	1.00	1.35	none	6.83	2.97	8 h
2	211/m	p.o.	3	0.1962	0.99	1.35	none	6.83	2.97	24 h
2	212/m	p.o.	3	0.2007	1.00	1.35	none	6.74	2.93	72 h
2	213/m	p.o.	3	0.1967	1.00	1.35	none	6.88	2.99	168 h
3	214/f	p.o.	3	0.1853	0.94	1.35	none	6.86	2.98	2 h
3	215/f	p.o.	3	0.1881	0.95	1.35	none	6.83	2.97	24 h
4	216/m*	p.o.	3	0.1811	0.92	1.35	none	6.87	2.99	24 h
5	217/m**	p.o.	3	0.1987	1.00	1.35	none	6.81	2.96	4 h

* = pigmented rat (Long Evans)
** = test on volatility

Results

Table 14: Qualitative Distribution of Regorafenib

Organ/tissue	Animal #/Sex										
	206 M	207M	208M	209M	210M	211M	212M	213 M	214 F	215 F	216 M
Adrenal cortex	H	H	H	H	H	H	M	L	M	H	H
Liver	H	H	M	H	H	H	M	M	M	H	H
Myocard	H	M	M	M	M	M	L	L	M	M	
Acc. genital gland	M	M	M			M	L	BG			M
Adrenal medulla	M	M	M	M	M	M	L	L		M	M
Aorta wall	M	M	M	M	M	M	L	BG		M	M
Bile ducts, contents	M	M	M	M	H	H	L	BG	L	M	M
Blood	M	M	M	M	M	M	L	L	L	M	M
Bone marrow	M	M	M	M	M	M	L	BG	L	M	M
Brown adipose tissue	M	H			M	M	L	L	M	M	M
Choroid plexus	M	M	M	M	M	M	L	BG	L	M	M
Ciliary body	M	L	BG	BG	BG	L	BG		BG	L	L
Trachea/esophagus	M	M		M		M	L	BG		M	M
Eye wall	M	M	M	M	M	M	L	BG	L	M	H
G.I.T. mucosa	M	M	M	M	M	M		L		M	M
Kidney	M	M	M	M	M	M	M	M	M	M	M
Lacrimal gland	M	M	M	M	M	M			L		M
Lung	M	M	M	M	M	M	L		L	M	M
Pancreas	M	M	M	M	M	M	L		M	H	M
Pharyngeal mucosa	M	M	M			M					M
Pineal body/ hypophysis	M		M	M	M	M	L	L	L	M	M
Salivary gland	M	M	M	M	M	M	L	L	L	M	M
Skeletal muscle	M	M	M	M	M	M	L	L	L		M
Skin/hair follicle	M	M	L		M	M	L	L	L	M	H
Spleen	M		M			M		L	L		
Thymus/lymph nodes	M	M	L	M		M	L				M

Organ/tissue	Animal #/Sex										
	206 M	207M	208M	209M	210M	211M	212M	213 M	214 F	215 F	216 M
Thyroid	M	M	M	M	M	M		L	L	M	M
Brain/spinal cord	L	L	L	L	L	L		BG	BG	L	L
Lymph	L										
Nasal mucosa	L	L		M	M	L	L	L	L	M	L
S. intestinal contents	L	H	EH	L	EH		M	L	H		S
Testis/Epididymis	L	L	L	M	M	M	L	L			M
White adipose tissue	L	M	H	M				L		M	
Cartilaginose tissue	BG	BG		L	L	L	L	BG	BG	L	L
Compact bone	BG	L	L	L	L	BG	BG		BG	L	L
L. intestinal contents	BG	L	BG		L		H		BG		H
Stomach, contents	BG	M	S	H	H	H	L	L	S	H	EH
Urine (bladder)	BG	L			M	M	L	BG		M	M
Vitreous body/eyelens	BG	BG	BG	BG	BG	BG	BG	BG	BG	BG	BG
Incisor pulp											M
Incisor peridental											M

ACC. genital glands = seminal vesicles + prostate gland + coagulation gland + Cowperian gland + preputial gland
Adipose tissues = white and brown adipose tissue
Lacrimal glands = Harderian gland + infraorbital gland
Salivary glands = submandibular gland + parotid gland + sublingual gland
Lymphatic system = lymph + lymph nodes + spleen + thymus

Luminographic intensity:
S – Saturated; EH – Extra high; H – High; M – Medium; L – Low;
BG – Back ground

Study title: **Quantitative Whole-body Autoradiography. Distribution of Radioactivity and Elimination from Blood, Organs, Tissues, and Fetuses after Single Oral Administration of [^{14}C]BAY 73-4506 to Pregnant Rats**

Study no.:	I 5145-5
Report no.:	A53543
Study report location:	Nonclinical Pharmacokinetics Building 468, Bayer Pharma AG 42096 Wuppertal, Germany
Conducting laboratory and location:	Bayer Pharma AG BPH GDD-GED-DMPK 42096 Wuppertal, Germany
Date of study initiation:	November 27, 2009
GLP compliance:	No
QA statement:	Yes
Drug, lot #, and % purity:	[^{14}C]BAY 73-4506, batch # GCM 1569-1-11A, 99.2% radiochemical purity
Specific radioactivity:	2.47 MBq/mg
Labeling position:	4-{4-[({[4-chloro-3-(trifluoromethyl)phenyl]amino}carbonyl)amino]-3-fluorophenoxy}-N-methylpyridine-2-[^{14}C]carboxamide
Formulation:	60% PEG 400, 40% demin. water (v/v)
Species/strain:	Hsd Cpb:WU Wistar rat, pregnant, Day 19 of gestation
Age:	~14 weeks
Weight:	303-353 g
Doses:	3 mg/kg, see table below for details

Animal No./ sex	Target dose	Body weight (admin.)	Body weight (sacrifice)	Admin. volume	Admin. solution	Indiv. rad. dose	Individual dose	Sacrifice after admin.
	[mg/kg]	[kg]	[kg]	[mL]	[MBq/mL]	[MBq/kg]	[mg/kg]	[h]
203/f	3	0.3286	0.3293	1.71	1.43	7.43	3.01	2
204/f	3	0.3530	0.3531	1.84	1.43	7.44	3.01	4
205/f	3	0.3032	0.2970	1.58	1.43	7.44	3.01	8
206/f	3	0.3098	0.3063	1.61	1.43	7.42	3.00	24

Key Study Findings

- The highest maximum concentrations of radioactivity were seen in the maternal liver and adrenal gland.
- Fetal brain exposure was approximately 2-fold higher than maternal brain exposure

Methods

The radioactivity was determined by radioluminographic methods (whole-body autoradiography) and by liquid scintillation counting. It was not possible to distinguish

between unchanged compound and radioactive metabolites using these analytical methods

Results

(Tables Excerpted from Applicant's submission)

Table 15: Pharmacokinetic parameters of pregnant Wistar rats

Organs/tissues	Ceq_{max} [µg-eq/L]	Ceq_{max} ratio organ/blood	t_{max} [h]	AUC(0-24) [µg-eq·h/L]	$t_{1/2}$[a] [h]
Adipose tissue, brown	2972	2.02	8.00	43913	13.3
Adipose tissue, white	1629	1.11	8.00	25339	21.0
Adrenal cortex	7574	5.14	8.00	146894	131
Adrenal medulla	6725	4.56	8.00	115520	32.5
Adrenal glands	7281	4.94	8.00	139990	104
Amnion	3480	2.36	8.00	45820	11.7
Amniotic fluid	149	0.101	8.00	2580	39.5
Aorta wall	1751	1.19	8.00	27990	17.4
Blood (heart)	**1474**	**1.00**	**8.00**	**23267**	**18.1**
Brain	195	0.132	8.00	3381	27.0
Cardiac muscle	3499	2.37	8.00	51613	14.8
Kidney, cortex	3181	2.16	8.00	57135	39.8
Kidney, inner medulla	2409	1.64	8.00	39451	22.4
Kidney, outer medulla	2931	1.99	8.00	55660	70.6
Kidneys	2866	1.94	8.00	50092	31.2
Liver	8589	5.83	8.00	150867	31.0
Lungs	2421	1.64	8.00	40008	23.3
Mammary glands	2298	1.56	8.00	39035	28.3
Ovaries	3220	2.19	8.00	53849	23.4
Placentae	1518	1.03	8.00	24742	20.8
Skeletal muscle (dorsal)	1229	0.834	8.00	19400	20.6
Skin (dorsal)	1236	0.839	8.00	18037	14.4
Spleen	1792	1.22	8.00	28419	18.5
Submandibulary gland	3224	2.19	8.00	49189	16.8
Uterus	1918	1.30	8.00	27375	14.5
Fetal adrenal glands	2175	1.48	8.00	34634	21.9
Fetal blood	868	0.589	8.00	11859	12.5
Fetal brain	400	0.272	8.00	5313	10.9
Fetal kidneys	807	0.547	8.00	12160	17.5
Fetal liver	1073	0.728	8.00	16158	16.4
Fetal skeletal muscles	887	0.602	8.00	12439	13.0
Fetal skin	987	0.670	8.00	14026	14.2
Fetus (average)	902	0.612	8.00	12544	12.9

Table 16: Equivalent concentrations (µg-eq/L) of radioactivity of pregnant Wistar rats and fetuses at various observation times

Time point [h]	2	4	8	24
Animal No.	203	204	205	206
Organs/tissues				
Adipose tissue, brown	1338	1027	2972	1288
Adipose tissue, white	333	388	1629	961
Adrenal cortex	3062	3142	7574	6960
Adrenal medulla	1678	2524	6725	4779
Adrenal glands	2858	3071	7281	6545
Amnion	614	571	3480	1344
Amniotic fluid	35.6	45.9	149	112
Aorta wall	1114	534	1751	927
Blood (heart)	**666**	**460**	**1474**	**798**
Brain	131	61.2	195	129
Cardiac muscle	1255	900	3499	1657
Kidney, cortex	1322	1240	3181	2407
Kidney, inner medulla	821	865	2409	1468
Kidney, outer medulla	1338	1241	2931	2505
Kidneys	1050	1220	2866	2010
Liver	3797	3519	8589	6007
Lungs	1020	774	2421	1504
Mammary glands	978	693	2298	1554
Ovaries	1432	1169	3220	2006
Placentae	668	514	1518	892
Skeletal muscle (dorsal)	458	282	1229	717
Skin (dorsal)	435	304	1236	572
Spleen	779	575	1793	983
Submandibulary gland	1294	817	3224	1664
Uterus	524	353	1919	892
Fetal adrenal glands	721	513	2175	1311
Fetal blood	214	162	868	358
Fetal brain	125	78.6	400	145
Fetal kidneys	256	160	807	428
Fetal liver	348	279	1073	546
Fetal skeletal muscles	298	178	887	378
Fetal skin	232	212	987	451
Fetus (average)	258	182	902	382

Table 17: Ratios of equivalent concentrations and AUCs of radioactivity in selected organs / body fluids of pregnant Wistar rats and fetuses

Time point [h]		2	4	8*	24	AUC(0-24)
Animal No.		203	204	205	206	
Ratios						Ratios
Placentae	/maternal blood	1.00	1.12	1.03	1.12	1.06
Amniotic fluid	/maternal blood	0.0535	0.0998	0.101	0.141	0.111
Fetus	/maternal blood	0.388	0.396	0.612	0.478	0.539
Fetus	/placentae	0.387	0.354	0.594	0.428	0.507
Fetus	/amniotic fluid	7.26	3.97	6.07	3.40	4.86
Fetal blood	/maternal blood	0.322	0.353	0.589	0.449	0.510
Fetal liver	/maternal liver	0.0918	0.0792	0.125	0.0909	0.107
Fetal brain	/maternal brain	0.955	1.28	2.06	1.13	1.57

Metabolism

Study Title: [^{14}c]Bay 73-4506: Species Comparison Based On Phase I Metabolism In Liver Microsomes Of Different Species Including Man.

Report No.:	Ph-33760
Study Report Location:	Nonclinical Pharmacokinetics Building 468, Bayer Pharma Ag 42096 Wuppertal, Germany
Conducting Laboratory And Location:	Ph-R&D-Pd-P-Mic, Building 466 Bayer Health Care Ag D-42096 Wuppertal, Germany

Date Of Study Initiation: March 11, 2004
Glp Compliance: No
Qa Statement: Yes
Drug, Lot #, And % Purity: Bay 73-4506

Key Study Findings

- Formation of **M-3** by N-methyl hydroxylation predominated in rat and dog liver microsomal incubations, whereas in man, monkey, mouse, and rabbit N-oxidation of the drug to **M-2** was the preferred biotransformation reaction.

Methods:

[^{14}C]BAY 73-4506 was incubated with liver microsomes from man (pool), Rhesus monkey, Beagle dog, Himalaya rabbit, Wistar rat, CD-1 mouse, or NMRI mouse for 60 or 180 minutes. The metabolic profiles of regorafenib were analyzed by LC-^{14}C and LC-^{14}C-UV-MS/MS.

Results

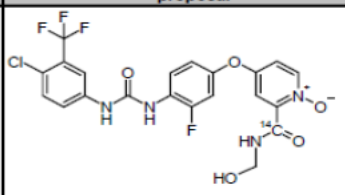
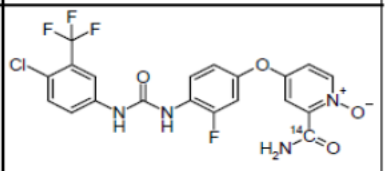
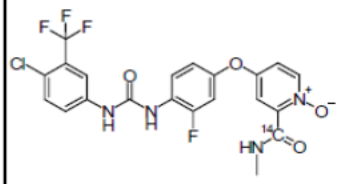
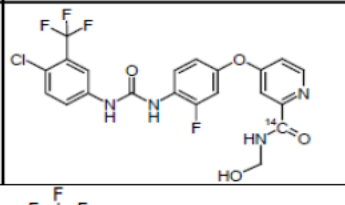
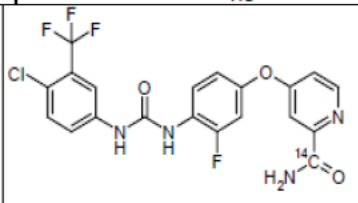
Table 18: Metabolite profiles after 60 minute incubations with liver microsomes of different species

Assignment (%)	Man	Rhesus monkey	Wistar rat	CD-1 mouse	NMRI mouse	Beagle dog	Himalaya rabbit
M-1/M-5	3.4	7.7	-	3.2	3.2	-	3.1
M-2	29.7	26.1	5.4	28.1	26.9	3.6	26.0
M-3	7.7	15.3	19.0	10.7	12.0	25.6	13.1
M-4	-	3.6	-	1.5	-	-	-
Drug	59.1	47.3	75.7	56.4	57.8	70.8	57.8
Metabolites balance	100	100	100	100	100	100	100

Table 19: Metabolite profiles after 180 minute incubations with liver microsomes of different species

Assignment (%)	Man	Rhesus monkey	Wistar rat	CD-1 mouse	NMRI mouse	Beagle dog	Himalaya rabbit
M-1/M-5	8.1	20.5	-	8.6	6.9	1.6	7.2
M-2	41.2	28.3	8.6	33.1	33.9	2.9	28.2
M-3	10.1	18.9	31.8	18.2	16.6	41.9	19.6
M-4	2.3	9.0	-	4.1	2.7	7.2	1.8
Drug	38.3	21.2	59.6	36.0	39.9	46.4	43.3
Metabolites balance	100	100	100	100	100	100	100

Figure 3: Assumed metabolic fate of the parent drug

R_t (^{14}C) [min]	^{14}C-mass $[M+H]^+$	Assign-ment	Structure proposal
23.9	517	M-1	
24.4	487	M-5	
26.0	501	M-2, BAY 75-7495	
26.5	501	M-3	
27.1	471	M-4	

(b) (4)

Excretion:

Study Title: Secretion Of Radioactivity Into Milk Of Lactating Rats After Single Oral Administration Of [^{14}c]Bay 73-4506

Report No.:	A53331
Study Report Location:	Nonclinical Pharmacokinetics Building 468, Bayer Pharma Ag 42096 Wuppertal, Germany
Conducting Laboratory And Location:	Bayer Pharma Ag Gdd-Ged-Dmpk 42096 Wuppertal Germany
Date Of Study Initiation:	May 11, 2011
Glp Compliance:	No
Qa Statement:	Yes
Drug, Lot #, And % Purity:	[^{14}c]Bay 73-4506, Batch # Gcm 1569-1-14b, 99% Purity
Labeling Position	4-{4-[({[4-Chloro-3-(Trifluoromethyl)Phenyl]Amino}Carbonyl)Amino]-3-Fluorophenoxy}-N-Methylpyridine-2-[^{14}c]Carboxamide
Dosage Form	10% Ethanol, 40% Solutol Hs 15, 50% Demineralized Water (V/V/V)
Species/Strain	Wistar Rat (Hsdcpb:Wu), Female Lactating Approx. 8-10 Days Post Partum
Age	> 12 Weeks
Body Weight	290-326 G

Key Study Findings

- Approximately 50% of [^{14}C]BAY 73-4506 radioactivity penetrated from blood circulation into the milk of lactating Wistar rats within 48 hours of administration.
- It was not possible to distinguish between unchanged compound and radioactive metabolites with the analytical method used.

Methods:

[^{14}C]BAY 73-4506 was administered by gavage to lactating rats (8 to 10 days after parturition) at a dose level of 2 mg/kg (mean radioactive dose of 5 MBq/kg). Blood samples were collected at different time points. Blood cells and plasma were separated by centrifugation. The radioactivity was determined by liquid scintillation counting. The milk flow was estimated on the base of an average body weight of 307 g and a daily milk production of 20% of the body weight. Thus, a milk flow of 2.56 mL/h was assumed [Grigor *et al., J. Nutrition 117, 1247-1258* (1987)]. The pharmacokinetic parameters were calculated from the geometric mean plasma concentrations using the TOXKIN program.

Results

Equivalent concentration *vs.* time curves of total radioactivity in plasma and milk of lactating Wistar rats after a single oral administration of 2 mg/kg [^{14}C]BA 73-4506. [Geometric means and standard deviations (n=3)]

(Excerpted from Applicant's submission)

Figure 4: Radioactivity from Regorafenib in Rat Plasma and Milk

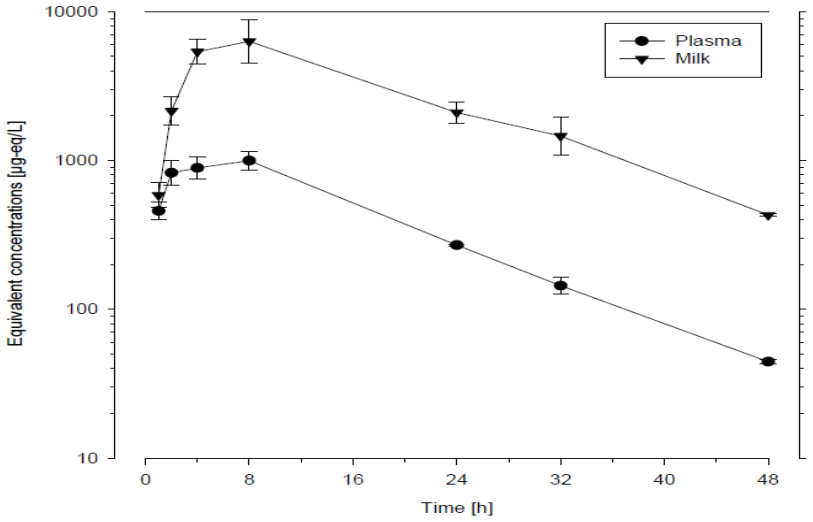


Table 20: Equivalent concentrations [µg-eq/L] of total radioactivity in milk and plasma.

	Time [h]	Equivalent concentration [µg-eq/L]			Mean geom.	S.D. geom.	Mean arithm.	S.D. arithm.
Milk	1	523	726	524	584	1.21	591	117
	2	1700	2240	2610	2150	1.25	2180	462
	4	4880	6660	4760	5370	1.21	5430	1070
	8	7780	7540	4280	6310	1.40	6530	1960
	24	2270	1730	2360	2100	1.18	2120	337
	32	1700	1760	1040	1460	1.34	1500	400
	48	441	425	424	430	1.02	430	9.40
Plasma	1	429	536	417	458	1.15	461	65.6
	2	899	666	942	826	1.21	836	148
	4	810	1080	807	890	1.18	898	155
	8	1110	847	1050	997	1.15	1000	138
	24	272	269	269	270	1.01	270	1.95
	32	164	143	127	144	1.14	145	18.7
	48	46.0	44.3	43.1	44.5	1.03	44.5	1.45
Ratio milk/plasma	1	1.22	1.35	1.26	n.c.	n.c.	1.28	0.0699
	2	1.89	3.36	2.77	n.c.	n.c.	2.68	0.741
	4	6.02	6.17	5.90	n.c.	n.c.	6.03	0.134
	8	7.01	8.90	4.08	n.c.	n.c.	6.66	2.43
	24	8.35	6.43	8.77	n.c.	n.c.	7.85	1.25
	32	10.4	12.3	8.19	n.c.	n.c.	10.3	2.06
	48	9.59	9.59	9.84	n.c.	n.c.	9.67	0.143

Table 21: Summary PK parameters of total radioactivity derived from equivalent concentrations [µg-eq/L] in milk and plasma, and corresponding milk/plasma concentration ratios

		Milk	Plasma	Ratio milk/plasma
Number of animals		3	3	
AUC	[µg-eq·h/L]	128000	18800	6.81
AUC_{norm}	[kg·h/L]	63.9	9.41	6.79
$AUC(0\text{-}t_{last})$	[µg-eq·h/L]	121000	18200	6.65
$AUC(0\text{-}t_{last})_{norm}$	[kg·h/L]	60.6	9.11	6.65
t_{last}	[h]	48	48	n.c.
AUC(0-24)	[µg-eq·h/L]	93700	15300	6.12
$\%AUC(t_{last\text{-}inf})$	[%]	5.15	3.13	n.c.
C_{max}	[µg-eq/L]	6310	997	6.33
$C_{max,norm}$	[kg/L]	3.15	0.498	6.33
t_{max}	[h]	8.00	8.00	1.00
$t_{1/2}$	[h]	10.3	9.25	1.11

Study Title: Absorption And Excretion Of The Radioactivity In Male Wistar Rats After Single Administration Of [^{14}c]Bay 73-4506

Study No.:	I 5001752, I 4558-2
Report No.:	Ph-33884
Study Report Location:	Ph-R&D-P Preclinical Pharmacokinetics Of Bayer Healthcare Ag D-42096 Wuppertal, Germany
Conducting Laboratory And Location:	Ph-R&D-Ppk-Dk, Building 468 Bayer Healthcare Ag, D-42096 Wuppertal, Germany
Date Of Study Initiation:	April 14, 2004
Glp Compliance:	No
Qa Statement:	Yes
Drug, Lot #, And % Purity:	[^{14}c]Bay 73-4506, Batch # Pls 0650-1-04 C, 99.5% Purity
Labeling Position	4-{4-[({[4-Chloro-3-(Trifluoromethyl) Phenyl]Amino}Carbonyl)Amino]- 3-Fluorophenoxy}-N-Methylpyridine-2-[^{14}c]Carboxamide
Species/Strain	Wistar Rat
Age	Approximately 8 Weeks
Weight	187 – 272 G

Key Study Findings

- In intact rats, approximately 87% of the radioactivity was found in feces until Day 7 after intravenous or oral administrations.

- After intravenous administration to bile duct-cannulated rats, biliary excretion amounted to 43% of the administered dose.

Methods

Study design of [^{14}C]BAY 73-4506 administration in rats for pharmacokinetics

Experimental animal numbers	Mean body weight [g] [Min - Max]	Route of admin.	Chemical dose [mg/kg]	Dilution labeled + non-labeled	Actual radioactive dose [MBq/kg]	Admin. volume [mL/kg]	Period of observ./ objectives [h p. appl.]
79 - 83	227 ± 3 [223 - 230]	p.o.[a]	2	none	4.77 ± 0.059	5[b]	0 - 24 Excretion, residues
84 - 88	197 ± 7 [187 - 206]	i.v. bolus	2	none	4.87 ± 0.161	5[b]	0 - 168 Excretion, residues
89 - 93	242 ± 2 [240 - 243]	p.o.	2	none	4.76 ± 0.030	5[b]	0 - 168 Excretion, residues
732 - 736	260 ± 10 [250 - 272]	i.v.[a] bolus	2	none	4.79 ± 0.181	5[b]	0 - 24 Excretion, residues

Results

(Excerpted from Applicant's submission)

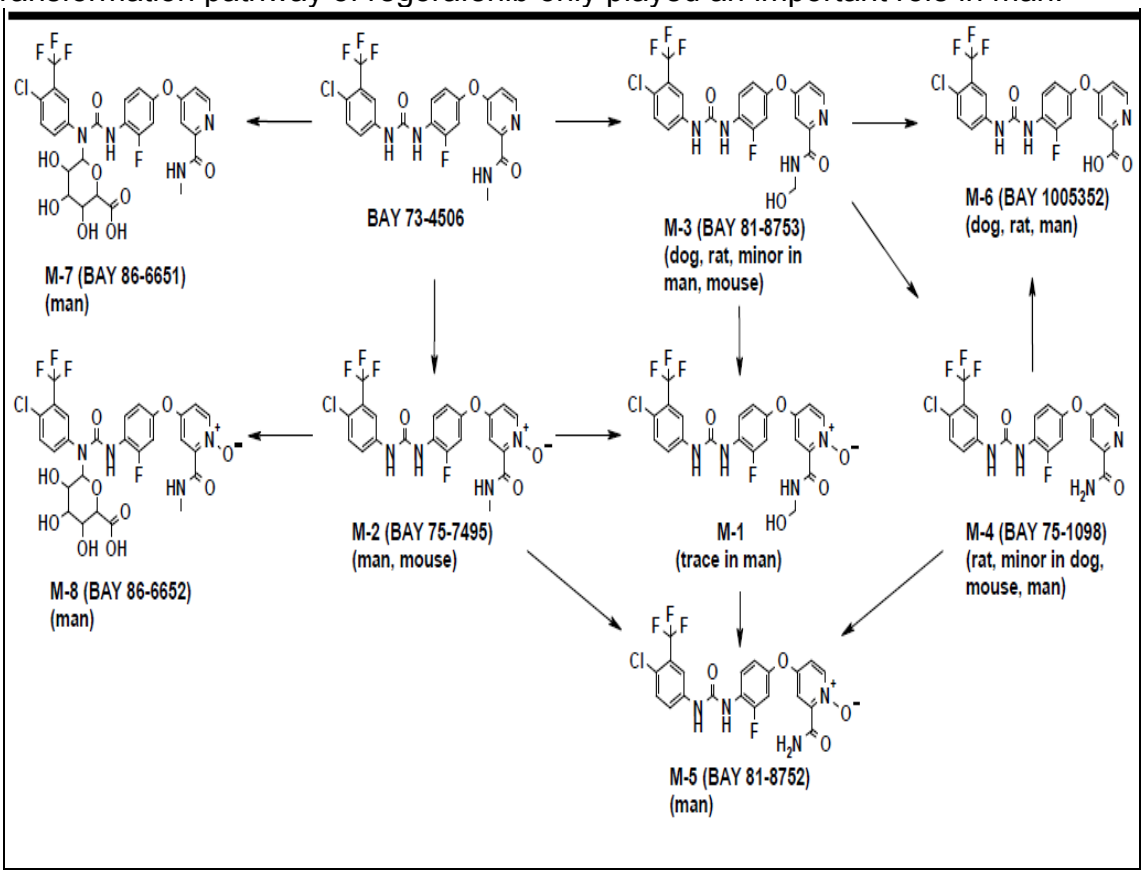
Table 22: Comparison of cumulative excretion data of radioactivity in per cent of the administered dose

Route	Intravenous		Oral		Oral[A]		Intravenous[A]	
Animals per point of time	n = 5		n = 5		n = 5		n = 4	
Duration [h]	168		168		24		24	
	Mean arithm.	C.V. [%]	Mean arithm.	C.V. [%]	Mean arithm.	C.V. [%]	Mean arithm.	C.V. [%]
Expired Air								
8	n.d.	n.d.	0.00449	18.1	n.d.	n.d.	n.d.	n.d.
24	n.d.	n.d.	0.0222	11.2	n.d.	n.d.	n.d.	n.d.
Urine								
8	1.06	25.9	0.969	42.8	0.529	101	0.414	36.2
24	3.75	24.5	3.60	9.09	1.94	86.5	1.44	9.44
48	5.08	17.3	4.72	8.56	n.d.	n.d.	n.d.	n.d.
72	5.49	16.1	5.09	7.74	n.d.	n.d.	n.d.	n.d.
96	5.72	14.6	5.25	7.95	n.d.	n.d.	n.d.	n.d.
120	5.89	14.4	5.39	7.93	n.d.	n.d.	n.d.	n.d.
144	6.01	14.0	5.47	8.06	n.d.	n.d.	n.d.	n.d.
168	6.09	13.7	5.51	8.17	n.d.	n.d.	n.d.	n.d.
Bile								
4	n.d.	n.d.	n.d.	n.d.	2.09	43.0	8.13	6.51
8	n.d.	n.d.	n.d.	n.d.	8.62	35.6	18.5	4.44
24	n.d.	n.d.	n.d.	n.d.	33.6	24.0	43.4	3.78
Feces								
24	44.4	5.90	50.2	13.3	10.1	34.8	8.23	23.0
48	80.9	5.01	82.6	3.69	n.d.	n.d.	n.d.	n.d.
72	84.7	5.19	86.9	3.09	n.d.	n.d.	n.d.	n.d.
96	85.4	5.14	87.6	2.94	n.d.	n.d.	n.d.	n.d.
120	85.7	5.09	87.9	2.94	n.d.	n.d.	n.d.	n.d.
144	85.9	5.04	88.1	2.86	n.d.	n.d.	n.d.	n.d.
168	86.0	5.02	88.2	2.86	n.d.	n.d.	n.d.	n.d.
Cage Rinse								
24	n.d.	n.d.	n.d.	n.d.	0.399	28.4	0.231	15.5
168	0.0833	50.7	0.0743	24.1	n.d.	n.d.	n.d.	n.d.
Sum	92.2	4.21	93.8	2.52	46.0	19.9	53.3	6.21

A = bile duct-cannulated rats
n.d. = not determined

5.2 Toxicokinetics (included in toxicity studies)

Comparison of the biotransformation of regorafenib in man and animal species revealed significant differences in phase 1 as well as in phase 2 reactions *in vitro* and *in vivo*. In man, N-oxidation of the pyridine (M-2 formation) was much more pronounced than methyl hydroxylation (M-3 formation) *in vitro* and *in vivo*. Glucuronidation as a primary biotransformation pathway of regorafenib only played an important role in man.



Proposed metabolic pathway of regorafenib *in vitro* and *in vivo* studies

Toxicokinetics: (included in general toxicology studies)

6 General Toxicology

6.1 Single-Dose Toxicity

See Section 3.2. Acute studies were not fully reviewed for this NDA submission.

6.2 Repeat-Dose Toxicity

Study title: **Chronic Oral Toxicity Study in Rats (6-Months Administration by Gavage)**

Study no.:	T2078241
Report no.:	PH-35874
Study report location:	Bayer Schering Pharma AG
Conducting laboratory and location:	Bayer Schering Pharma AG GDD-GED General Toxicology 42096 Wuppertal, Germany
Date of study initiation:	September 24, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BAY 73-4506 GRAN 10% 010, batch 050817-010, 97% purity

Key Study Findings

- BAY 73-4506 up to 2 mg/kg/day for 26 weeks did not cause any mortality.
- Red blood cells were increased in high dose animals.
- Histopathological changes were seen in the kidneys, liver, spleen, heart, and thyroid gland.
- C_{max} and AUC_{0-24} values increased dose-dependently.
- The contribution of metabolites M-2 and M-5 to overall exposure was very low.

Methods

Doses:	0.1, 0.5 or 2.0 mg/kg/day (see table below)	
Frequency of dosing:	Once daily	
Route of administration:	Oral by gavage	
Dose volume:	10 mL/kg	
Formulation/Vehicle:	Water	
Species/Strain:	Wistar rats (Hsd Cpb:WU)	
Number/Sex/Group:	20	
Age:	About 5 weeks	
Weight:	Males: 134-184 g	Females: 110-154 g
Satellite groups:	Yes, see table below	
Unique study design:	None	
Deviation from study protocol:	None	

	Group	Dose (mg/kg/day)	Sex	Number of animals
1	Main group	0.0	M	20
2	Main group	0.1	M	20
3	Main group	0.5	M	20
4	Main group	2.0	M	20
5	Main group	0.0	F	20
6	Main group	0.1	F	20
7	Main group	0.5	F	20
8	Main group	2.0	F	20
9	Satellite group	0.0	M	3
10	Satellite group	0.1	M	6
11	Satellite group	0.5	M	6
12	Satellite group	2.0	M	6
13	Satellite group	0.0	F	3
14	Satellite group	0.1	F	6
15	Satellite group	0.5	F	6
16	Satellite group	2.0	F	6

Observations and Results

Mortality: (Twice daily)

Control group – One female died in week 3 due to moderate purulent meningoencephalitis.

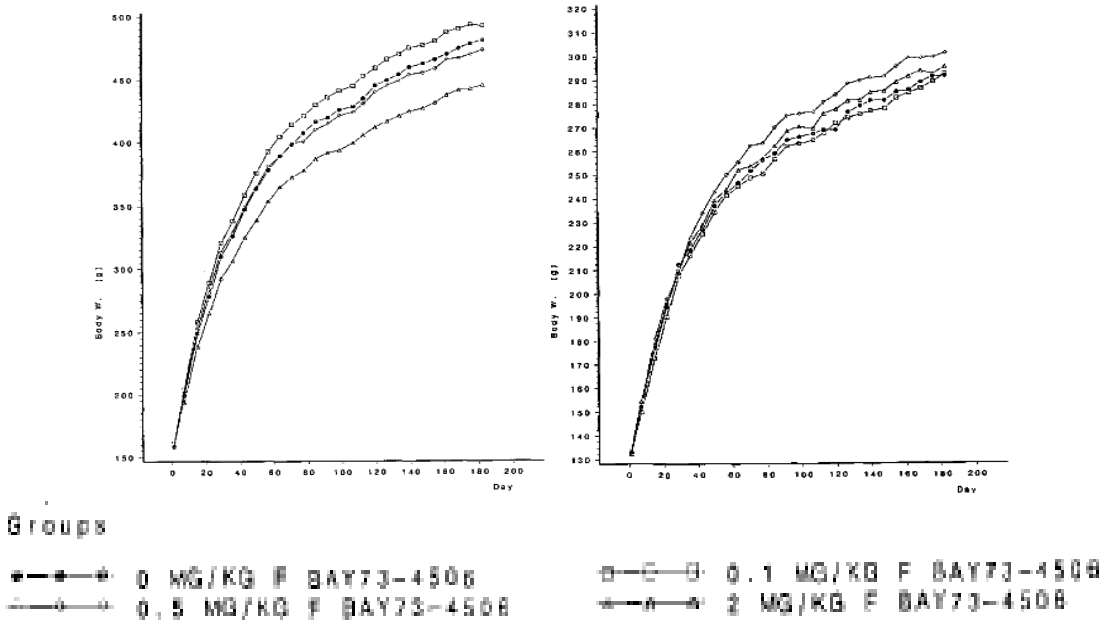
0.5 mg/kg group – One male killed moribund in week 23 likely due to gavage error

Clinical Signs: (Daily) 2 mg/kg – Ungroomed coat

Body Weights: (Daily) 2 mg/kg – Males (~ 8% ↓ vs. control)

Table 23: Body Weights (26 Week Rat, Main Groups)

Male Female



(excerpted from the Applicant's submission)

Feed Consumption: (Weekly) No effect

Ophthalmoscopy: (Before start of study and on day 170)
No treatment-related effects

Hematology: Main Groups

Table 24: Main Group Hematology (26 Week Rat)

Dose mg/kg	ERY 10E12/l	HB g/l	HCT l/l	MCV fl	MCH pg	MCHC g/l ERY
males	Day 23/ 31 [1]					
0	7.69	148	0.460	59.9	19.3	323
0.1	7.82	150	0.465	59.5	19.2	323
0.5	7.69	151	0.463	60.3	19.6	325
2	8.29 ++	160 ++	0.490 ++	59.1	19.3	326
males	Day 86/ 101 [2]					
0	8.73	154	0.501	57.4	17.7	308
0.1	8.67	153	0.489	56.4	17.6	313 +
0.5	8.84	157	0.503	57.0	17.8	313 +
2	9.11 +	165 ++	0.524 ++	57.6	18.0	314 ++
males	Day 176/ 185 [3]					
0	8.89	154	0.506	56.9	17.3	304
0.1	8.75	150	0.490	56.1	17.2	306
0.5	9.03	159	0.511	56.7	17.6	310 ++
2	9.01	164 ++	0.518	57.4	18.2 ++	316 ++
females	Day 23/ 31 [1]					
0	7.37	143	0.435	59.0	19.4	329
0.1	7.26	145	0.434	59.8	19.9	333
0.5	7.85 ++	153 ++	0.464 ++	59.2	19.5	329
2	7.84 +	158 ++	0.459 +	58.5	20.2 +	346 ++
females	Day 86/ 101 [2]					
0	8.05	154	0.488	60.6	19.1	315
0.1	8.08	154	0.495	61.3	19.1	311
0.5	8.33	159	0.508	61.0	19.1	314
2	8.59 ++	164 ++	0.521 ++	60.7	19.1	316
females	Day 176/ 185 [3]					
0	7.98	149	0.477	59.9	18.8	314
0.1	8.16	152	0.490	60.1	18.6	310
0.5	8.45 ++	157 ++	0.503 +	59.6	18.6	313
2	8.61 ++	162 ++	0.516 ++	60.0	18.8	314

TS 1% - ++ statistically significant at the 99% level
TS 5% - + - statistically significant at the 95% level
(excerpted from the Applicant's submission)

Clinical Chemistry: Main groups

Table 25: Main Group Clinical Chemistry (26 Week Rat)

Dose mg/kg	ASAT (GOT) U/l	ALAT (GPT) U/l
males	Day 30/ 31 [1]	
0	81.3	29.8
0.1	79.2	29.6
0.5	83.9	33.4
2	98.2 ++	45.9 ++
males	Day 100/ 101 [2]	
0	71.9	30.6
0.1	68.4	29.3
0.5	80.2	35.2 +
2	80.3	46.7 ++
males	Day 184/ 185 [3]	
0	62.4	30.7
0.1	62.1	29.0
0.5	80.7	43.4 ++
2	71.9	50.0 ++
females	Day 30/ 31 [1]	
0	67.8	24.9
0.1	76.0 +	26.9
0.5	77.4 +	27.3
2	87.3 ++	38.3 ++
females	Day 100/ 101 [2]	
0	72.5	27.9
0.1	76.9	29.0
0.5	76.2	29.2
2	81.9	40.4 ++
females	Day 184/ 185 [3]	
0	79.6	35.8
0.1	79.3	33.8
0.5	86.0	42.6
2	80.9	45.8

1) in the text summarized under week 4 + 5
2) in the text summarized under week 13 + 15
3) in the text summarized under week 26 + 27{= termination)
TS 1% - ++ statistically significant at the 99% level
TS 5% - + - statistically significant at the 95% level

(excerpted from the Applicant's submission)

No dose- and duration-related effects on triiodothyronine (T3), thyroxine (T4) and thyroid stimulating hormone (TSH).

Urinalysis: (Days 30/31, 100/101 and 184/185)
No compound-related effects

Gross Pathology: No gross pathological findings

Organ Weights: 2 mg/kg – Decreased absolute heart (13%) and liver (13%) in males and liver (10%) in females as compared to control animals

Histopathology:

Adequate Battery: Yes
Peer Review: No

Table 26: Histological Findings (26-Week Rat)

	Sex	Males				Female			
	No. of animals	20	20	19	20	19	20	20	20
Organ/Finding	Dose (mg/kg)	0	0.1	0.5	2.0	0	0.1	0.5	2.0
Heart, valvular thickening		2	3	3	8	1	2	3	12
Kidneys, glomerulopathy		-	-	1	14	-	-	-	3
tubular degeneration		-	-	-	4	1	-	-	-
Liver, cytopl. basophilia PP		-	-	-	13	-	-	-	7
rounded hepatocytes		-	-	11	12	-	-	-	3
pigment storage K.C.		-	-	-	1	4	3	6	10
Mes. lymph nodes, incr. mast cells		1	1	2	16	1	1	1	17
act. germinal centers		1	2	1	3	-	-	1	4
Ovaries, mean No. of corpora lutea		-	-	-	-	31	30	38	46
luteal cysts		-	-	-	-	6	7	10	1
Spleen, incr. hematopoiesis		5	7	11	14	9	9	8	8
incr. pigment storage		8	7	6	11	15	17	18	17
Thyroid glands, flattened foll. epithelium		-	1	-	7	-	-	-	-

- = no finding act. =activated cytopl. = cytoplasmic
foll. = follicular incr. = increased K.C. = Kupffer cells
PP = periportal

Toxicokinetics: (Satellite group on day 1 and main group in week 26 at predose, 1, 2, 4, 7, and 24 hours after dose administration)

Since differences in the males and females exposure were slight, data were combined.

Table 27: Summary of Pharmacokinetic Parameters on Day 1 and 182 (Rat 26-Week)

		BAY 73-4506			BAY 75-7495 (M-2)			BAY 81-8752 (M-5)		
Dose: BAY 73-4506 [mg/kg]		0.1	0.5	2	0.1	0.5	2	0.1	0.5	2
Equivalent Dose: M-2 [mg/kg]					0.103	0.517	2.07			
Equivalent Dose: M-5 [mg/kg]								0.100	0.502	2.01
AUC(0-t_n)	[µg·h/L]	40.4	1303	7232	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
AUC(0-t_n)$_{norm}$	[kg·h/L]	0.404	2.61	3.62	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
AUC(0-24)	[µg·h/L]	n.c.	1303	7232	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
AUC(0-24)$_{norm}$	[kg·h/L]	n.c.	2.61	3.62	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
C_{max}	[µg/L]	7.35	114	750	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
$C_{max,norm}$	[kg/L]	0.0735	0.228	0.375	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
C(24)/C_{max}	[%]	n.c.	10.9	7.30	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
t_{max}	[h]	7.00	4.00	4.00	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.

		BAY 73-4506			BAY 75-7495 (M-2)			BAY 81-8752 (M-5)		
Dose: BAY 73-4506 [mg/kg]		0.1	0.5	2	0.1	0.5	2	0.1	0.5	2
Equivalent Dose: M-2 [mg/kg]					0.103	0.517	2.07			
Equivalent Dose: M-5 [mg/kg]								0.100	0.502	2.01
AUC(0-24)	[µg·h/L]	511	3149	12489	n.c.	n.c.	n.c.	n.c.	n.c.	130
$AUC(0-24)_{norm}$	[kg·h/L]	5.11	6.30	6.24	n.c.	n.c.	n.c.	n.c.	n.c.	0.0649
C_{max}	[µg/L]	33.7	234	956	n.c.	n.c.	2.42	n.c.	n.c.	7.15
$C_{max,norm}$	[kg/L]	0.337	0.468	0.478	n.c.	n.c.	0.00117	n.c.	n.c.	0.00356
$C(24)/C_{max}$	[%]	27.2	21.6	20.7	n.c.	n.c.	n.c.	n.c.	n.c.	64.0
t_{max}	[h]	2.00	4.00	2.00	n.c.	n.c.	4.00	n.c.	n.c.	0.00
R_{A1}	[%]	459	205	128	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
R_{A3}	[%]	n.c.	242	173	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
MR-1	[%]				n.c.	n.c.	0.244	n.c.	n.c.	0.744
MR-2	[%]				n.c.	n.c.	n.c.	n.c.	n.c.	1.04

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n.c. = not calculated
R_{A1} = $C_{max,norm}$_Day182 / $C_{max,norm}$_Day 1 in [%]
R_{A3} = $AUC(0-24)_{norm}$_Day 182 / $AUC(0-24)_{norm}$_Day 1 in [%]
MR-1: metabolic ratio in terms of $C_{max,norm}$ in [%]: $C_{max,norm}$ (M-2) or (M-5) / $C_{max,norm}$ (BAY 73-4506)
MR-2: metabolic ratio in terms of $AUC(0-24)_{norm}$ in [%]: $AUC(0-24)_{norm}$ (M-2) or (M-5) / $AUC(0-24)_{norm}$_(BAY 73-4506)

(excerpted from the Applicant's submission)

Stability and Homogeneity: Stable and homogenously distributed in the vehicle.

Study title: **Subchronic Oral Toxicity Study in Beagle Dogs (13 Week Administration by Gavage)**

Study no.:	T3076046
Report n.:	PH-34580
Study report location:	Toxicology of Bayer HealthCare AG
Conducting laboratory and location:	Bayer HealthCare AG PH-GDD Toxicology 42096 Wuppertal Germany
Date of study initiation:	October 31, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(1) BAY 73-4506 GRAN 10%010 Batch # 050817-010 Active ingredient 9.9% (2) BAY 73-4506 PLAC POWD 000 Batch # 050314-000

Key Study Findings

- Oral gavage administration of BAY 73-4506 at 20 and 80 mg/kg/day for 13 weeks caused dentin alterations in the teeth, degeneration and lymphangiectasia in the small intestine in dogs
- Clinical observations included alopecia, vomiting, liquid feces and swelling of eyelids in mid and high dose animals.

- Kidneys, spleen, thymus and reproductive system were the main target organs of toxicity.

Methods

Doses:	5, 20,and 80 mg/kg/day
Frequency of dosing:	Daily for 13 weeks
Route of administration:	Oral
Dose volume:	10 mL/kg
Formulation/Vehicle:	Tap water
Species/Strain:	Purebred beagles
Number/Sex/Group:	4
Age:	25-29 weeks
Weight:	8.1 to 11.0 kg
Satellite groups:	No
Unique study design:	None
Deviation from study protocol:	None

Observations and Results

Mortality: (Daily) None

Clinical Signs: (Daily)

Group 2 – Alopecia, liquid feces and vomiting

Groups 3 & 4 – Alopecia, liquid feces and vomiting, whitish mucus and bloody particles/blood in the feces, gum bleeding/anemic gum and swelling of eyelids.

Body Weights: (Weekly)

Groups 3 & 4 - Reduced compared to control group

Table 28: Mean body weights (kg) 13 week dog

Period	Sex		0 mg/kg	5 mg/kg	20 mg/kg	80 mg/kg
Week -1	Males	Mean	9.35	9.28	9.32	9.25
		S.D.	0.72	0.51	1.05	0.7
		N	4	4	4	4
	Females	Mean	9.38	9.45	9.43	9.35
		S.D.	0.93	1.11	0.90	0.67
		N	4	4	4	4
Week 13	Males	Mean	13.25	12.25	11.50	9.73
		S.D.	0.66	0.60	1.67	1.50
		N	4	4	4	4
	Females	Mean	12.57	11.65	10.60	9.43
		S.D.	0.67	1.11	1.29	1.23
		N	4	4	4	4

Feed Consumption: (Daily)

Groups 3 & 4 – Food intake was reduced. Dosing was interrupted for 1 male group 2 and all males group 3 during weeks 9 to 12 for several days

Ophthalmoscopy: (Weeks -3, 6 and 13)

Group 3 - Swelling of eyelids (1 M & 1 F) at week 13
Group 4 – swelling of the eyelids (1 M) at week 6

ECG: (Week -3, before and 2 hours after administration in weeks 6 and 13)

No relevant drug-related changes in blood pressures, heart rate, and Q-T interval,

Hematology: (Weeks -3, 6 and 13)

No drug-related dose dependent effects

Clinical Chemistry: (Weeks -3, 6 and 13)

Table 29: Clinical chemistry (Med ± S.D)

Dose (mg/kg)	Sex	Week	ASAT (U/L)	ALAT (U/L)
20	M	-3	28±8.7	39±15.1
		6	40±12.6	44±58.7
		13	31±8.9	43±22.0
80	M	-3	19 ±2.4	36±8.0
		6	47 ±9.0	156±41.5
		13	45 ±13.5	157±89.8

Dose (mg/kg)	Sex	Week	ASAT (U/L)	ALAT (U/L)
20	F	-3	25 ±1.7	45 ±7.1
		6	35 ±4.5	54 ±31.5
		13	34 ±13.5	58 ±15.9
80	F	-3	19±5.3	37±4.7
		6	40±24.2	248±183
		13	40±9.2	115±55

Urinalysis: (Weeks -3, 6 and 13)

No drug-related changes

Gross Pathology: **(**At necropsy, week 14)

All treated animals showed alterations in the skin (hairless, sparse hair coat)

Organ Weights:

Table 30: Summary of organ-to-body weight ratios (Mean±S.D.) (13 week dog)

Organ	Group 2 (5 mg/kg)		Group 3 (20 mg/kg)		Group 4 (80 mg/kg)	
	Male	Female	Male	Female	Male	Female
Final body wt (kg)	12.0±0.5	12±1.2	11.4±1.6	10.4±1.3	9.6±1.7	9.6±1.5
Adrenals ratio	0.01±0.002	0.015±0.004	0.012±0.002	0.015±0.003	0.016±0.002	0.019±0.004
Gallbladder ratio	0.020±0.004	0.023±0.005	0.022±0.003	0.024±0.006	0.029±0.008	0.024±0.004
Heart ratio		0.80±0.06		0.82±0.09		0.90±0.07
Kidneys ratio		0.44±0.02		0.53±0.12		0.58±0.17
Liver, ratio		3.12±0.35		3.29±0.60		3.27±0.3
Ovaries ratio		0.015±0.007		0.0090.005		0.010±0.002
Pancreas ratio	0.22±0.02	0.30±0.04	0.3±0.06	0.29±0.03	0.31±0.039	0.35±0.07
Prostate ratio	0.07±0.02		0.07±0.15		1.0±0.03	
Spleen ratio	0.27±0.06	0.37±0.9	0.25±0.06	0.30±0.03	0.32±0.04	0.32±0.08
Testes ratio	0.15±0.04		0.13±0.03		0.11±0.04	
Thymus	0.13±0.03		0.08±0.03		0.090.05	

Histopathology:

Adequate Battery: Yes
Peer Review: Yes

Table 31: 13 Week Dog Histological Findings

Group	1		2		3		4	
Dose (mg/kg)	0		5		20		80	
Sex	M	F	M	F	M	F	M	F
Organ/Finding No.	4	4	4	4	4	4	4	4
Adrenal glands, incr. eosin. / Z. fasc., grade 1					1		2	
Bone marrow cylinder, activation, grade 1							1	
grade 2				1			2	
Canine tooth, dentin alteration, grade 1					1	3	3	2
Cecum, atrophy/lymph. foll., grade 2							3	1
grade 3							1	
Colon, incr. cell. infiltr., grade 2							2	
Duodenum, degeneration/villi, grade 2							1	
grade 3							1	
lymphectasia, grade 1					2	1	1	1
grade 2							2	
Epididymides, aspermia					1		2	
oligospermia, grade 4							1	
Femur, persist. growth pl., grade 1	1				1	1		1
grade 2		1					2	1
grade 3					1		1	1

Group	1		2		3		4	
Dose (mg/kg)	0		5		20		80	
Sex	M	F	M	F	M	F	M	F
Organ/Finding No.	4	4	4	4	4	4	4	4
grade 4							1	1
incr. fat marrow, grade 2						2	1	1
grade 3						1	3	1
incr. hemato. / condyle, grade 1							2	1
growth pl. thick, grade 2							1	1
grade 3							1	1
Gallbladder, inspissation		1	1	1	3	1	2	-
Heart, degeneration, grade 2			1					
infiltr. mononuclear, grade 1			1				1	
Ileum, degeneration/villi, grade 1							1	2
grade 2							1	
Jejunum, degeneration/villi, grade 2							1	
mucosal cyst /s, grade 1						1		
grade 2								1
Kidneys, tub., degen. /reg., grade 1					3	3	2	2
grade 2						1	1	1
grade 3							1	1
glomerulopathy, grade 1					3	1	3	2
grade 2						1	1	2
tubular dilation, grade 1						1	3	
grade 2								1
Larynx, atrophy/lymph. tissue, grade 2							1	
grade 3					1	1	3	1
grade 4						2		3
Liver, centrilob. hypertr., grade 1					1	1	1	2
grade 2					1		2	
incr. pigment depos., grade 1					1		1	
grade 2					1		1	
hepatitis, periportal hemorrh., grade 2							1	
fat accumul. / cl, grade 1							3	
incr. pigment depos., grade 1					2	3	1	1
grade 2						1	1	
grade 3								1
infilt. mononuclear, grade 1				1		1		1
grade 2								2
Mesent. lymph node, abscess, grade 4							1	
follicul. necr. /deg., grade 1					1		1	1
Ovaries, red. devel. follicles, grade 1								2
grade 2								1
grade 3								1
inc. foll. degener., grade 1						1		2
grade 2						1		1
grade 3								1
Pancreas, degen./atrophy, grade 1								2
grade 2							3	2
grade 3						1	1	
Parotid gland, infiltr. mononuclear, grade 1	1			1	2	1	1	
Parathroid glands, cyst /s, grade 1						1	1	2
grade 3							1	

Group	1		2		3		4	
Dose (mg/kg)	0		5		20		80	
Sex	M	F	M	F	M	F	M	F
Organ/Finding No.	4	4	4	4	4	4	4	4
Peyer's patches, atrophy/degeneration, grade 1							2	
grade 2					3	1		
grade 3						2	1	3
grade 4							1	1
Pituitary gland, infiltr. monon. /pv, grade 1							3	1
Prostate, interst. fibrosis, grade 2							1	
Skin, dyskeratosis, grade 1					1			
grade 3							1	
hyperkeratosis, grade 3					1		1	
acanthosis, grade 3					2		1	
hair growth arrest, grade 3			1	2		3	4	4
grade 4			2		4			
dermatitis, grade 2			1					
grade 4					2			
Spinal cord, gliosis, grade 1								1
Spleen, incr. hematopoiesis, grade 1					3	1		
grade 2					1			1
grade 3							4	3
protein. mat. /white, grade 2							1	1
grade 3							1	
follicular hemorrh, grade 2	1	1				2	1	2
grade 3							1	
focal necr. /abscess, grade 3							2	
incr. blood content, grade 2							2	
grade 3							1	
peri -/vasculitis, grade 2							1	
Sternum, incr. fat marrow, grade 2	1		1	3	2	2	2	1
grade 3					1	1	2	2
incr. myelopoiesis, grade 1							1	
grade 2							1	
Sublingual gland, atrophy, grade 1						2		3
grade 2						1	3	
grade 4							1	
inflammatory infil., grade 1							1	
Teeth/incisor, dentin alteration, grade 1					1	2	1	2
grade 2						1	3	2
Testes, retarded maturation, grade 2					1		1	
grade 3							2	
grade 4					1			
spermat. giant cells, grade 2							3	
grade 3							1	
grade 4					1			
sertoli cells, grade 2							1	
Thymus, atrophy, grade 2					1		3	2
grade 3					1			
grade 4							1	
incr. starry sky m., grade 1						1		1
Thyroid gland, atrophy, grade 1					1			
grade 2						1	2	2

Group	1		2		3		4	
Dose (mg/kg)	0		5		20		80	
Sex	M	F	M	F	M	F	M	F
Organ/Finding No.	4	4	4	4	4	4	4	4
grade 3							2	1
Tonsils atrophy, grade 2							1	2
grade 3							1	
grade 4							2	
mineralization, grade 1						1		1
Urinary bladder, inflammation, grade 2							1	1
Uterus, juvenile appearance		1		1		1		2
Vagina, juvenile appearance		1		1		1		2

Grade 1 - Minimal/very few / very small
Grade 2 - Slight / few / small
Grade 3 - Moderate / moderate number / moderate size
Grade 4 - Marked / many / large

Special Evaluation: None

Toxicokinetics: Day 1 and in Week 13 at 0, 1, 2, 4, 7, and 24 hours after administration of the drug)

There was no evidence of sex-related differences in exposure, therefore, combined PK data are shown below in 2 tables.

Table 32: Toxicokinetics (13-week Dog)

		Day 1					
Analyte		BAY 73-4506			BAY 75-7495 (M-2)		
Dose: BAY 73-4506 [mg/kg]		5	20	80	5*	20*	80
		Mean geom.	Mean geom.	Mean geom.	Mean geom.	Mean geom.	Mean geom.
AUC(0-t_n)	[µg·h/L]	14090	41777	101033	87.4	530	1452
AUC(0-t_n)$_{norm}$	[kg·h/L]	2.82	2.09	1.26	0.0169	0.0256	0.0176
AUC(0-24)	[µg·h/L]	14090	41777	101033	109	651	1452
AUC(0-24)$_{norm}$	[kg·h/L]	2.82	2.09	1.26	0.0211	0.0315	0.0176
C_{max}	[µg/L]	2764	6118	14012	30.9	125	280
$C_{max,norm}$	[kg/L]	0.553	0.306	0.175	0.00599	0.00606	0.00339
C(24)/C_{max}	[%]	3.44	7.22	8.89	n.c.	1.82	2.63
t_{max}	[h]	2.00	2.00	1.83	2.00	2.18	1.83
MR-2 (0-t_n)	[%]				0.620	1.27	1.44
MR-1	[%]				1.12	2.05	2.00
MR-2	[%]				0.773	1.56	1.44

		Week 13					
		BAY 73-4506			BAY 75-7495 (M-2)		
Dose: BAY 73-4506 [mg/kg]		5	20	80	5*	20*	80*
		Mean geom.	Mean geom.	Mean geom.	Mean geom.	Mean geom.	Mean geom.
AUC(0-t_n)	[µg·h/L]	11723	25250	48214	58.7	272	621
AUC(0-t_n)$_{norm}$	[kg·h/L]	2.34	1.26	0.603	0.0113	0.0131	0.00752
AUC(0-24)	[µg·h/L]	11723	25250	48214	77.6	307	685
AUC(0-24)$_{norm}$	[kg·h/L]	2.34	1.26	0.603	0.0150	0.0148	0.00829
C_{max}	[µg/L]	1923	3742	7036	17.7	66.6	131
$C_{max,norm}$	[kg/L]	0.385	0.187	0.0880	0.00342	0.00322	0.00159
C(24)/C_{max}	[%]	6.74	6.65	8.45	n.c.	3.87	3.98
t_{max}	[h]	1.68	1.41	1.30	1.83	1.83	1.54
MR-2 (0-t_n)	[%]				0.500	1.08	1.29
MR-1	[%]				0.919	1.78	1.87
MR-2	[%]				0.662	1.22	1.42
R_{A1}	[%]	69.6	61.2	50.2	57.1	53.1	47.0
R_{A3}	[%]	83.2	60.4	47.7	71.3	47.2	47.1

n.c. = not calculated
MR-1: metabolic ratio in terms of C_{max} in [%]: C_{max} (BAY 75-7495 (M-2)) / C_{max} (BAY 73-4506)
MR-2 (0-t_n): metabolic ratio in terms of AUC(0-t_n) in [%]: AUC(0-t_n)_(BAY 75-7495 (M-2)) / AUC(0-t_n)_(BAY 73-4506)
MR-2: metabolic ratio in terms of AUC(0-24) in [%]: AUC(0-24)_(BAY 75-7495 (M-2)) / AUC(0-24)_(BAY 73-4506)
R_{A1} = C_{max}_Week 13 / C_{max}_Day 1 in [%]
R_{A3} = AUC(0-24)_Week 13 / AUC(0-24)_Day 1 in [%]
* = in some cases no sample concentrations at 24 h or earlier time points were available therefore AUC(0-24) was obtained by extrapolation (see raw data)

Stability and Homogeneity: Stable and homogenous during the study period.

Study title: **Systemic Toxicity Study in Dogs (M+F) with Daily Intragastric Administration Over a Period of Approx. 52 Weeks**

Study no.:	TXST20070037 (T6077787)
Report no.:	A45739
Study report location:	Bayer Schering Pharma AG Nonclinical Drug Safety 13342 Berlin, Germany
Conducting laboratory and location:	Bayer Schering Pharma AG Nonclinical Drug Safety 13342 Berlin, Germany
Date of study initiation:	March 14, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BAY 73-4506, batch # 061120-010, 98% purity

Key Study Findings

- Regorafenib up to 16 mg/kg/day for 52 weeks did not cause any mortality in dogs.
- Clinically, fur and skin/mucosa alteration (hair loss, abscess like lesions) and gastrointestinal disorders were observed in a dose-dependent manner.
- Compound related gross finding were observed in the gallbladder, kidney, skeletal muscle and skin.

- Mineralization of thyroid glands and ovaries was observed.
- Histopathological examination revealed kidney (tubular degeneration and regeneration), liver (microgranulomas and perivascular mononuclear cell infiltration), gallbladder (epithelial hyperplasia) and reproductive tract were the target organs of toxicity.
- The observed increase in $AUC_{(0-24)}$ and C_{max} of high dose animals was slightly less than dose-proportional.
- The contributions of BAY 75-7495 (M-2 metabolite of BAY 73-4506) and BAY 81-8752 (M-5 metabolite of BAY 73-4506) to the total $AUC_{(0-24)}$ on day 354 were below 2.5%.

Methods

Doses:	1.0, 4.0 and 16.0 mg/kg (see table below for details)
Frequency of dosing:	Once daily
Route of administration:	Intragastric
Dose volume:	10 mL/kg
Formulation/Vehicle:	BAY 73-4506 PLAC POWD 000 (contains PVP 25 and Ac-Di-Sol (4+5))
Species/Strain:	Beagle dog
Number/Sex/Group:	4
Age:	11 to 12 months
Weight:	Male: 6.6 -8.7 kg Female: 6.8 – 9.0 kg
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None

Group	No. of animals/ group	Animal nos.	Compound	Concentration BAY 73-4506 [mg/mL]	Dose per day BAY 73-4506 [mg/kg]	Administration volume [mL/kg]
1 (control)	4 M / 4 F	201M – 204M 205F – 208F	vehicle*	0.0	0.0	10.0
2 (low)		209M –212M 213F – 216F	BAY 73-4506	0.1	1.0	
3 (mid)		217M – 220M 221F – 224F		0.4	4.0	
4 (high)		225M – 228M 229F – 232F**		1.6	16.0	

M: male animals
F: female animals
*: Polyvinylpyrolidon 25/Ac-Di-Sol 4:5
**: Due to impaired general condition of animal 230F; dosing of this animal was interrupted between days 26 and 28

Observations and Results

Mortality: (twice daily) No mortality

Clinical Signs: Dose and duration dependent increase in hair loss, skin/mucosa reddening, diarrhea, discolored feces, halitosis, vomiting, hyperactivity, head shaking, tremor, and irregular respiration were observed.

Body Weights: (Twice weekly)

Group	Body weight **relative to control** animals on day 365 [body weight on day 365 relative to start weight on day 1]	
	Male	Female
Control	N.A. [115 %]	N.A. [120 %*]
1.0 mg/kg BAY 73-4506	98 % [111 %]	107 % [118 %]
4.0 mg/kg BAY 73-4506	105 % [103 %]	104 % [117 %]
16.0 mg/kg BAY 73-4506	84 % [94 %]	91 % [96 %]

*: n = 3 animals

Feed Consumption: (daily) No compound related effect

Group	Food consumption relative to control animals	
	Males on day 364 (week 51)	Females on day 356 (week 51)
1.0 mg/kg BAY 73-4506	76 % (84 %)	87 % (91 %)
4.0 mg/kg BAY 73-4506	122 % (135 %)	92 % (98 %)
16.0 mg/kg BAY 73-4506	113 % (127 %)	120 % (102 %*)

*: n = 3 animals; excluding animal 230F which received canned dog food supplementation

Water Consumption: (Weekly) No effect

Ophthalmoscopy: (Weeks -3/-2(pre-dosing), 6, 12, 25, 38, and 52)
No compound-related effect

Blood Pressure: (Pre- and 2 hr- post administration in weeks 5, 12, 25, 37, and 51 as well as 24 h after administration in weeks 6, 13, 28, 38, and 52)

No compound-related effect on blood pressure as shown below.

Table 33: Blood pressure (mmHg) on week 51 of treatment (52 Week Dog Study)

Group	1		2		3		4	
Dose (mg/kg)	0		1		4		16	
Sex	M	F	M	F	M	F	M	F
Syst. Mean	185	187	208	175	193	194	202	180
S.D.	24	13	12	14	21	12	23	6
Diast. Mean.	91	88	99	86	88	95	96	87
S.D.	7	5	11	8	10	10	6	9

ECG: (Pre and 2 h after administration in weeks 5, 12, 25, 37, and 51 as well as 24 h after administration in weeks 6, 13, 28, 38, and 52)

Though at the high dose there was a trend towards a decreased heart rate in animals of both sexes that correlated with a prolonged RR interval compared to controls, there was no clear dose and/or time–dependent effect observed on other parameters including QTc interval.

Table 34: ECG Parameters (Week 51 of treatment relative to start date-52 Week Dog Study)

Group		1		2		3		4	
Dose (mg/kg)		0		1		4		16	
Sex		M	F	M	F	M	F	M	F
RR-1'2 (ms)	Mean	681	714	654	864	707	656	881	837
	S.D.	49	71	155	45	179	143	104	36
HR'2 (bts/min	Mean	88	85	96	70	91	96	69	72
	S.D.	6	8	23	3	30	25	9	3
PWdth'2 (ms)	Mean	52	52	50	52	49	47	52	55
	S.D.	2	1	3	2	3	4	1	2
PR-1'2 (ms)	Mean	97	109	88	100	96	102	95	108
	S.D.	4	16	3	7	3	10	4	12
QRS'2 (ms)	Mean	45	41	36	41	40	39	40	40
	S.D.	5	3	4	4	6	4	3	3
QT-1'2 (ms)	Mean	201	204	211	223	197	197	217	206
	S.D.	5	8	23	3	14	18	13	7
QTcb'2 (ms)	Mean	244	241	265	241	239	245	232	225
	S.D.	7	9	36	9	20	8	13	9

Clinical Test of Nervous System Function:

(Weeks -2/-1 (pre-values), 6, 12, 25, 37 and 51 (before administration). The function of the nervous system is tested by stimulation of certain effectors-organs and evaluation of brain reflexes ((pupillary light reflex, consensual light reflex, palpebral reflex, corneal reflex, gag and cough reflexes), spinal nerve reflexes (flexor reflex, patellar reflex, supratarsal & supracarpal reflex, and anal reflex), as well as attitudinal reactions (extensor postural thrust reaction, hopping reaction, placing reaction, Schuster reflex, righting reaction, tonic neck reaction). Sensitivity of the skin of the torso and extremities were also evaluated.

No compound-related findings as shown below.

Table 35: Clinical test of nervous system function in the dog

Group 1	Test of brain reflexes					Test of spinal nerve reflexes					Attitude reactions						Sensitivity	
Animal	PuR	PuCR	CR/LR	COR	GRX	FR	PR	STR	SCR	AR	EPTR	SchR	HR	PLR	RR	TNR	E	T
201 M	+	+	+	-	+	+	+	+	-	+	+	F-H+	+	+	+	+	-	-
202 M	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+
203 M	+	+	+	-	+	+	+	+	-	-	+	+	+	+	+	+	+	-
204 M	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+
205 F	+	+	+	-	+	+	+	+	-	-	+	F-H+	+	+	+	+	+	+
206 F	+	+	+	-	+	+	+	-	-	-	+	+	+	+	+	+	+	+
207 F	+	+	+	-	+	+	+	+	-	-	+	+	+	+	0+	+	+	+
208 F	+	+	+	-	+	+	+	+	-	-	+	+	+	+	+	+	+	+

Group 2	Test of brain reflexes					Test of spinal nerve reflexes					Attitude reactions						Sensitivity	
Animal	PuR	PuCR	CR/LR	COR	GRX	FR	PR	STR	SCR	AR	EPTR	SchR	HR	PLR	RR	TNR	E	T
209 M	+	+	+	-	+	+	+	+	-	-	+	F-H+	+	+	+	+	+	+
210 M	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+
211 M	+	+	+	-	+	+	+	+	-	-	+	F-H+	+	+	+	+	+	+
212 M	+	+	+	-	+	+	+	+	-	-	+	+	+	+	+	+	+	+
213 F	+	+	+	-	+	+	+	+	-	+	+	F-H+	+	+	+	+	+	+
214 F	+	+	+	-	+	+	+	-	-	+	+	F-H+	+	+	+	+	+	+
215 F	+	+	+	-	+	+	+	+	-	-	+	+	+	+	+	+	+	+
216 F	+	+	+	-	+	+	+	+	-	-	+	+	+	+	+	+	+	-

Group 3	Test of brain reflexes					Test of spinal nerve reflexes					Attitude reactions						Sensitivity	
Animal	PuR	PuCR	CR/LR	COR	GRX	FR	PR	STR	SCR	AR	EPTR	SchR	HR	PLR	RR	TNR	E	T
217 M	+	+	+	+	+	+	+	+	-	-	+	F-H+	+	+	+	+	+	+
218 M	+	+	+	-	+	+	+	-	-	-	F-H+	+	+	+	+	+	+	+
219 M	+	+	+	-	-	+	+	+	+	-	+	F-H+	+	+	+	+	+	+
220 M	0+	+	+	-	+	+	+	+	-	-	+	+	+	+	+	+	+	+
221 F	+	+	+	-	+	+	+	+	-	-	+	+	+	+	+	+	+	+
222 F	+	+	+	-	+	+	+	+	-	-	+	F-H+	+	+	+	+	+	-
223 F	+	+	+	-	+	+	+	+	-	-	+	F-H+	+	+	-	+	+	+
224 F	+	+	+	-	+	+	+	+	-	-	+	+	+	+	+	+	+	+

Group 4	Test of brain reflexes					Test of spinal nerve reflexes					Attitude reactions						Sensitivity	
Animal	PuR	PuCR	CR/LR	COR	GRX	FR	PR	STR	SCR	AR	EPTR	SchR	HR	PLR	RR	TNR	E	T
225 M	+	+	+	-	+	+	+	+	-	-	+	+	+	+	+	+	+	+
226 M	+	+	+	-	+	+	+	+	-	-	+	+	+	+	+	+	+	+
227 M	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-
228 M	+	+	+	-	+	+	+	-	-	-	+	+	+	+	+	+	+	+
229 F	+	+	+	-	+	+	+	+	-	-	+	+	+	+	+	+	+	+
230 F	+	+	+	-	+	+	+	+	-	-	+	+	+	+	+	+	+	+
231 F	+	+	+	-	+	+	-	-	-	-	+	+	+	+	+	+	+	+
232 F	+	+	+	-	-	+	+	-	+	-	+	+	+	+	+	+	+	-

PuR - pupillary light reflex
PuCR - consensual pupillary light reflex
CR/LR - corneal and palpebral reflex
COR - cough reflex
GRX - gag reflex
FR - flexor reflex
PR - patellar reflex
STR - supratarsal reflex
SCR - supracarpal reflex
AR - anal reflex
EPTR - extensor postural thrust reaction
SchR - Schuster reflex (tactile reflex)
HR - hopping reaction
PLR - placing reaction
RR - righting reaction
TNR - tonic neck reaction
E - sensitivity of skin (extremities)
T - sensitivity of skin (torso)

\- - no response
\+ - normal response
+- - unclear (ambivalent) response
+! - exaggerated response
0+ - delayed response
+/+ - additional response of the opposite reflex arc
(?)Ct - (?) response with tonic convulsion
(?)Cc - (?) response with clonic convulsion
r - right
l - left
F - forelimb
H - hindlimb
E - limbs
T - trunk
h - head

(excerpted from the Applicant's submission)

Body Temperature: (Weeks -1 (pre-value), 6, 12, 25, 37, and 51 (before administration)

No compound-related effects.

Hematology: (Weeks -3 (pre-value), 6, 13, 28, 38, and 52)

Monocyte counts were increased in high dose females (16 mg/kg) from week 13 onwards.

Table 36: Hematology Findings (52-Week Dog)

Group		1		2		3		4	
Dose (mg/kg/day)		0		1		4		16	
Sex		M	F	M	F	M	F	M	F
Monocyte*	Mean	0.42	0.43	0.50	0.75	0.87*	0.57	0.91*	1.43
	S.D.	0.16	0.19	0.13	0.46	0.27	0.07	0.26	1.41

*Week 52

The following statistically significant differences in hematological parameters were not considered compound related.

Parameter	Increase ↑ Decrease ↓	Group	Sex	Week	Statistical significance	Reason
RBC	↓	4	M	13	$p < 0.05$	B, T
HGB	↓	4	M	13, 28	$p < 0.05$	B, T
MCHC	↓	2, 3, 4	F	13	$p < 0.05$ to $p < 0.01$	B, T
MCHC	↓	4	M	28	$p < 0.05$	B, T
WBC	↑	3	M	13	$p < 0.05$	B, D
Neutrophil count	↑	3	M	13	$p < 0.05$	B, D
Eosinophil count	↑	2	M	38	$p < 0.05$	B, D
Eosinophil count	↓	4	F	52	$p < 0.05$	B
Basophil count	↓	4	M	38, 52	$p < 0.05$	B
Basophil count	↓	3, 4	F	52	$p < 0.05$ to $p < 0.01$	B

B: the difference to control animals is too small to be biologically relevant
D: lack of dose-dependence
T: lack of time-dependence
M: male
F: female

(excerpted from the Applicant's submission)

Clinical Chemistry: (Weeks -3 (pre-value), 6, 13, 28, 38, and 52)

Table 37: Clinical Chemistry-Week 52 Values (52 Week Dog)

Group	1		2		3		4	
Dose (mg/kg)	0		1		4		16	
Sex	M	F	M	F	M	F	M	F
AST (u/L)	31±10	35±6	35±4	33±9	40±5	33±4	65±21**	48±15
ALT (u/L)	48±14	41±9	39±8	38±9	41±9	42±14	71±21	44±18
ALP (u/L)	79±48	128±48	61±27	79±32	121±56	130±65	111±41	147±27
GLU (mg/100Ml)	84±8	83±10	90±8	94±11	100±6*	99±9	99±2*	103±11*
CREA(mg/100 mL)	1.1±0.1	0.8±0.1	1.0±0.1	0.8±0.1	0.8±0.1**	0.8±0.2	0.7±0.1**	0.7±0.2

Group	1		2		3		4	
Dose (mg/kg)	0		1		4		16	
Sex	M	F	M	F	M	F	M	F
Na (mmol/L)	149±1	149±2	148±1	147±2	146±1**	148±1	145±1**	145±2*

Statistics Test: Dunnett Test: * - 5% significance level;
** - 1% significance level;

Thyroid Hormones: (Weeks -3 (pre-value), 6, 13, 28, 38, and 52)

Table 38: Mean values of thyroid hormone analysis, males and females combined (52 Week Dog)

Dose	T3		T4		TSH	
	nmol/l		nmol/l		mcg/l	
Week 6						
0 mg/kg	1.26		20		0.09	
1 mg/kg	1.32	-	23	-	0.16	-
4 mg/kg	1.33	-	27	-	0.10	-
16 mg/kg	1.27	-	23	-	**0.17**	+
Week 13						
0 mg/kg	1.40		23		0.16	
1 mg/kg	1.43	-	30	-	0.26	-
4 mg/kg	1.25	-	26	-	0.16	-
16 mg/kg	1.37	-	29	-	**0.42**	-
Week 28						
0 mg/kg	1.48		25		0.14	
1 mg/kg	1.46	-	29	-	0.24	-
4 mg/kg	1.49	-	29	-	0.22	-
16 mg/kg	1.41	-	26	-	**0.52**	+
Week 38						
0 mg/kg	1.48		29		0.17	
1 mg/kg	1.40	-	30	-	0.22	-
4 mg/kg	1.59	-	33	-	0.20	-
16 mg/kg	1.41	-	29	-	**0.63**	+
Week 52						
0 mg/kg	1.52		26		0.17	
1 mg/kg	1.44	-	29	-	0.22	-
4 mg/kg	1.48	-	27	-	0.22	-
16 mg/kg	1.32	-	26	-	**1.17**	++

+:p≤0.05, ++:p≤0.01 -:no significance ,TSH:Thyroid stimulating hormone

Bone Marrow: Week 52 relative to start date

Table 39: Bone Marrow Smear Findings (52-Week Dog)

Group	Dose (mg/kg)	Sex	Erythro immature	Erythro mature	Eosino immature	Eosino mature
1	0	M	95±12	249±29	4±3	5±2
		F	96±21	228±11	12±2	8±4
2	1	M	59±6*	233±18	7±8	7±10
		F	85±14	234±32	4±2**	8±6

Group	Dose (mg/kg)	Sex	Erythro immature	Erythro mature	Eosino immature	Eosino mature
3	4	M	87±7	201±39	7±3	9±4
		F	84±7	242±38	4±3**	11±9
4	16	M	70±17*	223±24	4±1	4±4
		F	72±8	186±39	9±3	9±4

Statistics Test: Dunnett Test: * - 5% significance level;
** - 1% significance level;

Urinalysis: (Weeks -1 (pre-value), 6, 13, 28, 38, and 51)
No dose and duration-related effects.

Gross Pathology: (End of treatment)

Table 40: Gross Pathology (52 Week Dog)

Group		1		2		3		4	
Dose (mg/kg)		0		1.0		4.0		16.0	
Sex		M	F	M	F	M	F	M	F
General observations	Animal emaciated							1	1*
Gall bladder	Contents discolored, jelly like					1		1	
Kidney	Discoloration							1	
	Enlarged							1	
Skeletal muscle	Atrophic					1			2
Skin/subcutis	Abscess-like lesion							1	
	Alopecia				1	4	4	4	4
	Thinning of fur			1		4	4	4	4
	Scab formation					2	4	4	4
	Thickened								2

* = Finding recorded as “retarded body growth

Organ Weights: (At necropsy)

16.0 mg/kg – Decreased thymus weight, increased kidney weight and decreased ovary weight

Histopathology:

Adequate Battery: Yes
Peer Review: Yes

Histological Findings:

Group		1		2		3		4	
Dose (mg/kg/day)		0.0		1.0		4.0		16.0	
No. of animals		4	4	4	4	4	4	4	4
Organ	Sex	M	F	M	F	M	F	M	F
Adrenal cortices, vacuolar degener. total affected		-	-	-	1	-	2	2	1
mean severity		-	-	-	1.0	-	2.0	2.0	2.0
Epididymides, lymphoid c. infiltr., total affected		1	-	1	-	2	-	3	-
mean severity		2.0		2.0		2.0	-	2.0	
tubular mineralizat., total affected		-	-	1	-	1	-	2	-
mean severity		-	-	2.0	-	2.0	-	2.0	-
cellular debris, total affected		1	-	2	-	4	-	1	-
mean severity		1.0	-	1.0	-	1.5	-	1.0	
epith. vacuole. degen., total affected		-	-	3	-	-	-	1	-
mean severity		-	-	2.3	-	-	-	3.0	-
Gallbladder, lymphoid follicles, total affected		1	2	1	2	3	3	3	4
mean severity		1.0	1.0	1.0	1.0	1.3	1.0	2.7	2.0
epithelial hyperplasia, total affected		-	-	-	-	3	-	4	3
mean severity		-	-	-	-	1.7	-	2.8	2.3
inspissation, total affected		-	-	1	2	3	-	2	3
mean severity		-	-	1.0	1.0	1.0	-	1.0	1.7
calculus, total affected		-	-	1	2	3	-	2	3
mean severity		-	-	1.0	-	-	1.0	-	1.0
Ileum, eosinoph. homogenous material, total affected -		-	-	1	-	1	1	3	-
mean severity		-	-	1.0	-	1.0	1.0	1.7	-
Jejunum, eosinoph. homogenous material, total affected		-	-	-	-	-	-	2	-
mean severity		-	-	-	-	-	-	2.0	-
Kidneys, glomerulosclerosis, total affected		-	-	-	-	2	-	4	4
mean severity		-	-	-	-	1.0	-	2.5	1.0
tub. dege./regen, total affected		-	-	-	-	4	4	4	4
mean severity		-	-	-	-	2.0	2.0	2.5	2.3
hyaline casts, total affected		-	-	2	2	3	3	4	2
mean severity		-	-	1.0	1.0	1.0	1.0	1.5	1.0
interstit. fibrosis, total affected		-	-	-	1	-	-	2	1
mean severity		-	-	-	1.0	-	-	2.0	1.0
cortical mineralization, total affected		-	1	-	-	-	2	3	2
mean severity		-	1.0	-	-	-	1.0	1.3	1.0
glomerulopathy, total affected		1	-	1	1	2	4	3	4
mean severity		1.0	-	1.0	1.0	2.0	2.3	2.0	2.0
Liver, microgranuloma, total affected		3	3	4	3	2	3	4	4
mean severity		1.0	1.0	1.0	1.0	1.5	1.7	1.5	2.5
infiltration mononuclear, total affected		-	3	1	1	3	4	4	4
mean severity		-	1.0	1.0	1.0	1.3	1.8	2.3	2.5
Lung, alveolar/foamy, macrophages, total affected		-	-	1	1	2	-	1	2
mean severity		-	-	1.0	1.0	1.0	-	1.0	2.0

	Group	1		2		3		4	
	Dose (mg/kg/day)	0.0		1.0		4.0		16.0	
	No. of animals	4	4	4	4	4	4	4	4
Organ	Sex	M	F	M	F	M	F	M	F
Ovaries, cystic corpora lutea, total affected		-	-	-	-	-	-	-	4
mean severity		-	-	-	-	-	-	-	2.5
follicular cyst, total affected		-	-	-	-	-	-	-	2
mean severity		-	-	-	-	-	-	-	3.5
red. devel. follicle, total affected		-	-	-	-	-	-	-	4
mean severity		-	-	-	-	-	-	-	3.3
inc. foll. degener, total affected		-	-	-	-	-	3	-	3
mean severity		-	-	-	-	-	1.0		2.0
mineralization, total affected		-	-	-	2	-	3	-	-
mean severity		-	-	-	1	-	1	-	-
Pituitary gland, foamy macrophages, total affected		-	-	-	-	-	-	-	2
mean severity		-	-	-	-	-	-	-	2.0
Skin/subcutis, inflammation, total affected		-	-	-	-	-	-	1	1
mean severity		-	-	-	-	-	-	3	3
Hair growth arrest, total affected		-	-	1	1	4	4	4	4
mean severity		-	-	2.0	1.0	2.8	3.0	4.0	4.0
hyperkeratosis, total affected		-	-	2	-	4	4	4	4
mean severity		-	-	1.5	-	1.3	1.8	2.0	2.8
follicular keratosis, total affected		-	4	3	4	4	4	4	4
mean severity		-	1.0	2.0	1.0	2.8	2.5	4.0	4.0
hypergranulosis, total affected		-	2	1	3	-	3	3	4
mean severity		-	1.0	1.0	1.0	-	1.7	2.3	2.5
lymphoid C. infiltr., total affected		1	1	2	1	1	3	2	3
mean severity		1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.7
Spleen, increased hematopoiesis, total affected		-	-	-	-	2	1	4	4
mean severity		-	-	-	-	1.5	2.0	2.0	2.0
lymphoid hyperplasia, total affected		-	-	-	-	-	-	4	4
mean severity		-	-	-	-	-	-	2.0	2.0
incr. pigment deposit, total affected		1	-	2	-	2	4	4	4
mean severity		1.0	-	1.0	-	2.0	1.8	2.0	2.5
Testes, spermat. giant cells, total affected		1	-	2	-	3	-	3	-
mean severity		1.0		1.5		1.0		1.0	
Thymus, atrophy, total affected		2	2	3	3	3	1	3	4
mean severity		1.0	2.0	1.7	1.7	1.7	1.0	3.0	2.3
Thyroid gland, mineralization, total affected		-	-	-	2	2	-	1	3
mean severity		-	-	-	1.0	1.5	-	2.0	1.0
Tonsils, mineralization, total affected		-	-	-	-	-	-	1	1
mean severity		-	-	-	-	-	-	1.0	1.0
Uterus, cystic gld. dilatat., total affected		-	-	-	-	-	1	-	4
mean severity		-	-	-	-	-	3.0	-	2.0

Grading: 1 = very slight, minimal; 2 = slight; 3 = moderate; 4 = marked; 5 = massive

Toxicokinetics: (Before and 1, 2, 4, 7, and 24 h after administration on days 1, 81, 172, and 354)

Table 41: The exposure to BAY73-4506 on day 354 (52-week dog)

Dose: BAY 73-4506 [mg/kg]		1.0		4.0		16.0	
		Mean geom.	S.D. geom.	Mean geom.	S.D. geom.	Mean geom.	S.D. geom.
$AUC_{(0-24)}$	[µg·h/L]	4265	1.27	14441	1.35	34822	1.21
$AUC_{(0-24)norm}$	[kg·h/L]	4.27	1.27	3.61	1.35	2.18	1.21
C_{max}	[µg/L]	672	1.17	2210	1.38	4936	1.18
$C_{max,norm}$	[kg/L]	0.672	1.17	0.552	1.38	0.308	1.18
$C(24)/C_{max}$	[%]	6.18	2.06	5.52	1.58	4.18	1.35
t_{max}	[h]	1.68	1.38	1.68	1.63	2.38	1.38
R_{A1}	[%]	112		89.9		95.8	
R_{A3}	[%]	118		97.1		97.0	

$R_{A1} = C_{max,norm}$_Day 354 / $C_{max,norm}$_Day 1 in [%]

$R_{A3} = AUC(0-24)_{norm}$_Day 354 / $AUC(0-24)_{norm}$_Day 1 in [%]

Stability and Homogeneity: Stable and homogenous

Special Toxicology Studies

Study title: **BAY81-8752 (Metabolite M-5):Subacute Oral Toxicity Study in CD-I**

Study no.: T4079477
Report no.: PH-35852
Study report location: Toxicology of Bayer Schering Pharma AG, BSP-GDD-GED-GTOX, Wuppertal, Germany
Conducting laboratory and location: Bayer Schering Pharma AG
GDD-GED General Toxicology
42096 Wuppertal
Germany
Date of study initiation: August 26, 2008
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BAY 81-8752, batch # BXR3TVA, 98% purity

Key Study Findings

- Gavage administration of BAY 81-8752 (metabolite M-5 of BAY 73-4506) up to 5 mg/kg/day for 4 months did not cause any toxicity/mortality in main groups.
- One kinetic group male at 5 mg/kg was found dead on day 14.
- There was an increase in dentin alteration in 20 mg/kg females
- Histopathology revealed slightly dilated bone marrow sinuses and a slight hypocellularity (femur and sternum) in two high dose males.

- AUC_{0-24} and C_{max} values increased dose-dependently with no evidence of sex-related differences in exposure.

Methods

Doses:	1, 5 and 20 mg/kg/day (see table below)
Frequency of dosing:	Daily for 4 weeks
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	Ethanol/solutol HS 15/demineralized water
Species/Strain:	Crl:CD-1
Number/Sex/Group:	10
Age:	6-7 weeks
Weight:	Males – 26 to 34 g Females – 23 to 27 g
Satellite groups:	Yes
Unique study design:	None
Deviation from study protocol:	None

Group No.	Dose (mg/kg)	Sex	Number of animals	Animal No.
Main toxicity groups				
1	0	M	10	1-10
2	1	M	10	11-20
3	5	M	10	21-30
4	20	M	10	31-40
5	0	F	10	41-50
6	1	F	10	51-60
7	5	F	10	61-70
8	20	F	10	71-80
Satellite groups for toxicokinetics				
9	0	M	4	81-84
10	1	M	19	85-103
11	5	M	19	104-122
12	20	M	19	123-141
13	0	F	4	142-145
14	1	F	19	146-164
15	5	F	19	165-183
16	20	F	19	184-202

Observations and Results

Mortality: (twice daily)
5 mg/kg satellite (kinetic) group – One male (found dead on Day 14)
20 mg/kg satellite group – one male (day 14), one female (Day 21)
[post-mortem investigations were not performed]

Clinical Signs: (daily)
20 mg/kg satellite group – One female had piloerection, reduced motility and labored breathing probably due to gavage error

Body Weights: (daily)
No effect up to 20 mg/kg/day

Feed Consumption: (weekly)

Table 42: Cumulative and mean daily food intake (M-5 Study)

Dose mg/kg	Days	g/animal total	g/animal per day	g/kg body weight total	g/kg body weight per day
			Main Groups		
males					
0	27	209	7.7	7003	259.4
1	27	228	8.4	7474	276.8
5	27	232	8.6	7463	276.4
20	27	199	7.4	6788	251.4
females					
0	27	222	8.2	8935	330.9
1	27	299	11.1	10976	406.5
5	27	245	9.1	9116	337.6
20	27	267	9.9	10598	392.5

Ophthalmoscopy: Not reported

ECG: Not done

Hematology: (day 17/18 main groups)
No toxicologically relevant changes

Clinical Chemistry: (day 24/25 main groups)

Dose (mg/kg)	0		1		5		20	
Sex	M	F	M	F	M	F	M	F
Total bilirubin (mcmol/l)	3.1	3.2	3.4	2.3	4.9	2.9	10.4++	8.5++

Urinalysis: Not done

Gross Pathology: days 29-31 (main groups)
High dose - Diminished size of seminal vesicle in 2 males

Organ Weights: No significant difference in absolute and relative organ weights of brain, heart, liver (with gall bladder), spleen, kidneys (both), ovaries (both), testes (both), and uterus (with cervix).

Histopathology:

Adequate Battery: Yes
Peer Review: Yes

Table 43: Histological Findings - Terminal sacrifice (M-5 Study)

Group	1		2		3		4	
Dose (mg/kg)	0		1		5		20	
Sex	M	F	M	F	M	F	M	F
Organ/findings Animal examined	10	10	10	10	10	10	10	10
Duodenum, elongated villi, grade 2	-		-		-		1	
Epididymides, mononuclear infiltr. grade 1	-		-		-		1	
Esophagus, focal inflammation, grade 1	1		-		-		3	
grade 2	-		-		-		1	
Femur, dilated sinuses, grade 2	-		-		-		2	1
Heart, degener./mineral, grade 2		-		-		-		1
inflammat. infiltr., grade		-		-		-		1
Ileum, elongated villi, grade 3	-		-		-		1	
Jejunum, elongated villi, grade 3	-		-		-		1	
Liver, distinct lobulation	-	-	-	-	-	-	-	1
Kupffer cell foci, grade 1		1		-		-		3
Lungs, adhesion	-	-	-	-	-	1	-	-
discoloration	-	-	-	-	-	-	-	1
pleuritis, grade 3		-		-		1		-
pleural fibrosis, grade 3		-		-		1		-
incompl. instillation		-		-		-		1
Seminal ves./coag., diminished in size	-	-	-	-	-	-	2	-
artifact, grade 3	-		-		-		1	
Spleen, atrophy, grade 3		-		-		-		1
lymphocytolysis, grade 3		-		-		-		1
Sternum, dilated sinuses, grade 2	-		-		-		2	
grade 3								1
Stomach, erosion, grade 1	-		-		-		1	
vacuolization, grade 1	-		-		-		1	
Teeth, dentin alteration, grade 1								3
grade 2								1
grade 3								1
Testes, vacuolation, grade 1	-		-		-		1	
Thymus, adhesion	-	-	-	-	-	1	-	-
edematous	-	-	-	-	-	-	-	1
Uterus, inflammat. infiltr., grade 2		-		-		-		1

GRADE 1 = Minimal / very few / very small
GRADE 2 = Slight / few / small
GRADE 3 = Moderate / moderate number / moderate size
GRADE 4 = Marked / many / large
GRADE 5 = Massive / extensive number / extensive size
P = Finding present, severity not scored
(= Finding unilateral in paired organs

Special Evaluation: None

Toxicokinetics: (days 1 and 21 at 0.5, 1, 2, 4, 7 and 24 hours post administration, satellite groups)

Table 44: Toxicokinetics (M-5 Study)

Dose: BAY 81-8752 (M-5) [mg/kg]		1	5**	20**
		Mean geom.	Mean geom.	Mean geom.
$AUC(0\text{-}t_n)$	[µg·h/L]	1100	14951	77805
$AUC(0\text{-}t_n)_{norm}$	[kg·h/L]	1.10	2.99	3.89
AUC(0-24)	[µg·h/L]	1600*	14951	77805
$AUC(0\text{-}24)_{norm}$	[kg·h/L]	1.60*	2.99	3.89
C_{max}	[µg/L]	218	2134	9808
$C_{max,norm}$	[kg/L]	0.218	0.427	0.490
$C(24)/C_{max}$	[%]	n.c.	0.178	0.282
t_{max}	[h]	4.00	4.00	4.00
R_{A1}	[%]	76.2	86.4	53.3
R_{A3}	[%]	55.8	84.0	65.3

n.c. = not calculated
* = obtained by extrapolation
R_{A1} = $C_{max,norm}$_Day 21 / $C_{max,norm}$_Day 1 in [%]
R_{A3} = $AUC(0\text{-}24)_{norm}$_Day 21 / $AUC(0\text{-}24)_{norm}$_Day 1 in [%]
** = n = 5, 6

(excerpted from the Applicant's submission)

Stability and Homogeneity: Stable and homogenous

Study title: **BAY 75-7495 (metabolite M-2): Repeated Dose Systemic Toxicity Study in CD-1 Mice (4-Weeks Administration by Gavage)**

Study no.:	T0077259
Report no.:	PH-35885
Study report location:	Toxicology of the Bayer Schering Pharma AG Wupperta1, Germany.
Conducting laboratory and location:	Bayer Schering Pharma AG GDD-GED General Toxicology 42096 Wuppertal Germany.
Date of study initiation:	September 15, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BAY 75-7495 (metabolite M-2), batch # BXR3BSX, 91 % purity

Key Study Findings

- Oral administration of BAY 75-7495 (metabolite of BAY 73-4506) up to 20 mg/kg for 4 weeks did not cause any mortality in mice.
- Incisor teeth of the lower and upper jaw displayed dentin alteration in both sexes at 20 mg/kg.

- AUC_{0-24} and C_{max} values increased more than dose proportionally.
- NOAEL for BAY 75-7495 was 5 mg/kg/day in both sexes.

Methods

Doses:	1, 5 or 20 mg/kg/day (see table below for details)
Frequency of dosing:	Daily
Route of administration:	Oral by gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	Ethanol/Solutol HS15/demineralized water (1/4/5 w/w/w)
Species/Strain:	Crl CD-I(ICR)BR mice
Number/Sex/Group:	10
Age:	6 weeks
Weight:	Males – 27 to 31 g females – 24 to 29 g
Satellite groups:	Yes
Unique study design:	None
Deviation from study protocol:	None

Group No	Dose (mg/kg)	Sex	No of Animals	Animal-No		
Main Groups						
1	0	m	10	1	-	10
2	1	m	10	11	-	20
3	5	m	10	21	-	30
4	20	m	10	31	-	40
5	0	f	10	41	-	50
6	1	f	10	51	-	60
7	5	f	10	61	-	70
8	20	f	10	71	-	80
Satellite Groups for Toxicokinetics/Cytogenetics (males)						
9	0	m	4	81	-	84
10	1	m	19	85	-	103
11	5	m	19	104	-	122
12	20	m	19	123	-	141
13	0	f	4	142	-	145
14	1	f	19	146	-	164
15	5	f	19	165	-	183
16	20	f	19	184	-	202

Observations and Results

Mortality: (twice daily) Control (0 mg/kg/day) – 1 M & 1 F
1 mg/kg/day – 1 M

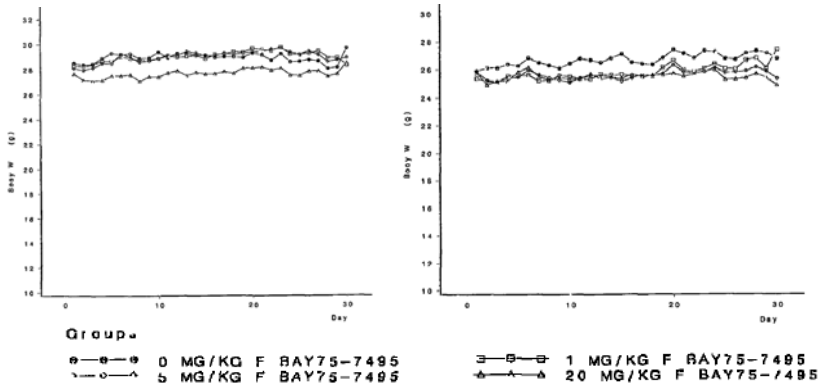
Clinical Signs: (daily) 20 mg/kg/day – Loss of hair in 1 female

Body Weights: (daily)

20 mg/kg/day – 7% lower vs. control (both sexes)

Figure 5: Body Weight (M-2 Study)

Males Females



Feed Consumption: (weekly)

Table 45: Mean Food Intake (M-2 Study)

Mean food intake					
Dose (mg/kg)	Day	G/animal		G/kg body weight	
		Total	Per day	Total	Per day
Males					
0	1-29	261	9.33	9183	327.95
1	1-29	297	10.62	10219	364.96
5	1-29	239	8.55	8271	295.41
20	1-29	213	7.66	7738	276.37
Females					
0	1-29	330	11.78	12270	438.23
1	1-29	336	11.99	13067	466.68
5	1-29	290	10.34	11543	412.25
20	1-29	284	10.15	11190	399.65

5 mg/kg/day – Decreased 10% (males) and 6% (females) per kg body weight
20 mg/kg/day – Decreased 16% (males) and 9% (females) per kg body weight

Ophthalmoscopy: Not done

ECG: Not done

Hematology: (day 24/25)

Table 46: Hematology Findings (M-2 Study)

Dose mg/kg	ERY 10E12/l		HB g/l		HCT l/l		MCV fl	MCH pg	MCHC g/l ERY		RETI o/oo		THRO 10E9/l
m	Day 24												
0	9 97		157		0 504		50 6	15 7	311		25		1349
1	9 72		148	++	0 483	+	49 8	15 3	307		27		1265
5	9 54		147	++	0 471	++	49 5	15 5	313		28		1306
20	9 60		154		0 484	+	50 4	16 0	318	+	34	++	1400
f	Day 25												
0	9 93		155		0 490		49 4	15 7	317		31		1255
1	9 53	+	152		0 486		51 1	16 0	312		33		1207
5	9 63		150		0 475	+	49 3	15 6	316		26	+	1212
20	9 59		155		0 483		50 4	16 1	319		34		1187

Legends + p ≤ 0 05, ++ p ≤ 0 01

Clinical Chemistry: (day 28/29)

Table 47: Clinical Chemistry (M-2 Study)

Dose (mg/kg/day)	Sex	AST (GOT) U/L	ALT (GPT) U/L	BILI-t (mcmol/L)
0	M	41.4	38.5	2.1
1		46.3	43.5	2.6
5		55.4^{++}	48.2	2.0
20		50.1^{+}	50.7^{+}	3.5^{++}
0	F	54.7	39.1	1.9
1		58.9	44.4	2.1
5		66.0	39.9	2.0
20		65.9	38.7	2.7^{++}

+ - p≤0.05 ++ - p≤0.01

Urinalysis: Not done

Gross Pathology: (days 29-31) No effect

Organ Weights: 20 mg/kg/day – Absolute and relative spleen weight increased

Histopathology:

Adequate Battery: Yes
Peer Review: No

Histological Findings:

Sex	Males				Females			
Number of animals	9	9	10	10	9	10	10	10
Organ/Finding Dose (mg/kg)	0	1	5	20	0	1	5	20
Liver, hematopoiesis, grade 1	0	0	0	1	2	1	3	5
grade 2	0	0	0	0	0	0	0	2
Spleen, lymphoid depletion, grade 1	0	0	0	1	0	0	0	0
Teeth, lower jaw, dentin alteration, grade 1	0	0	0	4	0	0	0	3
Teeth, upper jaw, dentin alteration, grade 1	0	0	0	4	0	0	0	7
grade 2	0	0	0	5	0	0	0	3
Testes, hypoplastic tubules, grade 3	0	0	0	3				

Cytogenetics: (Day 35 of satellite males)

Microscopic slides were not evaluated for cytogenetics.

Toxicokinetics: (Days 1/2 and 29/30 at 0 5, 1, 2, 4, 7, and 24 after administration)

Table 48: Toxicokinetics (M-2 Study)

Dose BAY 75-7495 (M-2) [mg/kg]		1 mean geometric	5 mean geometric	20 mean geometric
AUC(0-24)	[µg h/L]	1686	11533	46867
$AUC(0-24)_{norm}$	[kg h/L]	1 69	2 31	2 34
C_{max}	[µg/L]	220	1889	7047
$C_{max\ norm}$	[kg/L]	0 220	0 378	0 352
$C(24)/C_{max}$	[%]	1 85	0 509	0 349
t_{max}	[h]	4 00	2 00	4 00
R_AAUC	[%]	66 3	73 4	69 2
R_AC_{max}	[%]	52 5	64 2	51 0

$R_AAUC = AUC(0-24)_{norm}$ Day 30 / $AUC(0-24)_{norm}$ Day 1 in [%]
$R_AC_{max} = C_{max\ norm}$ Day 30 / $C_{max\ norm}$ Day 1 in [%]

Stability and Homogeneity: Acceptable. Formulation is stable for 8 days.

Histopathology inventory:

Study	T4079477	T0077259	T2078241	T3076046	TXST20070037
Species	Mouse	Mouse	Rat	Dog	Dog
Adrenals	X	X	X*	X*	X
Aorta	X	X	X	X	X
Bone Marrow smear			X	X	X
Bone (femur)	X	X	X	X	X
Brain	X*	X*	X*	X*	X*
Cecum	X	X	X	X	X
Cervix	X*	X*			X
Colon	X	X	X	X	X
Duodenum	X	X	X	X	X
Epididymis	X	X	X*	X*	X*
Esophagus	X	X	X	X	X
Eye	X	X	X	X	X
Fallopian tube					
Gall bladder	X*	X*		X*	X
Gross lesions	X	X	X	X	X
Harderian gland	X	X	X		
Heart	X*	X*	X*	X*	X*
Ileum	X	X	X		X
Injection site					
Jejunum	X	X	X	X	X
Kidneys	X*	X*	X*	X*	X*
Lachrymal gland	X	X	X		X
Larynx	X	X	X	X	X

Study	T4079477	T0077259	T2078241	T3076046	TXST20070037
Species	Mouse	Mouse	Rat	Dog	Dog
Liver	X*	X*	X*	X*	X*
Lungs	X	X	X	X*	X*
Lymph nodes, cervical					
Lymph nodes mandibular	X	X	X	X	X
Lymph nodes, mesenteric	X	X	X	X	X
Mammary Gland		X			X
Nasal cavity	X	X		X	X
Optic nerves	X	X	X	X	X
Ovaries	X*	X*	X*	X*	X*
Pancreas	X	X	X	X*	X
Parathyroid	X		X*	X*	X*
Peripheral nerve					X
Pharynx	X	X	X	X	
Pituitary	X	X	X	X*	X
Prostate	X	X	X*	X*	X*
Rectum	X	X	X	X	X
Salivary gland	X	X	X		X
Sciatic nerve	X	X	X	X	X
Seminal vesicles	X	X	X*		
Skeletal muscle	X	X	X	X	X
Skin	X	X	X	X	X
Spinal cord	X	X	X	X	X
Spleen	X*	X*	X*	X*	X*
Sternum	X	X	X	X	X
Stomach	X	X	X	X	X
Teeth	X	X		X	
Testes	X*	X*	X*	X*	X*
Thymus	X	X	X*	X*	X*
Thyroid	X	X	X*	X*	X*
Tongue	X	X	X	X	X
Trachea	X	X	X	X	X
Urinary bladder	X	X	X	X	X
Uterus	X*	X*	X*	X*	X*
Vagina	X	X	X	X	X
Zymbal gland	X	X	X		

X, histopathology performed
*, organ weight obtained

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: **Salmonella/Microsome Test. Plate Incorporation and Preincubation Method.**

Study no.:	T 1074307
Report no.:	PH-33605
Study report location:	Toxicology International of Bayer HealthCare AG, Wuppertal, Germany
Conducting laboratory and location:	Bayer HealthCare AG PH-R&D-PD Toxicology International Molecular and Genetic Toxicology 42096 Wuppertal Germany
Date of study initiation:	June 28, 2004
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BAY 73-4506, batch # BX01K3D, 96% purity

Key Study Findings

- BAY 73-4506 did not induce mutation in the Ames test at concentrations up to 3 mg/plate with or without metabolic activation (S9).

Methods

Strains:	TA 1535, TA 100, TA 1537, TA 98, and TA102
Concentrations in definitive study:	50, 100, 200, 400, 800, 1500, 3000 µg/plate
Basis of concentration selection:	Solubility
Negative control:	DMSO
Positive control:	sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine, mitomycin C, cumene hydroperoxide and 2-aminoanthracene

Study Validity:

- The negative control values were within the expected range.
- The positive controls showed sufficient effects.
- BAY 73-4506 up to 500 microgram per plate did not show any bacteriotoxic effect.

Results

Test 1 Summary of mean values with and without S9

Compound	Concentration (µg/plate)	S9	TA1535	TA100	TA1537	TA98	TA102
BAY 73-4506	0	-	36	138	14	27	193
		+	15	147	13	44	213
	16	-	37	132	13	27	202
		+	10	122	12	43	201
	50	-	31	114	10	29	180
		+	14	105	9	40	183
	158	-	24	112	8	33	223
		+	13	113	12	37	190
	500	-	23	104	7	25	219
		+	9	98	8	44	197
	1581	-	24	124	7	26	207
		+	10	110	7	38	205
	5000	-	-	-	-	-	-
		+	8	-	-	-	-
Na-azide	10	-	458				
NF	0.2	-		318			
4-NPDA	10	-			107	153	
MMC	0.2	-					535
2-AA	3	+	119	1375	261	1042	542

Na-azide – sodium azide NF - nitrofurantoin

4-NPDA – 4-nitro-1,2-phenylene diamine MMC – mitomycin C

2-AA – 2-aminoanthracene

Test 2 Summary of mean values with and without S9

Compound	Concentration (µg/plate)	S9	TA1535	TA100	TA1537	TA98	TA102
BAY 73-4506	0	-	20	132	9	31	184
		+	11	135	8	46	240
	50	-	9	81	8	15	129
		+	7	105	6	42	240
	100	-	10	92	5	15	155
		+	7	119	5	35	174
	200	-	11	90	6	21	167
		+	7	127	5	38	211
	400	-	12	70	4	18	166
		+	7	101	4	31	188
	800	-	9	82	3	14	150
		+	8	106	3	17	185
	1500	-	8	75	4	14	158
		+	9	109	3	22	182
	3000	-	-	64	-	12	140
		+	9	95	-	14	169
Na-azide	10	-	484				
NF	0.2	-		515			
4-NPDA	10	-			153	160	
Cumene	50	-					420
2-AA	3	+	135	1501	237	1139	625

Na-azide – sodium azide NF - nitrofurantoin

4-NPDA – 4-nitro-1,2-phenylene diamine MMC – mitomycin C

Cumene – cumene hydroperoxide 2-AA – 2-aminoanthracene

7.2 *In Vitro* Assays in Mammalian Cells

Study title: ***In Vitro* Chromosome Aberration Test with Chinese Hamster V79 Cells.**

Study no.:	T 2074308
Report no.:	PH-33732
Study report location:	Bayer HealthCare AG, Wuppertal, Germany
Conducting laboratory and location:	Bayer HealthCare AG PH-R&D-PD Toxicology International Molecular and Genetic Toxicology 42096 Wuppertal Germany
Date of study initiation:	September 27, 2004
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BAY 73-4506, batch # BX01K3D, 95% purity

Key Study Findings

- BAY 73-4506 in the absence and presence of S9 mix did not induce biologically relevant or statistically significant increased numbers of aberrant metaphases.
- The positive controls mitomycin C and cyclophosphamide induced clastogenic effects.

Methods

Cell line:	Chinese hamster V79
Concentrations in definitive study:	15, 30, 60, 75, 90, µg/mL (see tables below)
Basis of concentration selection:	Preliminary cytotoxicity assays
Negative control:	DMSO
Positive control:	Mitomycin C and cyclophosphamide
Formulation/Vehicle:	DMSO for BAY 73-4506. Hanks balanced salt solution (b) (4) for mitomycin C and cyclophosphamide
Incubation & sampling time:	Harvest times – 18 and 30 hours Treatment times – 4 and 18 hours(see tables below for details)

Study Validity:

The positive controls (mitomycin C and cyclophosphamide) demonstrated the sensitivity of the test system.

Negative controls were in the expected range from the published studies.

Results

Surviving cells in the screening test, 4 hours treatment

Experimental Group	Concentration in µg/ml	Harvest Time in Hours	Survival Index in %
without metabolic activation			
DMSO	0	8	100.0
BAY 73-4506	75	8	63.6#
BAY 73-4506	90	8	62.8#
BAY 73-4506	105	8	71.1#
BAY 73-4506	120	8	63.6#
BAY 73-4506	135	8	65.3#
DMSO	0	18	100.0
BAY 73-4506	75	18	69.4#
BAY 73-4506	90	18	58.2#
BAY 73-4506	105	18	55.2#
BAY 73-4506	120	18	53.7#
BAY 73-4506	135	18	47.0#
with metabolic activation			
DMSO	0	8	100.0
BAY 73-4506	30	8	67.3#
BAY 73-4506	45	8	67.3#
BAY 73-4506	60	8	59.3#
BAY 73-4506	75	8	60.2#
BAY 73-4506	90	8	53.1#
DMSO	0	18	100.0
BAY 73-4506	30	18	65.3#
BAY 73-4506	45	18	56.2#
BAY 73-4506	60	18	62.0#
BAY 73-4506	75	18	46.3#
BAY 73-4506	90	18	31.4#

Surviving cells in the pre-test, 18 hours treatment

Experimental Group 18 Hours Treatment	Concentration in µg/ml	Harvest Time in Hours	Survival Index in %
without metabolic activation			
DMSO	0	18	100.0
BAY 73-4506	1	18	74.6
BAY 73-4506	5	18	37.9
BAY 73-4506	10	18	28.7
BAY 73-4506	20	18	29.0
BAY 73-4506	40	18	30.9
BAY 73-4506	60	18	20.6
BAY 73-4506	80	18	26.5

Mitotic index in the screening test, 4 hours treatment

Experimental Group	Concentration in µg/ml	Harvest Time in Hours	Mitotic Nuclei in 2000 Cells absolute	in %
without metabolic activation				
DMSO	0	8	180	100.0
BAY 73-4506	75	8	51	28.3**
BAY 73-4506	90	8	19	10.6**
BAY 73-4506	105	8	27	15.0**
BAY 73-4506	120	8	15	8.3**
BAY 73-4506	135	8	16	8.9**
DMSO	0	18	198	100.0
BAY 73-4506	75	18	108	54.5**
BAY 73-4506	90	18	94	47.5**
BAY 73-4506	105	18	79	39.9**
BAY 73-4506	120	18	68	34.3**
BAY 73-4506	135	18	35	17.7**
with metabolic activation				
DMSO	0	8	93	100.0
BAY 73-4506	30	8	50	53.8**
BAY 73-4506	45	8	23	24.7**
BAY 73-4506	60	8	17	18.3**
BAY 73-4506	75	8	21	22.6**
BAY 73-4506	90	8	18	19.4**
DMSO	0	18	152	100.0
BAY 73-4506	30	18	136	89.5
BAY 73-4506	45	18	91	59.9**
BAY 73-4506	60	18	50	32.9**
BAY 73-4506	75	18	20	13.2**
BAY 73-4506	90	18	0	0.0**

* $p < 0.05$
** $p < 0.01$

Mitotic index in the pre-test, 18 hours treatment

Experimental Group 18 Hours Treatment	Concentration in µg/ml	Harvest Time in Hours	Mitotic Nuclei in %
without metabolic activation			
DMSO	0	18	100.0
BAY 73-4506	1	18	46.1
BAY 73-4506	5	18	0.0
BAY 73-4506	10	18	0.0
BAY 73-4506	20	18	0.0
BAY 73-4506	40	18	0.0
BAY 73-4506	60	18	0.0
BAY 73-4506	80	18	0.0

Final concentrations of BAY 73-4506 in the main study were based on the results of the surviving cells and mitotic index in the screening tests.

Mitotic index – Permanent treatment

Experimental Group	Concentration in µg/ml	Harvest Time in Hours	Mitotic Nuclei in 2000 Cells absolute	in %
without metabolic activation				
DMSO	0	8	174	100.0
BAY 73-4506	0.8	8	148	85.1
BAY 73-4506	1.6	8	35	20.1**
BAY 73-4506	2.4	8	18	10.3**
DMSO	0	18	144	100.0
BAY 73-4506	0.2	18	135	93.8
BAY 73-4506	0.4	18	139	96.5
BAY 73-4506	0.8	18	103	71.5**
BAY 73-4506	1.6	18	52	36.1**
BAY 73-4506	2.4	18	6	4.2**
Mitomycin C	0.03	18	149	103.5

* $p < 0.05$
** $p < 0.01$

Survival index – Permanent treatment

Experimental Group	Concentration in µg/ml	Harvest Time in Hours	Survival Index in %
without metabolic activation			
DMSO	0	8	100.0
BAY 73-4506	0.8	8	88.7
BAY 73-4506	1.6	8	81.7
BAY 73-4506	2.4	8	63.4#
DMSO	0	18	100.0
BAY 73-4506	0.2	18	107.8
BAY 73-4506	0.4	18	114.0
BAY 73-4506	0.8	18	96.1
BAY 73-4506	1.6	18	68.7#
BAY 73-4506	2.4	18	46.4#
Mitomycin C	0.03	18	92.2

relevant reduction of survival index

Chromosomal aberrations without metabolic activation – 18 hours treatment

Experimental Group and Concentration in µg/ml	Harvest Time in Hours	Culture Number	Cells scored	Gaps		Classes of Aberrations: Chromatid Type			Chromosome Type			other				Metaphases with Aberrations (%)		
				g	ig	b	f	d	ib	if	id	ex	maE	ma	cd	incl. gaps	excl.gaps	exchanges
DMSO	18	55	100	1	0	0	0	0	0	0	0	0	0	0	0	1.0	0.0	0.0
0		56	100	0	0	0	0	0	2	0	0	0	0	0	0	2.0	2.0	0.0
			200	1	0	0	0	0	2	0	0	0	0	0	0	1.5	1.0	0.0
BAY 73-4506	18	53	100	0	0	1	0	0	0	0	0	0	0	0	0	1.0	1.0	0.0
0.4		50	100	1	0	2	0	0	1	1	0	0	0	0	0	4.0	3.0	0.0
			200	1	0	3	0	0	1	1	0	0	0	0	0	2.5	2.0	0.0
BAY 73-4506	18	57	100	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0	0.0
0.8		47	100	0	0	0	0	0	0	0	2	0	0	0	0	2.0	2.0	0.0
			200	0	0	0	0	0	0	0	2	0	0	0	0	1.0	1.0	0.0
BAY 73-4506	18	49	100	0	0	0	0	0	0	0	0	2	0	0	0	1.0	1.0	1.0
1.6		51	100	0	0	0	1	0	0	0	0	0	0	0	0	1.0	1.0	0.0
			200	0	0	0	1	0	0	0	0	2	0	0	0	1.0	1.0	0.5
Mitomycin C	18	45	100	3	3	24	5	0	10	3	0	12	0	0	0	39.0	38.0	10.0
0.03		54	100	4	1	18	1	0	12	0	2	8	0	0	0	30.0	29.0	8.0
			200	7	4	42	6	0	22	3	2	20	0	0	0	34.5**	33.5**	9.0**

* $p < 0.05$
** $p < 0.01$

(Tables excerpted from Sponsor's submission)

b – break
d – deletion
f – fragment
ig – isogap
ib – isobreak
id - isodeletion
if – isofragment
ma – multiple aberrations
maE – multiple aberration with exchange
cd – chromosome disintegration
ex – exchange
g – gap

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: **Micronucleus-Test on the Male Mouse**

Study no.:	T 3074309
Report no.:	PH-33682
Study report location:	Toxicology International of Bayer HealthCare AG, Wuppertal
Conducting laboratory and location:	Bayer HealthCare AG PH-R&D-PD Toxicology International Molecular and Genetic Toxicology 42096 Wuppertal Germany
Date of study initiation:	August 4, 2004
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BAY 73-4506 Gran 10% 010, batch # 040607-010, 99% purity

Key Study Findings

- Intraperitoneal administration of BAY 73-4506 up to 2000 mg/kg did not show any clastogenic effect in the *in vivo* mouse micronucleus test.
- Cyclophosphamide demonstrated clastogenic effect.

Methods

Doses in definitive study:	500, 1000, 2000 mg/kg (see table below)
Frequency of dosing:	Two doses separated by 24 hours
Route of administration:	Intraperitoneal
Dose volume:	20 mL/kg
Formulation/Vehicle:	0.5% aqueous Cremophor
Species/Strain:	Hsd/Win: NMRI male mice
Number/Sex/Group:	5 Males
Satellite groups:	No
Age:	6 to 12 weeks
Weight:	39-45 g
Basis of dose selection:	Pilot study
Negative control:	0.5% aqueous Cremophor
Positive control:	Cyclophosphamide

Experimental Group	Dose in mg/kg	Route and Number of Applications	
Negative Control	0	i.p.	2
BAY 73-4506 GRAN 10% 010	500	i.p.	2
BAY 73-4506 GRAN 10% 010	1000	i.p.	2
BAY 73-4506 GRAN 10% 010	2000	i.p.	2
BAY 73-4506 GRAN 10% 010	2000	replacement group	
Positive Control Cyclophosphamide	20	i.p.	1

Study Validity:

The positive control cyclophosphamide demonstrated the sensitivity of the test system.

Results

Mortality: None

Clinical Signs: Rough fur, apathy, loss of weight, difficulty in breathing and slitted eyes observed in all test compound treated animals.

On May 31, 2012 the FDA sent an information request to the Sponsor requesting all information from the full study report for Study PH-33682. On June 5, 2012 the sponsor provided the following tables to demonstrate the dose and time dependent clinical signs of the test compound in animals.

(Excerpted from Sponsor's submission)

Table 49: Clinical Observations after the First Administration of Regorafenib

Dose	**500 mg/kg**	**1000 mg/kg**	**2000 mg/kg**	**2000 mg/kg (replacement animals)**
Animal No.	4, 20, 21, 27, 28	2, 7, 8, 10, 23	1, 6, 11, 15, 25	5, 12, 19, 29, 30
Time after administration				
5 minutes	Apathy (mild, partially)	Apathy (mild, partially)	Apathy (medium)	Apathy (medium)
15 minutes	Apathy (mild); Difficulty in breathing (medium)	Apathy (medium); Difficulty in breathing (medium); Roughened fur (mild)	Apathy (medium); Difficulty in breathing (medium); Roughened fur (mild)	Apathy (medium); Difficulty in breathing (medium); Roughened fur (mild)
1 hour	Apathy (mild); Roughened fur (mild); Difficulty in breathing (medium)	Apathy (medium); Difficulty in breathing (medium); Roughened fur (mild)	Apathy (medium); Difficulty in breathing (medium); Roughened fur (mild)	Apathy (medium); Difficulty in breathing (medium); Roughened fur (mild)
2 hours	Apathy (mild); Roughened fur (mild); Difficulty in breathing (medium)	Apathy (medium); Difficulty in breathing (medium); Roughened fur (mild)	Apathy (medium); Difficulty in breathing (medium); Roughened fur (mild)	Apathy (medium); Difficulty in breathing (medium); Roughened fur (mild)

Dose	500 mg/kg	1000 mg/kg	2000 mg/kg	2000 mg/kg (replacement animals)
Animal No.	4, 20, 21, 27, 28	2, 7, 8, 10, 23	1, 6, 11, 15, 25	5, 12, 19, 29, 30
Time after administration				
3 hours	Apathy (mild); Roughened fur (mild); Difficulty in breathing (medium)	Apathy (medium, partially); Difficulty in breathing (medium); Roughened fur (mild); Slitted eyes (isolated cases)	Apathy (medium); Difficulty in breathing (medium); Roughened fur (mild); Slitted eyes (isolated cases)	Apathy (medium); Difficulty in breathing (medium); Roughened fur (mild); Slitted eyes (isolated cases)
24 hours	Reduced body weight (partially)	Roughened fur (mild, partially); Spasm (mild, partially); Reduced body weight	Roughened fur (mild; Spasm (mild, partially); Reduced body weight (isolated cases)	Roughened fur (mild); Spasm (mild, partially); Reduced body weight (isolated cases)

Table 50: Clinical Observations after the Second Administration of Regorafenib

Dose	500 mg/kg	1000 mg/kg	2000 mg/kg	2000 mg/kg (replacement animals)
Animal No.	4, 20, 21, 27, 28	2, 7, 8, 10, 23	1, 6, 11, 15, 25	5, 12, 19, 29, 30
Time after administration				
5 minutes	Apathy (mild, partially)	Apathy (mild, partially); Roughened	Apathy (mild); Roughened fur (mild);	Apathy (mild); Roughened fur (mild);

Dose	**500 mg/kg**	**1000 mg/kg**	**2000 mg/kg**	**2000 mg/kg (replacement animals)**
Animal No.	4, 20, 21, 27, 28	2, 7, 8, 10, 23	1, 6, 11, 15, 25	5, 12, 19, 29, 30
Time after administration				
		fur (mild, partially); Spasm (mild, partially)	Spasm (mild)	Spasm (mild)
15 minutes	Apathy (mild, partially)	Apathy (mild, partially); Roughened fur (mild, partially); Spasm (mild, partially)	Apathy (medium, partially); Roughened fur (mild); Spasm (mild); Difficulty in breathing (medium, partially)	Apathy (medium, partially); Roughened fur (mild); Spasm (mild); Difficulty in breathing (medium, partially)
1 hour	Apathy (mild) Difficulty in breathing (mild, partially)	Apathy (mild); Roughened fur (mild); Spasm (mild); Difficulty in breathing (mild, partially)	Apathy (medium, partially); Roughened fur (mild); Spasm (mild); Difficulty in breathing (medium, partially)	Apathy (medium, partially); Roughened fur (mild); Spasm (mild); Difficulty in breathing (medium, partially)

Dose	500 mg/kg	1000 mg/kg	2000 mg/kg	2000 mg/kg (replacement animals)
Animal No.	4, 20, 21, 27, 28	2, 7, 8, 10, 23	1, 6, 11, 15, 25	5, 12, 19, 29, 30
Time after administration				
2 hours	Apathy (mild); Roughened fur (mild)	Apathy (medium, partially); Roughened fur (mild); Spasm (mild); Difficulty in breathing (mild, partially)	Apathy (medium, partially); Roughened fur (medium); Spasm (mild); Difficulty in breathing (medium, partially)	Apathy (medium, partially); Roughened fur (medium); Spasm (mild); Difficulty in breathing (medium, partially)
3 hours	Apathy (mild); Roughened fur (mild)	Apathy (medium, partially); Roughened fur (mild); Spasm (mild); Difficulty in breathing (mild, partially)	Apathy (medium, partially); Roughened fur (medium); Spasm (mild); Difficulty in breathing (medium, partially); Slitted eyes (medium, partially)	Apathy (medium, partially); Roughened fur (medium); Spasm (mild); Difficulty in breathing (medium, partially) Slitted eyes (medium, partially)
24 hours	Roughened fur (mild, isolated cases)	Roughened fur (mild, isolated cases); Spasm (mild, isolated cases)	Roughened fur (mild, partially); Spasm (mild, partially)	Roughened fur (mild, partially); Spasm (mild, partially)

Table 51: Mouse micronucleus assay - Negative control

random number and sex	body weight in g	number of evaluated PCE	number of NCE per 2000 PCE	MNNCE per 2000 NCE	MNPCE per 2000 PCE
13 ♂	42	2000	2331	2.6	5
16 ♂	39	2000	1666	1.2	2
17 ♂	39	2000	1375	2.9	11
18 ♂	44	2000	1903	4.2	7
24 ♂	40	2000	2455	5.7	4
Mean	41	2000	1946	3.3	5.8
1s	2	na	451	1.7	3.4

Table 52: Mouse micronucleus assay - 2 x 500 mg/kg BAY 73-4506

random number and sex	body weight in g	number of evaluated PCE	number of NCE per 2000 PCE	MNNCE per 2000 NCE	MNPCE per 2000 PCE
4 ♂	44	2000	1178	5.1	3
20 ♂	39	2000	1759	6.8	6
21 ♂	42	2000	2029	3.9	3
27 ♂	39	2000	1267	0	4
28 ♂	40	2000	455	8.8	3
Mean	41	2000	1338	4.9	3.8
1s	2	na	605	3.3	1.3

Table 53: Mouse micronucleus assay - 2 x 1000 mg/kg BAY 73-4506

random number and sex	body weight in g	number of evaluated PCE	number of NCE per 2000 PCE	MNNCE per 2000 NCE	MNPCE per 2000 PCE
2 ♂	41	2000	1332	0	4
7 ♂	39	2000	951	4.2	6
8 ♂	41	2000	884	2.3	1
10 ♂	45	2000	953	6.3	5
23 ♂	42	2000	647	12.4	10
Mean	42	2000	953	5.0	5.2
1s	2	na	246	4.7	3.3

Table 54: Mouse micronucleus assay - 2 x 2000 mg/kg BAY 73-4506

random number and sex	body weight in g	number of evaluated PCE	number of NCE per 2000 PCE	MNNCE per 2000 NCE	MNPCE per 2000 PCE
1 ♂	40	2000	1219	4.9	3
6 ♂	43	2000	3164	1.9	2
11 ♂	39	2000	1169	1.7	5
15 ♂	42	2000	3821	1.0	0
25 ♂	39	2000	1412	1.4	5
Mean	41	2000	2157	2.2	3.0
1s	2	na	1244	1.6	2.1

Table 55: Mouse micronucleus assay – 1 x 20 mg/kg i.p. cyclophosphamide

random number and sex	body weight in g	number of evaluated PCE	number of NCE per 2000 PCE	MNNCE per 2000 NCE	MNPCE per 2000 PCE
3 ♂	40	2000	3228	0.6	28
9 ♂	41	2000	2415	1.7	26
14 ♂	41	2000	1172	0	29
22 ♂	42	2000	968	8.3	30
26 ♂	43	2000	1210	0	16
Mean	41	2000	1799	2.1	25.8
1s	1	na	981	3.5	5.7

Table 56: Summary of *In Vivo* Micronucleus assay results

Group	PCE evaluated	NCE per 2000PCE	MNNCE per 2000NCE	MNPCE per 2000 PCE
Negative control	10, 000	1946±451	3.3±1,7	5.8±3.4
BAY 73-4506 – 2 x 500 mg/kg	10, 000	1338±605	4.9±3.3	3.8±1.3
BAY 73-4506 – 2 x 1000 mg/kg	10,000	953±246	5.0±4.7	5.2±3.3
BAY 73-4506 – 2 x 2000 mg/kg	10,000	2157±1244	2.2±1.6	3.0±2.1
Positive control cyclophosphamide – 20 mg/kg	10,000	1799±981	2.1±3.5	25.8±5.7*

*P < 0.01 in non-parametric Wilcoxon ranking test

C - Cremophor in water MNNCE - micronucleated NCE
MNPCE - micronucleated PCE NCE - normochromatic erythrocytes
PCE - polychromatic erythrocytes PEG 400 - polyethylene glycol 400
T - Tylose in water NaCl - NaCl in water na - not applicable

Toxicokinetics: Not done

7.4 Other Genetic Toxicity Studies

Study title: **BAY 75-7495: Salmonella/Microsome Test Plate Incorporation and Preincubation Method.**

Study no.:	T 0077772
Report no:	PH-35197
Study report location:	Toxicology of Bayer HealthCare AG, Wuppertal, Germany
Conducting laboratory and location:	Bayer HealthCare AG BSP-GDD-GED-GTOX Genetic Toxicology 42096 Wuppertal Germany
Date of study initiation:	July 6, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BAY 75-7495, batch # BXR3BSX, 95% purity

Key Study Findings

- BAY 75-7495 (Metabolite M-2 of BAY 73-4506) up to 5000 µg/plate did not show any mutagenic activity.
- The positive controls demonstrated marked mutagenic effects.

Methods

Strains:	TA 1535, TA 100, TA 1537, TA 98, and TA 102
Concentrations in definitive study:	16, 50, 158, 500, 1581, 5000 µg/plate
Basis of concentration selection:	Initial plate incorporation test
Negative control:	DMSO
Positive control:	Sodium azide nitrofurantoin, 4-nitro-1,2-phenylene diamine, mitomycin C, cumene hydroperoxide, and 2-aminoanthracene

Study Validity:

The negative and positive controls were within the expected ranges.

Titer showed sufficient bacterial density in the suspension.

Results

Table 57: Summary of mean values without S9 in M-2 Ames Assay

Table and Group	Strain TA 1535	TA 100	TA 1537	TA 98	TA 102
µg/Tube					
0	12	110	12	20	267
16	11	99	8	17	213
50	5	95	7	10	185
158	5	95	4	13	204
500	5	95	2	14	275
1581	4	124	3	14	262
5000	0	142	0	0	309
Na-azide	1151				
NF		575			
4-NPDA			131	166	
Cumene					543

Table 58: Summary of mean values with S9 in M-2 Ames Assay

Table and Group	Strain TA 1535	TA 100	TA 1537	TA 98	TA 102
µg/Tube					
0	10	118	9	26	312
16	9	113	10	28	302
50	10	110	6	17	337
158	7	132	6	22	300
500	7	117	6	25	232
1581	7	133	4	21	222
5000	8	123	5	19	242
2-AA	169	1886	365	1254	554

Na-azide – sodium azide

NF - nitrofurantoin

4-NPDA – 4-nitro-1,2-phenylene diamine

MMC – mitomycin C

Cumene – cumene hydroperoxide

2-AA – 2-aminoanthracene

Study title: **BAY 75-7495: *In Vitro* Chromosome Aberration Test With Chinese Hamster V79 Cells**

Study no.:	T 9077771
Report no.:	PH-35189
Study report location:	Toxicology of Bayer HealthCare AG
Conducting laboratory and location:	Bayer HealthCare AG BSP-GDD-GED-GTOX Genetic Toxicology 42096 Wuppertal Germany
Date of study initiation:	August 9, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BAY 75-7495, batch # BXR3BSX, 95 % purity

Key Study Findings

- BAY 75-7495 (Metabolite M-2 of BAY 73-4506) in the absence of S9 mix did not show clastogenic effects in Chinese hamster V9 cells.
- BAY 75-7495 in the presence of S9 mix induced biologically relevant and statistically significant increases of metaphases with aberrations.

Methods

Cell line:	Chinese hamster V79
Concentrations in definitive study:	See table below
Basis of concentration selection:	Preliminary experiment
Negative control:	DMSO
Positive control:	Mitomycin C, cyclophosphamide
Incubation & sampling time:	4 hours incubation 18 and 30 hours sampling time

Study Validity:

- Positive controls induced biological relevant increase in chromosome aberrations.
- Negative controls values were in the expected historical range.
- Test compound in the presence of S9 mix induced clastogenic effect

Results

Based on the survival index and mitotic index, the following concentration of BAY 75-7495 were selected

test groups	S9 Mix	concentration in µg/ml	harvest time in hours
BAY 75-7495	-	1	18
BAY 75-7495	-	3	18
BAY 75-7495	-	9	18
BAY 75-7495	+	6	18
BAY 75-7495	+	12	18
BAY 75-7495	+	24	18
BAY 75-7495	-	9	30
BAY 75-7495	+	24	30

Chromosomal Aberrations without metabolic Activation
4 Hours Treatment

Experimental Group and Concentration in µg/ml	Harvest Time in Hours	Culture Number	Cells scored	Gaps		Classes of Aberrations: Chromatid Type			Chromosome Type			other				Metaphases with Aberrations (%)	
				g	ig	b	f	d	ib	if	id	ex	maE	ma	cd	incl. gaps	excl.gaps
DMSO	18	34	100	0	0	0	0	0	0	0	1	0	0	0	0	1.0	1.0
0		17	100	0	0	2	0	0	0	0	0	0	0	0	0	2.0	2.0
			200	0	0	2	0	0	0	0	1	0	0	0	0	1.5	1.5
BAY 75-7495	18	16	100	0	0	1	0	0	1	0	1	1	0	0	0	4.0	4.0
1		9	100	0	0	0	0	0	0	0	4	0	0	0	0	4.0	4.0
			200	0	0	1	0	0	1	0	5	1	0	0	0	4.0	4.0
BAY 75-7495	18	23	100	0	0	0	0	0	0	0	0	1	0	0	0	1.0	1.0
3		39	100	0	0	0	0	0	2	1	0	0	0	0	0	2.0	2.0
			200	0	0	0	0	0	2	1	0	1	0	0	0	1.5	1.5
BAY 75-7495	18	35	100	0	0	0	0	0	1	0	1	0	0	0	0	2.0	2.0
9		19	100	0	0	0	0	0	1	1	1	2	0	0	0	5.0	5.0
			200	0	0	0	0	0	2	1	2	2	0	0	0	3.5	3.5
Mitomycin C	18	2	100	0	0	12	1	0	12	5	0	17	0	0	0	29.0	29.0
0.1		31	100	1	0	9	2	0	11	2	2	13	0	0	0	28.0	28.0
			200	1	0	21	3	0	23	7	2	30	0	0	0	28.5	28.5**

Chromosomal Aberrations with metabolic Activation
4 Hours Treatment

Experimental Group and Concentration in µg/ml	Harvest Time in Hours	Culture Number	Cells scored	Gaps		Classes of Aberrations: Chromatid Type			Chromosome Type			other				Metaphases with Aberrations (%)	
				g	ig	b	f	d	ib	if	id	ex	maE	ma	cd	incl. gaps	excl.gaps
DMSO	18	18	100	0	0	0	0	0	0	1	2	1	1	0	0	5.0	5.0
0		10	100	0	0	0	1	0	1	1	1	0	0	0	0	4.0	4.0
			200	0	0	0	1	0	1	2	3	1	1	0	0	4.5	4.5
BAY 75-7495	18	25	100	0	0	1	0	0	3	0	2	3	0	0	0	9.0	9.0
6		22	100	0	0	2	0	1	1	0	1	0	0	0	0	4.0	4.0
			200	0	0	3	0	1	4	0	3	3	0	0	0	6.5	6.5
BAY 75-7495	18	21	100	0	1	3	0	0	0	0	0	4	0	0	0	7.0	6.0
12		1	100	0	0	0	0	0	1	0	2	0	0	0	0	3.0	3.0
			200	0	1	3	0	0	1	0	2	4	0	0	0	5.0	4.5
BAY 75-7495	18	43	100	0	1	2	0	0	5	0	2	2	0	0	0	12.0	11.0
24		36	100	0	0	4	1	0	3	1	0	2	0	0	0	10.0	10.0
			200	0	1	6	1	0	8	1	2	4	0	0	0	11.0	10.5*
Cyclophosphamide	18	14	100	0	0	15	2	0	12	5	3	25	0	0	0	40.0	40.0
2		8	100	0	0	12	0	0	13	4	1	15	0	0	0	30.0	30.0
			200	0	0	27	2	0	25	9	4	40	0	0	0	35.0	35.0**

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		Classes of Aberrations: Chromatid Type			Chromosome Type			other				Metaphases with Aberrations (%)	
				g	ig	b	f	d	ib	if	id	ex	maE	ma	cd	incl. gaps	excl.gaps
without metabolic activation																	
DMSO	30	26	100	0	0	0	0	0	1	0	2	0	0	0	0	3.0	3.0
0		41	100	0	0	0	0	0	0	0	1	0	0	0	0	1.0	1.0
			200	0	0	0	0	0	1	0	3	0	0	0	0	2.0	2.0
BAY 75-7495	30	27	100	0	0	1	0	0	3	0	1	3	0	0	0	6.0	6.0
9		5	100	0	1	1	0	0	3	0	3	0	0	0	0	7.0	7.0
			200	0	1	2	0	0	6	0	4	3	0	0	0	6.5	6.5*
with metabolic activation																	
DMSO	30	32	100	0	0	0	0	0	1	0	2	0	0	0	0	3.0	3.0
0		3	100	1	0	0	0	0	1	0	1	0	0	0	0	2.0	2.0
			200	1	0	0	0	0	2	0	3	0	0	0	0	2.5	2.5
BAY 75-7495	30	38	100	1	0	5	0	0	7	2	1	11	0	0	0	18.0	18.0
24		11	100	0	1	8	5	0	9	2	0	8	0	0	0	17.0	17.0
			200	1	1	13	5	0	16	4	1	19	0	0	0	17.5	17.5**

*p<0.05 **p<0.01

b – break ib – isobreak cd – chromosome disintegration
d – deletion id - isodeletion ex – exchange
f – fragment if – isofragment g – gap
ig – isogap ma – multiple aberrations
maE – multiple aberration with exchange

Study title: **BAY 81-8752: Salmonella/Microsome Test Plate Incorporation and Preincubation Method**

Study no.:	T 1079285
Report no.:	PH-35596
Study report location:	Bayer HealthCare AG, Wuppertal, Germany
Conducting laboratory and location:	Bayer HealthCare AG BSP-GDD-GED-GTOX Genetic Toxicology 42096 Wuppertal Germany
Date of study initiation:	June 25, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BAY 81-8752, batch # BXR3TVA. 98% purity

Key Study Findings

- BAY 81-8752 (Metabolite M-5 of BAY 73-4506) at concentration of up to 5000 µg/plate with or without metabolic activation (S9 mix) did not show any mutagenic activity in Ames test.
- The positive controls demonstrated marked mutagenic effect.

Methods

Strains:	TA 1535, TA 100, TA 1537, TA 98 and TA 102.
Concentrations in definitive study:	See table below
Basis of concentration selection:	Results obtained from first experiment
Negative control:	DMSO
Positive control:	Sodium azide, nitrofurantoin, 4-nitro-l ,2-phenylene diamine, mitomycin C, cumene hydroperoxide and 2-aminoanthracene

Study Validity:

- The negative controls values were within the historical control values.
- The positive controls showed sufficient positive effects.
- Titer determination showed sufficient bacterial density in the suspension.

Results

Table 59: First experiment without S9 Mix (M-5 Ames)

Table and Group	Strain TA 1535	TA 100	TA 1537	TA 98	TA 102
µg/Plate					
0	10	144	6	13	244
16	9	209	5	15	273
50	11	209	5	16	248
158	10	197	6	16	260
500	28	191	5	17	235
1581	6	178	4	13	263
5000	6	128	2	8	175
Na-azide	942				
NF		387			
4-NPDA			62	129	
MMC					839

Table 60: First experiment with S9 Mix (M-5 Ames)

Table and Group	Strain TA 1535	TA 100	TA 1537	TA 98	TA 102
µg/Plate					
0	11	162	10	31	329
16	10	161	8	27	352
50	11	159	8	27	351
158	11	165	8	34	372
500	11	177	8	37	316
1581	11	155	7	28	306
5000	7	170	5	27	232
2-AA	128	2573	220	1890	753

Table 61: Final experiment without S9 Mix (M-5 Ames)

Table and Group	Strain TA 1535	TA 100	TA 1537	TA 98	TA 102
µg/Tube					
0	14	154	6	15	243
16	12	164	7	15	206
50	11	146	5	15	276
158	9	166	6	16	233
500	11	131	4	14	250
1581	9	132	5	11	233
5000	6	110	6	8	244
Na-azide	963				
NF		529			
4-NPDA			106	117	
Cumene					526

Table 62: Final experiment with S9 Mix (M-5 Ames)

Table and Group	Strain				
	TA 1535	TA 100	TA 1537	TA 98	TA 102
µg/Tube					
0	16	151	11	22	265
16	9	141	10	21	240
50	8	144	9	23	272
158	10	140	7	17	366
500	7	127	8	20	271
1581	7	125	5	13	251
5000	5	111	7	11	201
2-AA	154	2122	334	1805	656

Na-azide – sodium azide NF - nitrofurantoin

4-NPDA – 4-nitro-1,2-phenylene diamine MMC – mitomycin C

Cumene – cumene hydroperoxide 2-AA – 2-aminoanthracene

Study title: **BAY 81-8752: *In Vitro* Chromosome Aberration Test With Chinese Hamster V79 Cells.**

Study no.:	T 0079284
Report no.:	PH-35637
Study report location:	Toxicology of Bayer HealthCare AG
Conducting laboratory and location:	Bayer HealthCare AG BSP-GDD-GED-GTOX Genetic Toxicology 42096 Wuppertal Germany
Date of study initiation:	August 15, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BAY 81-8752, batch # BXR3TVA, 99% pure

Key Study Findings

- BAY 81-8752 (metabolite M-5 of BAY 73-4506) in the presence and in the absence of S9 mix did not show any biologically relevant or statistically significant increase in numbers of aberrant metaphases in Chinese hamster V79 cells.
- Mitomycin C and cyclophosphamide induced clastogenic effects.

Methods

Cell line:	Chinese hamster V79 cells
Concentrations in definitive study:	5, 10 and 20 µg/mL
Basis of concentration selection:	Results of the pre-test
Negative control:	DMSO
Positive control:	Mitomycin C and cyclophosphamide
Formulation/Vehicle:	DMSO
Incubation & sampling time:	4 &18 hours treatment and 18 & 30 hours harvest times

Study Validity:

- Positive controls induced biologically relevant increase in chromosome aberrations.
- Negative controls values were in the expected historical range.

Results

Table 63: Survival index – 4 hours treatment (M-5 CHO)

Experimental Group	Concentration in µg/ml	Harvest Time in Hours	Survival Index in %
4 Hours Treatment without metabolic Activation			
DMSO	0	8	100.0
BAY 81-8752	10	8	78.3#
BAY 81-8752	20	8	70.4#
BAY 81-8752	30	8	65.1#
DMSO	0	18	100.0
BAY 81-8752	2.5	18	96.2
BAY 81-8752	5	18	93.5
BAY 81-8752	10	18	70.3#
BAY 81-8752	20	18	66.5#
BAY 81-8752	30	18	58.2#
Mitomycin C	0.1	18	73.0#
DMSO	0	30	100.0
BAY 81-8752	10	30	58.6#
BAY 81-8752	20	30	44.7#
BAY 81-8752	30	30	38.5#
4 Hours Treatment with metabolic Activation			
DMSO	0	8	100.0
BAY 81-8752	10	8	76.7#
BAY 81-8752	20	8	86.6
BAY 81-8752	30	8	73.8#
DMSO	0	18	100.0
BAY 81-8752	2.5	18	103.4
BAY 81-8752	5	18	87.5
BAY 81-8752	10	18	72.7#
BAY 81-8752	20	18	66.3#
BAY 81-8752	30	18	51.5#
Cyclophosphamide	2	18	72.7#
DMSO	0	30	100.0
BAY 81-8752	10	30	86.0
BAY 81-8752	20	30	49.2#
BAY 81-8752	30	30	36.5#

relevant reduction of survival index

Table 64: Mitotic index – 4 hours treatment (M-5 CHO)

Experimental Group	Concentration in µg/ml	Harvest Time In Hours	Mitotic Nuclei in 2000 Cells absolute	in %
4 Hours Treatment without metabolic Activation				
DMSO	0	8	171	100.0
BAY 81-8752	10	8	182	106.4
BAY 81-8752	20	8	158	92.4
BAY 81-8752	30	8	167	97.7
DMSO	0	18	210	100.0
BAY 81-8752	2.5	18	373	177.6
BAY 81-8752	5	18	330	157.1
BAY 81-8752	10	18	308	146.7
BAY 81-8752	20	18	165	78.6**
BAY 81-8752	30	18	164	78.1**
Mitomycin C	0.1	18	271	129.0
DMSO	0	30	361	100.0
BAY 81-8752	10	30	238	65.9**
BAY 81-8752	20	30	170	47.1**
BAY 81-8752	30	30	149	41.3**
4 Hours Treatment with metabolic Activation				
DMSO	0	8	133	100.0
BAY 81-8752	10	8	167	125.6
BAY 81-8752	20	8	109	82.0
BAY 81-8752	30	8	140	105.3
DMSO	0	18	199	100.0
BAY 81-8752	2.5	18	233	117.1
BAY 81-8752	5	18	233	117.1
BAY 81-8752	10	18	348	174.9
BAY 81-8752	20	18	260	130.7
BAY 81-8752	30	18	241	121.1
Cyclophosphamide	2	18	218	109.5
DMSO	0	30	304	100.0
BAY 81-8752	10	30	361	118.8
BAY 81-8752	20	30	167	54.9**
BAY 81-8752	30	30	140	46.1**

* $p < 0.05$
** $p < 0.01$

Table 65: Aberrations without S9 mix – 4 hours treatment, 18 hours harvest (M-5 CHO)

Experimental Group and Concentration in µg/ml	Harvest Time in Hours	Culture Number	Cells scored	Gaps		Classes of Aberrations: Chromatid Type			Chromosome Type			other				Metaphases with Aberrations (%)	
				g	ig	b	f	d	ib	if	id	ex	maE	ma	cd	incl. gaps	excl.gaps
DMSO	18	36	100	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0
0		16	100	0	0	1	0	0	0	0	1	0	0	0	0	2.0	2.0
			200	0	0	1	0	0	0	0	1	0	0	0	0	1.0	1.0
BAY 81-8752	18	28	100	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0
5		34	100	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0
			200	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0
BAY 81-8752	18	24	100	0	0	0	0	0	1	0	0	1	0	0	0	2.0	2.0
10		2	100	0	0	0	0	0	1	0	0	1	0	0	0	1.0	1.0
			200	0	0	0	0	0	2	0	0	2	0	0	0	1.5	1.5
BAY 81-8752	18	44	100	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0
20		38	100	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0
			200	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0
Mitomycin C	18	26	100	2	0	13	4	0	17	4	3	40	0	0	0	54.0	54.0
0.1		42	100	0	1	29	4	0	8	2	3	20	0	0	0	43.0	43.0
			200	2	1	42	8	0	25	6	6	60	0	0	0	48.5	48.5**

Table 66: Aberrations with S9 mix – 4 hours treatment, 18 hours harvest (M-5 CHO)

Experimental Group and Concentration in µg/ml	Harvest Time in Hours	Culture Number	Cells scored	Gaps		Classes of Aberrations: Chromatid Type			Chromosome Type			other				Metaphases with Aberrations (%)	
				g	ig	b	f	d	ib	if	id	ex	maE	ma	cd	incl. gaps	excl.gaps
DMSO	18	39	100	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0
0		30	100	0	0	0	0	0	0	0	0	2	0	0	0	2.0	2.0
			200	0	0	0	0	0	0	0	0	2	0	0	0	1.0	1.0
BAY 81-8752	18	7	100	0	0	1	0	0	0	0	0	1	0	1	0	3.0	3.0
5		31	100	0	0	1	0	0	1	0	1	0	0	0	0	3.0	3.0
			200	0	0	2	0	0	1	0	1	1	0	1	0	3.0	3.0
BAY 81-8752	18	25	100	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0
10		21	100	0	0	0	0	0	1	0	0	1	0	0	0	2.0	2.0
			200	0	0	0	0	0	1	0	0	1	0	0	0	1.0	1.0
BAY 81-8752	18	27	100	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0
20		22	100	0	0	0	0	0	0	0	1	0	0	0	0	1.0	1.0
			200	0	0	0	0	0	0	0	1	0	0	0	0	0.5	0.5
Cyclophosphamide	18	14	100	2	1	11	1	0	14	5	2	21	0	0	0	42.0	42.0
2		17	100	1	0	12	2	0	15	3	3	18	0	0	0	37.0	37.0
			200	3	1	23	3	0	29	8	5	39	0	0	0	39.5	39.5**

*p<0.05 **p<0.01

b – break ib – isobreak cd – chromosome disintegration
d – deletion id - isodeletion ex – exchange
f – fragment if – isofragment g – gap
ig – isogap ma – multiple aberrations
maE – multiple aberration with exchange

Table 67: Aberrations without and with S9 mix – 4 hours treatment, 30 hours harvest (M-5 CHO)

Experimental Group and Concentration in µg/ml	Harvest Time in Hours	Culture Number	Cells scored	Gaps		Classes of Aberrations: Chromatid Type			Chromosome Type			other				Metaphases with Aberrations (%).	
				g	ig	b	f	d	ib	if	id	ex	maE	ma	cd	incl. gaps	excl.gaps
without metabolic activation																	
DMSO	30	8	100	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0
0		18	100	0	0	0	0	0	0	0	2	0	0	0	0	2.0	2.0
			200	0	0	0	0	0	0	0	2	0	0	0	0	1.0	1.0
BAY 81-8752	30	41	100	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0
20		1	100	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0
			200	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0
with metabolic activation																	
DMSO	30	20	100	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0
0		13	100	0	0	1	1	0	0	0	0	0	0	0	0	1.0	1.0
			200	0	0	1	1	0	0	0	0	0	0	0	0	0.5	0.5
BAY 81-8752	30	9	100	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0
20		11	100	0	0	1	0	0	0	1	1	1	0	0	0	3.0	3.0
			200	0	0	1	0	0	0	1	1	1	0	0	0	1.5	1.5

*p<0.05 **p<0.01

b – break ib – isobreak cd – chromosome disintegration
d – deletion id - isodeletion ex – exchange
f – fragment if – isofragment g – gap
ig – isogap ma – multiple aberrations
maE – multiple aberration with exchange

Table 68: Survival index – 18 hours treatment (M-5 CHO)

Experimental Group	Concentration in µg/ml	Harvest Time in Hours	Survival Index in %
8/18 Hours Treatment without metabolic Activation			
DMSO	0	8	100.0
BAY 81-8752	6	8	65.5#
BAY 81-8752	9	8	63.0#
BAY 81-8752	12	8	76.5#
DMSO	0	18	100.0
BAY 81-8752	1	18	83.7
BAY 81-8752	3	18	69.1#
BAY 81-8752	6	18	41.1#
BAY 81-8752	9	18	51.6#
BAY 81-8752	12	18	48.4#
Mitomycin C	0.03	18	73.2#

relevant reduction of survival index

Table 69: Mitotic index – 18 hours treatment (M-5 CHO)

Experimental Group	Concentration in µg/ml	Harvest Time In Hours	Mitotic Nuclei in 2000 Cells absolute	Mitotic Nuclei in 2000 Cells in %
8/18 Hours Treatment without metabolic Activation				
DMSO	0	8	141	100.0
BAY 81-8752	6	8	79	56.0**
BAY 81-8752	9	8	83	58.9**
BAY 81-8752	12	8	70	49.6**
DMSO	0	18	139	100.0
BAY 81-8752	1	18	132	95.0
BAY 81-8752	3	18	76	54.7**
BAY 81-8752	6	18	89	64.0**
BAY 81-8752	9	18	86	61.9**
BAY 81-8752	12	18	92	66.2**
Mitomycin C	0.03	18	94	67.6**

* $p < 0.05$
** $p < 0.01$

Table 70: Aberrations without S9 mix – 18 hours treatment (M-5 CHO)

Experimental Group and Concentration in µg/ml	Harvest Time in Hours	Culture Number	Cells scored	Gaps		Classes of Aberrations: Chromatid Type			Chromosome Type			other				Metaphases with Aberrations (%)	
				g	ig	b	f	d	ib	if	id	ex	maE	ma	cd	incl. gaps	excl.gaps
DMSO 0	18	51	100	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0
		47	100	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0
			200	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0
BAY 81-8752 1	18	52	100	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0
		49	100	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0
			200	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0
BAY 81-8752 3	18	55	100	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0
		53	100	0	0	0	0	0	0	0	1	0	0	0	0	1.0	1.0
			200	0	0	0	0	0	0	0	1	0	0	0	0	0.5	0.5
BAY 81-8752 6	18	45	100	0	0	0	1	0	0	0	1	0	0	0	0	2.0	2.0
		57	100	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0
			200	0	0	0	1	0	0	0	1	0	0	0	0	1.0	1.0
Mitomycin C 0.03	18	50	100	0	0	18	5	0	17	0	0	18	0	0	0	39.0	39.0
		56	100	1	1	16	3	0	17	1	1	11	0	0	0	33.0	32.0
			200	1	1	34	8	0	34	1	1	29	0	0	0	36.0	35.5**

*p<0.05 **p<0.01

b – break ib – isobreak cd – chromosome disintegration
d – deletion id - isodeletion ex – exchange
f – fragment if – isofragment g – gap
ig – isogap ma – multiple aberrations
maE – multiple aberration with exchange

Study title: **Evaluation of [redacted](b) (4) in a bacterial reverse mutation study using Salmonella typhimurium (Ames-Test)**

Study no.: TOXT7082944
Report no.: A57981
Study report location: [redacted](b) (4)
Conducting laboratory and location: [redacted](b) (4)
Date of study initiation: Nov. 4, 2011
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: [redacted](b) (4) batch # JSL 1149-2-2, 99% purity

Key Study Findings

- (b) (4) (impurity) did not show any mutagenic activity in the Ames test.
- Positive controls produced the expected increase in the number of revertant colonies

Methods

Strains:	TA1535, TA100, TA1537, TA98 and TA102
Concentrations in definitive study:	0.1, 0.25, 0.5, 1.0, 2.5, and 5.0 mg/plate
Basis of concentration selection:	Preliminary experiment
Negative control:	DMSO
Positive control:	Anthracene-2-amine, cumene hydroperoxide, mitomycin C, 2-nitro-9H-fluorene, 4-nitro-o-phenylenediamine, sodium azide
Formulation/Vehicle:	Phosphate buffer
Incubation & sampling time:	48 h for TA102; 72 h for TA1535, TA100, TA1537 and TA98

Results

Table 71: Direct plate incorporation without S9 ((b) (4)

Substance	Dose/plate	Strain				
		TA1535	TA100	TA1537	TA98	TA102
DMSO	50 µL	16	129	9	18	186
Test compound	0.1 mg	15	122	10	18	180
	0.25 mg	17	122	11	19	183
	0.5 mg	17	119	9	14	184
	1.0 mg	17	128	9	17	178
	2.5 mg	16	139	8	15	140
	5.0 mg	15	125	7	16	131
2NF	10.0 µg				609	
Na-azide	5.0 µg	837	663			
4-NPDA	10.0 µg			81		
MMC	0.2 µg					514

Table 72: Direct plate incorporation with S9 ((b) (4)

Substance	Dose/plate	Strain				
		TA1535	TA100	TA1537	TA98	TA102
DMSO	50 µL	14	132	13	39	225
Test compound	0.1 mg	15	130	15	40	244
	0.25 mg	14	140	14	41	228
	0.5 mg	14	139	14	49	205

Substance	Dose/plate	Strain				
		TA1535	TA100	TA1537	TA98	TA102
	1.0 mg	11	140	11	38	238
	2.5 mg	12	125	14	42	197
	5.0 mg	10	150	14	41	189
2AA	3.0 µg	210	1010	88	680	528

Table 73: Preincubation test without S9 ((b) (4)

Substance	Dose/plate	Strain				
		TA1535	TA100	TA1537	TA98	TA102
DMSO	50 µL	18	108	7	20	190
Test compound	0.1 mg	17	119	6	21	176
	0.25 mg	14	110	8	19	181
	0.5 mg	18	118	7	18	164
	1.0 mg	14	118	7	17	149
	2.5 mg	17	118	7	20	157
	5.0 mg	14	105	6	17	137
2NF	10.0 µg				508	
Na-azide	5.0 µg	701	864			
4-NPDA	10.0 µg			77		
MMC	0.2 µg					500
Cumene	50 µg					339

Table 74: Pre-incubation test with S9 ((b) (4)

Substance	Dose/plate	Strain				
		TA1535	TA100	TA1537	TA98	TA102
DMSO	50 µL	17	134	19	38	274
Test compound	0.1 mg	19	145	19	35	280
	0.25 mg	18	148	17	40	250
	0.5 mg	18	148	18	38	250
	1.0 mg	17	148	16	36	262
	2.5 mg	15	153	15	41	208
	5.0 mg	17	134	16	39	257
2AA	3.0 µg	119	443	57	187	511

Na-azide – sodium azide 2-NF – 2-nitro-9H-fluorene

4-NPDA – 4-nitro-1,2-phenylene diamine MMC – mitomycin C

Cumene – cumene hydroperoxide 2-AA – 2-aminoanthracene

Study title: (b) (4) **Salmonella/Microsome Test Plate Incorporation Method.**

Study no.:	T 8077761
Report no.:	PH-35108
Study report location:	Toxicology of Bayer HealthCare AG, Wuppertal, Germany
Conducting laboratory and location:	Bayer HealthCare AG BSP-GDD-GED-GTOX Genetic Toxicology 42096 Wuppertal Germany
Date of study initiation:	May 31, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) batch # BXR3K51

Key Study Findings

- (b) (4) (impurity) was positive for mutagenic potential in the bacterial reverse mutation assay in the presence of metabolic activation.

Methods

Strains:	TA 1535, TA 100, TA 1537, TA 98 and TA 102.
Concentrations in definitive study:	16, 50, 158, 500, 1581, and 5000 µg/plate
Basis of concentration selection:	Initial experiment
Negative control:	DMSO
Positive control:	sodium azide, nitrofurantoin, 4-nitro-1 ,2-phenylene diamine, mitomycin C and 2-aminoanthracene

Study Validity:

- The negative and positive control values were within the expected range.
- Titer determination demonstrated sufficient bacterial density in the suspension.

Results

Table 75: Summary of mean values without S9 mix ((b) (4)

Table and Group	Strain TA 1535	TA 100	TA 1537	TA 98	TA 102
µg/Plate					
0	14	148	7	15	273
16	13	145	7	18	243
50	15	143	6	14	258
158	10	139	7	18	268
500	16	130	7	16	265
1581	16	167	9	16	235
5000	14	155	7	18	193
Na-azide	871				
NF		357			
4-NPDA			92	180	
MMC					628

Summary of mean values with S9 mix ((b) (4)

Table and Group	Strain TA 1535	TA 100	TA 1537	TA 98	TA 102
µg/Plate					
0	13	211	8	28	326
16	12	219	10	29	321
50	15	265	7	47	275
158	17	344	7	44	307
500	23	457	10	58	330
1581	20	517	7	89	307
5000	15	493	9	97	254
2-AA	259	2178	276	947	568

Concentration (µg/plate)	TA 100	TA 98
0	149	29
300	358	56
600	322	63
1200	335	72
2400	744	97
3600	761	98
4800	707	98
2-AA	1966	1490

Na-azide – sodium azide NF - nitrofurantoin

4-NPDA – 4-nitro-1,2-phenylene diamine MMC – mitomycin C

Cumene – cumene hydroperoxide 2-AA – 2-aminoanthracene

Study title: (b) (4) **Salmonella/Microsome Test Plate Incorporation and Preincubation Method**

Study no.:	T 3077775
Report no.:	PH-35194
Study report location:	Toxicology of Bayer HealthCare AG
Conducting laboratory and location:	Bayer HealthCare AG BSP-GDD-GED-GTOX Genetic Toxicology 42096 Wuppertal Germany
Date of study initiation:	July 13, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) batch # BXR3N32, 90% purity

Key Study Findings

- (b) (4) (impurity) in the presence and absence of metabolic activation was not mutagenic in Ames test.

Methods

Strains:	TA 1535, TA 100, TA 1537, TA 98 and TA 102.
Concentrations in definitive study:	16, 50, 158, 500, 1581 and 5000 µg/plate
Basis of concentration selection:	Results of preliminary mutagenicity assay
Negative control:	DMSO
Positive control:	Sodium azide, nitrofurantoin, 4-nitro-l ,2-phenylene diamine, mitomycin C, cumene hydroperoxide and 2-aminoanthracene
Formulation/Vehicle:	DMSO
Incubation & sampling time:	17 hours

Study Validity:

- The negative and positive controls values were within the expected range.
- Titer determination demonstrated sufficient bacterial density in the suspension.

Results

Table 76: Experiment 1 - Direct plate incorporation - Mean vales with and without S9 mix ([redacted](b) (4))

Compound	Concentration (µg/plate	Strain					
		S9	TA 1535	TA 100	TA 1537	TA 98	TA 102
Test compound	0	-	14	104	7	20	285
	16	-	13	128	8	21	279
	50	-	8	103	7	22	288
	158	-	7	101	7	24	305
	500	-	6	117	5	23	255
	1581	-	5	128	5	25	256
	5000	-	5	131	6	26	180
Na-azide	10	-	679				
NF	0.2	-		366			
4-NPDA	10	-			84	135	
MMC	0.2	-					602
Test compound	0	+	10	149	12	34	329
	16	+	9	139	10	38	319
	50	+	9	179	12	34	345
	158	+	9	151	11	39	327
	500	+	7	145	10	30	305
	1581	+	9	142	8	36	225
	5000	+	4	121	6	30	173
2AA	3		159	2553	311	1769	770

Na-azide – sodium azide NF - nitrofurantoin

4-NPDA – 4-nitro-1,2-phenylene diamine MMC – mitomycin C

Cumene – cumene hydroperoxide 2-AA – 2-aminoanthracene

Table 77: Experiment 2 (preincubation for 20 min.) - Mean vales with and without S9 mix ((b) (4)

Compound	Concentration (µg/plate	Strain					
		S9	TA 1535	TA 100	TA 1537	TA 98	TA 102
Test compound	0	-	12	130	7	16	259
	16	-	11	137	7	15	223
	50	-	13	123	5	16	215
	158	-	12	134	6	15	218
	500	-	12	128	4	18	209
	1581	-	12	136	6	17	222
	5000	-	11	137	4	15	190
Na-azide	10	-	562				
NF	0.2	-		615			
4-NPDA	10	-			101	134	
Cumene	50	-					522
Test compound	0	+	11	174	8	22	319
	16	+	6	151	8	24	311
	50	+	9	163	7	27	310
	158	+	10	168	7	23	304
	500	+	8	174	6	22	286
	1581	+	8	171	7	21	288
	5000	+	8	142	6	26	171
2AA	3	+	126	2780	78	1753	500

Na-azide – sodium azide NF - nitrofurantoin

4-NPDA – 4-nitro-1,2-phenylene diamine MMC – mitomycin C

Cumene – cumene hydroperoxide 2-AA – 2-aminoanthracene

Study title: (b) (4) **BAY 73-4506. Salmonella/Microsome Test Plate incorporation Method**

Study no.:	T 3074778
Report no.:	PH-33994
Study report location:	Toxicology International of Bayer HealthCare AG
Conducting laboratory and location:	Bayer HealthCare AG PH-R&D Toxicology International Genetic Toxicology 42096 Wuppertal Germany
Date of study initiation:	January 21, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) batch # JSL 1102-10A, 99% purity

Key Study Findings

- (b) (4) (impurity) at 1600 µg/plate in the presence of S9 mix showed mutagenic activity in TA102 strain.

Methods

Strains:	TA 1535, TA 100, TA 1537, TA 98 and TA 102
Concentrations in definitive study:	800, 1200, 1600, 2000, 2400, 2800, and 3200 µg per plate
Basis of concentration selection:	Experiment 1
Negative control:	DMSO
Positive control:	Sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine, mitomycin C and 2-aminoanthracene
Formulation/Vehicle:	DMSO
Incubation & sampling time:	17 hours

Study Validity:

- A reproducible dose-related 2-fold increase over the negative controls.

Results

Table 78: Experiment 1 – Summary of mean values without and with S9 mix

((b) (4)

Compound	Concentration (µg/plate	Strain					
		S9	TA 1535	TA 100	TA 1537	TA 98	TA 102
Test compound	0	-	9	138	7	21	185
	16	-	9	143	5	17	216
	50	-	9	132	5	21	222
	158	-	11	120	8	19	215
	500	-	11	120	8	23	211
	1581	-	6	89	7	15	274
	5000	-	0	10	0	3	9
Na-azide	10	-	729				
NF	0.2	-		290			
4-NPDA	10	-			103	133	
MMC	0.2	-					542
Test compound	0	+	15	195	7	42	286
	16	+	20	179	6	41	275
	50	+	21	199	7	39	287
	158	+	16	178	6	44	260
	500	+	19	199	6	39	288
	1581	+	12	173	4	42	409
	5000	+	4	88	4	32	137
2AA	3	+	168	1240	200	1168	738

Na-azide – sodium azide NF - nitrofurantoin

4-NPDA – 4-nitro-1,2-phenylene diamine MMC – mitomycin C

2-AA – 2-aminoanthracene

Table 79: Experiment 2 - Summary of mean values without and with S9 mix ([redacted] (b) (4)

Compound	Concentration (µg/plate)	S9 mix	TA102
Test compound	0	-	231
	800	-	288
	1200	-	318
	1600	-	301
	2000	-	262
	2400	-	276
	2800	-	278
	3200	-	193
MMC	0.2	-	597
Test compound	0	+	313
	800	+	333
	1200	+	346
	1600	+	**426**
	2000	+	**488**
	2400	+	**537**
	2800	+	**559**
	3200	+	**436**
2-AA	3	+	994

MMC – mitomycin C 2-AA – 2-aminoanthracene

Note: Due to the test compound's mutagenicity seen in first experiment, doses ranging from 800 µg to 3200 µg per plate were chosen for the repeat tests.

Study title: (b) (4) ***In Vitro*** **Chromosome Aberration Test With Chinese Hamster V79 Cells.**

Study no.:	T 4074779
Report no.:	PH-33995.
Study report location:	Toxicology International of Bayer HealthCare AG, Wuppertal, Germany
Conducting laboratory and location:	Bayer HealthCare AG BSP-GDD-GED-GTOX Genetic Toxicology 42096 Wuppertal Germany
Date of study initiation:	April 4, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) batch No. JSL 1102-10A, 99 % purity

Key Study Findings

- (b) (4) (impurity) produced clastogenic effects in Chinese Hamster V79 Cells in the absence and presence of metabolic activation.

Methods

Cell line:	Chinese Hamster V79 Cells
Concentrations in definitive study:	10, 20 and 40 µg/mL in the absence of S9 mix 10, 20, and 50 µg/mL in the presence of S9 mix
Basis of concentration selection:	Cytotoxic effects in experiment 1
Negative control:	Solvent
Positive control:	Mitomycin C and cyclophosphamide
Formulation/Vehicle:	Ethanol
Incubation & sampling time:	4 hours treatment and 18 and 30 hours harvest time

Study Validity:

- Positive controls induced biological relevant increase in chromosome aberrations.
- Negative controls values were in the expected laboratory historical data.

Results

Table 80: Mitotic Index-4 hrs ((b) (4)

Experimental Group	Concentration in µg/ml	Harvest Time In Hours	Mitotic Nuclei in 2000 Cells absolute	in %
without metabolic activation				
Ethanol	0	8	155	100.0
(b) (4)	40	8	8	5.2**
	80	8	5	3.2**
	120	8	9	5.8**
Ethanol	0	18	146	100.0
(b) (4)	10	18	89	61.0**
	20	18	83	56.8**
	40	18	73	50.0**
	80	18	126	86.3
	120	18	77	52.7**
Mitomycin C	0.1	18	177	121.2
Ethanol	0	30	155	100.0
(b) (4)	40	30	40	25.8**
	80	30	73	47.1**
	120	30	97	62.6**
with metabolic activation				
Ethanol	0	8	102	100.0
(b) (4)	50	8	11	10.8**
	100	8	9	8.8**
	200	8	1	1.0**
Ethanol	0	18	199	100.0
(b) (4)	10	18	189	95.0
	20	18	193	97.0
	50	18	203	102.0
	100	18	245	123.1
	200	18	266	133.7
Cyclophosphamide	2	18	151	75.9**
Ethanol	0	30	132	100.0
(b) (4)	50	30	116	87.9
	100	30	130	98.5
	200	30	171	129.5

* $p < 0.05$
** $p < 0.01$

Mitosis rates were reduced at concentrations of ≥10 µg/mL and 50 µg/mL in the absence and presence of S9 mix, respectively.

Table 81: Survival Index-4 hrs ((b) (4)

Experimental Group	Concentration in µg/ml	Harvest Time in Hours	Survival Index in %
without metabolic activation			
Ethanol	0	8	100.0
(b) (4)	40	8	114.4
	80	8	93.9
	120	8	85.0
Ethanol	0	18	100.0
(b) (4)	10	18	87.9
	20	18	43.8#
	40	18	55.2#
	80	18	45.3#
	120	18	48.5#
Mitomycin C	0.1	18	42.8#
Ethanol	0	30	100.0
(b) (4)	40	30	68.2#
	80	30	64.7#
	120	30	34.6#
with metabolic activation			
Ethanol	0	8	100.0
(b) (4)	50	8	89.3
	100	8	87.2
	200	8	78.6#
Ethanol	0	18	100.0
(b) (4)	10	18	109.3
	20	18	82.2
	50	18	79.6#
	100	18	64.0#
	200	18	70.7#
Cyclophosphamide	2	18	67.1#
Ethanol	0	30	100.0
(b) (4)	50	30	64.6#
	100	30	38.9#
	200	30	49.6#

relevant reduction of survival index

The following concentrations were selected based on the survival index and mitotic index:

test groups	S9 Mix	concentration in µg/ml	harvest time in hours
(b) (4)	-/+	10	18
	-/+	20	18
	-	40	18
	+	50	18
	-	40	30
	+	50	30

Table 82: Chromosomal Aberration w/o metabolic Activation-4 hrs (b) (4)

Experimental Group and Concentration in µg/ml	Harvest Time in Hours	Culture Number	Cells scored	Gaps		Classes of Aberrations: Chromatid Type			Chromosome Type			other				Metaphases with Aberrations (%)		
				g	ig	b	f	d	ib	if	id	ex	maE	ma	cd	incl. gaps	excl.gaps	exchanges
Ethanol 0	18	18	100	0	1	1	0	0	0	0	3	0	0	0	0	5.0	4.0	0.0
		37	100	0	0	0	0	0	0	0	2	0	0	0	0	2.0	2.0	0.0
			200	0	1	1	0	0	0	0	5	0	0	0	0	3.5	3.0	0.0
(b) (4)	18	44	100	0	0	2	2	0	1	0	0	2	0	0	0	4.0	4.0	1.0
10		1	100	2	1	2	0	0	1	0	1	1	5	0	0	13.0	10.0	6.0
			200	2	1	4	2	0	2	0	1	3	5	0	0	8.5*	7.0	3.5*
(b) (4)	18	43	100	2	0	3	1	0	0	0	1	5	8	0	0	15.0	13.0	11.0
20		6	100	0	0	4	2	0	2	3	2	9	8	0	0	17.0	17.0	12.0
			200	2	0	7	3	0	2	3	3	14	16	0	0	16.0**	15.0**	11.5**
(b) (4)	18	35	100	2	0	5	1	0	3	3	4	22	14	1	0	33.0	31.0	24.0
40		17	100	1	0	4	5	0	2	0	1	5	10	0	0	19.0	19.0	14.0
			200	3	0	9	6	0	5	3	5	27	24	1	0	26.0**	25.0**	19.0**
Mitomycin C 0.1	18	22	100	5	11	35	5	0	14	1	0	16	2	1	0	53.0	45.0	15.0
		5	100	3	4	40	3	0	19	2	3	25	1	0	0	55.0	53.0	20.0
			200	8	15	75	8	0	33	3	3	41	3	1	0	54.0**	49.0**	17.5**

*p < 0.05
**p < 0.01

Table 83: Chromosomal Aberration with metabolic Activation-4 hrs ((b) (4)

Experimental Group and Concentration in µg/ml	Harvest Time in Hours	Culture Number	Cells scored	Gaps		Classes of Aberrations: Chromatid Type			Chromosome Type			other				Metaphases with Aberrations (%)		
				g	ig	b	f	d	ib	if	id	ex	maE	ma	cd	incl. gaps	excl.gaps	exchanges
Ethanol 0	18	16	100	0	0	1	0	0	1	0	1	1	0	0	0	4.0	4.0	1.0
		25	100	1	0	5	1	1	0	0	3	1	0	0	0	10.0	10.0	1.0
			200	1	0	6	1	1	1	0	4	2	0	0	0	7.0	7.0	1.0
(b) (4)	18	36	100	1	1	7	0	0	0	0	2	2	0	0	0	11.0	9.0	2.0
10		15	100	1	2	2	0	0	0	0	4	0	0	0	0	8.0	6.0	0.0
			200	2	3	9	0	0	0	0	6	2	0	0	0	9.5	7.5	1.0
(b) (4)	18	30	100	0	0	2	1	0	0	0	1	0	1	0	0	5.0	5.0	1.0
20		13	100	5	1	3	0	0	2	0	0	1	3	0	0	13.0	9.0	4.0
			200	5	1	5	1	0	2	0	1	1	4	0	0	9.0	7.0	2.5
(b) (4)	18	19	100	1	1	1	1	0	3	1	3	3	6	0	0	15.0	14.0	8.0
50		23	100	1	0	3	0	0	1	0	2	9	6	0	0	13.0	13.0	11.0
			200	2	1	4	1	0	4	1	5	12	12	0	0	14.0*	13.5*	9.5**
Cyclophosphamide 2	18	29	100	3	1	12	1	0	11	0	2	6	0	0	0	27.0	24.0	6.0
		27	100	4	0	19	1	0	11	2	5	8	0	1	0	41.0	38.0	8.0
			200	7	1	31	2	0	22	2	7	14	0	1	0	34.0**	31.0**	7.0**

*p < 0.05
**p < 0.01

b – break ib – isobreak cd – chromosome disintegration
d – deletion id - isodeletion ex – exchange
f – fragment if – isofragment g – gap
ig – isogap ma – multiple aberrations
maE – multiple aberration with exchange

Table 84: Chromosomal Aberrations with and without metabolic Activation-4 hrs ((b) (4) [redacted]

Experimental Group and Concentration in µg/ml	Harvest Time in Hours	Culture Number	Cells scored	Gaps		Classes of Aberrations: Chromatid Type			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																		
Ethanol	30	26	100	1	0	1	0	0	0	0	1	0	0	0	0	3.0	2.0	0.0
0		28	100	0	0	1	0	0	0	0	0	0	0	0	0	1.0	1.0	0.0
			200	1	0	2	0	0	0	0	1	0	0	0	0	2.0	1.5	0.0
(b) (4)	30	31	100	1	0	2	0	0	3	0	9	0	2	2	0	18.0	17.0	2.0
40		32	100	2	0	0	1	0	2	2	3	2	1	0	0	11.0	9.0	3.0
			200	3	0	2	1	0	5	2	12	2	3	2	0	14.5**	13.0**	2.5*
with metabolic activation																		
Ethanol	30	39	100	0	0	1	1	0	0	0	0	1	0	0	0	2.0	2.0	1.0
0		9	100	0	1	2	0	0	0	1	1	0	0	0	0	5.0	4.0	0.0
			200	0	1	3	1	0	0	1	1	1	0	0	0	3.5	3.0	0.5
(b) (4)	30	34	100	2	0	3	1	1	3	0	3	2	0	0	0	13.0	11.0	2.0
50		3	100	0	0	2	0	0	0	0	2	1	0	0	0	5.0	5.0	1.0
			200	2	0	5	1	1	3	0	5	3	0	0	0	9.0*	8.0*	1.5

*p < 0.05
**p < 0.01

b – break
d – deletion
f – fragment
ig – isogap
maE – multiple aberration with exchange
ib – isobreak
id - isodeletion
if – isofragment
ma – multiple aberrations
cd – chromosome disintegration
ex – exchange
g – gap

Study title: (b) (4) **- Bone Marrow Micronucleus Test and Liver Comet Assay In Male and Female Rats after Oral Administration over 3 Days.**

Study no.:	TOXT8081081
Report no.:	A48891
Study report location:	Archives of Nonclinical Drug Safety
Conducting laboratory and location:	Bayer Schering Pharma AG, Nonclinical Drug Safety, 13342 Berlin, Germany
Date of study initiation:	November 19, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) batch # BXR4J37, 99 % purity

Key Study Findings

- (b) (4) (an impurity) at concentrations of up to 500 mg/kg by intragastric administration did not show mutagenic potential in an *in vivo* micronucleus test in male and female rats.
- (b) (4) induced DNA damage in a liver comet assay at doses ≥125 mg/kg but not at doses up to 60 mg/kg.

Methods

Doses in definitive study:	High dose study – 125, 250 or 500 mg/kg Low dose study – 15, 30 or 60 mg/kg
Frequency of dosing:	Daily for three days
Route of administration:	Intragastrically
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.7 mL Polyethyleneglycol, 0.05 mL Ethanol abs., 0.25 mL Solutol HS15
Species/Strain:	Wistar (HsdCpb:WU), SPF rats
Age:	Approximately 8 weeks
Weight:	Males – 230 to 290 g Females – 150 to 201 g
Number/Sex/Group:	6 (see table below)
Satellite groups:	No
Basis of dose selection:	Pre-experiment, 500 mg/kg was MTD
Negative control:	Vehicle (PEG, ethanol and solutol HS 15)
Positive control:	Ethyl methane sulfonate (EMS) (200 mg/kg)

Experimental design

Group	Study	Treatment	Number of animals and sex	Dose (mg/kg)	Concentration (mg/mL)	Application Volume (mL/kg)
1	Combined bone marrow Micronucleus test / liver Comet Assay	Vehicle (negative control)	6M/6F	--	--	5
2		(b) (4)	6M/6F	125	25	5
3			6M/6F	250	50	5
4			6M/6F	500	100	5
5		EMS (positive control)	6M/6F	200	20	10
6	Follow-up liver Comet Assay	Vehicle (negative control)	6M/6F	--	--	5
7		(b) (4)	6M/6F	60	12	5
8			6M/6F	30	6	5
9			6M/6F	15	3	5
10		EMS (positive control)	6M/6F	200	20	10

EMS – Ethyl methane sulfonate

Study Validity:

- The positive control compound EMS gave the expected statistically significant increase in micronucleated PCE and NCE cell counts.
- Vehicle control values were in the historical data range.
- Both test systems were able to detect cytogenetic damage.

Results

Mortality: 500 mg/kg (b) (4) – One male died on the second treatment day
200 mg/kg EMS – one female died on third day of treatment

Clinical signs: 500 mg/kg – 5/11 showed adverse clinical signs

Table 85: Bone marrow micronucleus test ([redacted] (b) (4))

Treatment	Number of animals	Dose (mg/kg)	‰ MN-PCE (mean ± SD)	‰ MN-NCE (mean ± SD)	PCE/NCE (mean ± SD)
	Males				
Vehicle (negative control)	6	0	1.58 ± 0.66	1.00 ± 0.89	0.94 ± 0.11
(b) (4)	6	125	1.17 ± 0.41	0.67 ± 0.52	0.91 ± 0.06
	6	250	1.58 ± 0.38	1.00 ± 0.63	0.91 ± 0.01
	5	500	1.30 ± 0.84	0.80 ± 0.45	0.85 ± 0.11
EMS (positive control)	6	200	8.00 ± 1.67	2.50 ± 0.84	0.78 ± 0.06
	Females				
Vehicle (negative control)	6	0	1.50 ± 0.55	0.83 ± 0.98	0.84 ± 0.05
(b) (4)	6	125	1.08 ± 0.58	1.00 ± 0.63	0.85 ± 0.07
	6	250	1.33 ± 0.75	0.83 ± 0.75	0.79 ± 0.04
	6	500	1.67 ± 0.26	1.33 ± 0.52	0.84 ± 0.03
EMS (positive control)	5	200	9.20 ± 0.91	2.00 ± 1.22	0.13 ± 0.03
	Males + Females				
Vehicle (negative control)	12	0	1.54 ± 0.58	0.92 ± 0.90	0.89 ± 0.10
(b) (4)	12	125	1.13 ± 0.48	0.83 ± 0.58	0.88 ± 0.07
	12	250	1.46 ± 0.58	0.92 ± 0.67	0.85 ± 0.07
	11	500	1.50 ± 0.59	1.09 ± 0.54	0.84 ± 0.07 *
EMS (positive control)	11	200	8.55 ± 1.46 *	2.27 ± 1.01 *	0.48 ± 0.34 *

MN-PCE micronucleated polychromatic erythrocytes
MN-NCE micronucleated normochromatic erythrocytes
PCE/NCE ratio of polychromatic to normochromatic erythrocytes (calculated on basis of 1000 NCE scored per animal)
SD standard deviation
* statistically significant as compared to vehicle control, $p<0.05$
(positive control: statistical analysis was only performed with pooled values for both sexes)

(Tables excerpted from Applicant's submission)

Table 86: Liver Comet Assay with high doses of (b) (4)

Treatment	Dose (mg/kg)	Number of animals	Mean tail moment		Mean tail length		Mean tail intensity	
			mean±SD	-fold increase	mean±SD	-fold increase	mean±SD	-fold increase
		Males						
Vehicle	0	6	0.49±0.10	1	24.93±1.63	1	5.46±0.94	1
(b) (4)	125	6	0.71±0.13*	1.45	29.77±1.62**	1.19	7.82±1.25*	1.43
	250	6	0.68±0.17*	1.39	28.29±2.99	1.13	7.65±1.87*	1.40
	500	5	0.79±0.12**	1.61	30.88±2.98**	1.24	8.53±1.04**	1.57
EMS	200	6	13.74±4.67**	28.04	81.93±12.27**	3.29	53.84±9.10**	9.86
		Females						
Vehicle	0	6	0.50±0.06	1	25.80±0.56	1	5.44±0.90	1
(b) (4)	125	6	0.60±0.14	1.20	28.10±2.46	1.09	6.50±1.37	1.20
	250	6	0.59±0.15	1.18	29.02±2.27	1.12	6.20±1.30	1.14
	500	6	0.73±0.18*	1.46	30.21±3.20*	1.17	7.49±1.23*	1.38
EMS	200	5	18.76±3.53**	37.52	87.11±8.62**	3.38	64.79±5.98**	11.91
		Males + Females						
Vehicle	0	12	0.50±0.08	1	25.37±1.25	1	5.45±0.88	1
(b) (4)	125	12	0.65±0.14*	1.30	28.93±2.17**	1.14	7.16±1.43**	1.31
	250	12	0.63±0.16*	1.26	28.66±2.56**	1.13	6.92±1.71*	1.27
	500	11	0.76±0.15**	1.52	30.51±2.97**	1.20	7.96±1.22**	1.46
EMS	200	11	16.02±4.77**	32.04	84.29±10.60**	3.32	58.82±9.40**	10.79

SD standard deviation
EMS ethyl methane sulfonate
* statistically significant as compared to vehicle control, $p < 0.05$
** statistically significant as compared to vehicle control, $p < 0.01$

Table 87: Liver Comet Assay with low doses of [redacted] (b) (4)

Treatment	Dose (mg/kg)	Number of animals	Mean tail moment		Mean tail length		Mean tail intensity	
			mean±SD	-fold increase	mean±SD	-fold increase	mean±SD	-fold increase
		Males						
Vehicle	0	6	0.52±0.07	1	23.73±0.78	1	6.27±0.90	1
	15	6	0.55±0.17	1.06	24.07±1.00	1.02	6.06±1.66	0.97
[redacted] (b) (4)	30	6	0.47±0.06	0.90	25.16±0.62	1.06	5.55±0.72	0.89
	60	6	0.60±0.11	1.17	26.47±1.35**	1.16	7.02±1.04	1.12
EMS	200	6	9.68±1.48 **	18.62	78.65±7.36 **	3.31	44.39±3.45 **	7.08
		Females						
Vehicle	0	6	0.49±0.09	1	24.80±1.06	1	5.71±1.04	1
	15	6	0.46±0.09	0.94	25.39±1.04	1.05	5.38±0.97	0.94
[redacted] (b) (4)	30	6	0.56±0.10	1.14	26.36±1.07**	1.10	6.50±1.13	1.14
	60	6	0.57±0.09	1.16	27.31±0.59**	1.13	6.68±1.04	1.17
EMS	200	6	10.98±2.06 **	22.41	79.57±8.89 **	3.30	49.08±4.66 **	8.60
		Males + Females						
Vehicle	0	12	0.51±0.08	1	24.26±1.05	1	5.99±0.97	1
	15	12	0.50±0.14	0.98	24.73±1.19	1.02	5.72±1.35	0.95
[redacted] (b) (4)	30	12	0.51±0.09	1	25.76±1.04**	1.06	6.02±1.04	1.01
	60	12	0.59±0.10	1.16	26.89±1.09**	1.11	6.85±1.00	1.14
EMS	200	12	10.33±1.84 **	20.25	80.16±7.46 **	3.30	46.73±4.62 **	7.80

SD standard deviation
EMS ethyl methane sulfonate
** statistically significant as compared to vehicle control, $p < 0.01$

8 Carcinogenicity: Not conducted

9 Reproductive And Developmental Toxicology

9.1 Fertility and Early Embryonic Development

No studies conducted

Embryonic Fetal Development

No clinical data regarding embryofetal toxicity were reported. Regorafenib exhibited clear developmental toxicity and teratogenicity under the conditions tested nonclinically.

The applicant submitted two prenatal development studies, a pilot study in rats (not GLP) and a GLP-compliant study in rabbits (both reviewed below). Additionally, the applicant provided summaries of two range-finding studies (one in rats, one in rabbits), but did not provide these two studies in the NDA. Based on the results submitted, this reviewer concurs that submission of the two range-finding embryofetal studies is not warranted, and repetition of the non-GLP rat study (i.e. repeated under GLP) is not warranted, to inform safety.

Note regarding Report # T0074513
In report # 36547 (study report page 12), a rat dose-tolerability embryofetal study is described (report # T0074513, not submitted to the NDA; not summarized in the NDA's Toxicology Summary modules). Reportedly, groups of 2 female pregnant rats were dosed with 1, 5 or 20 mg/kg of regorafenib. All six of these treated rats revealed total resorptions. In the subsequent study (report # 36547, reviewed below), 0.3 mg/kg was the NOAEL for developmental toxicity, and the 1 mg/kg dose was relatively less toxic compared to the dose-tolerability study (i.e. did not cause total resorptions).

Study title: **BAY 73-4506: pilot prenatal developmental toxicity study in rats after oral administration**

Study no:	• PH-36547 • T4074517
Study report location:	NDA module 4.2.3.5.2.1 (317 pages)
Conducting laboratory and location:	Bayer Schering Pharma Ag GDD-GED Toxicology 42096 Wuppertal Germany
Report date	June 27, 2011
Date of study initiation:	February 27, 2006
End of in-life phase:	September 20, 2006
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Regorafenib [BAY 73-4506 GRAN 10% 010 (coprecipitate)]; batch #s 050413-010 and 050817-010; purity 9.9%

Key Study Findings

- The authors concluded, and this reviewer agrees, that this study identified:
  - a maternal toxicity NOAEL of 1 mg/kg/day (reduced weight gain $\geq$ 1.6 mg/kg)
  - a developmental toxicity of NOAEL of 0.3 mg/kg/day (reduced ossification $\geq$ 0.8 mg/kg); increased incidence of late resorptions was observed at higher doses ($\geq$ 1.6 mg/kg)
  - a teratogenicity NOAEL of 0.8 mg/kg/day
    - at $\geq$ 1 mg/kg: skeletal variations (14^{th} rib, additional lumbar vertebrae, combined malformations of vertebrae)
    - at > 1.6 mg/kg: increased postimplantation loss, decreased placental and fetal weight, increased incidence of visceral malformations (heart, blood vessels, diaphragm)
- Initially, three dose-levels were planned (0, 0.1 and 0.3 mg/kg of regorafenib); however, based on the results at those dose levels, additional (higher) dose groups were added (i.e. without concurrent negative control)
- No PK data reported

Methods

Doses:	• Placebo (BAY 73-4506 PLAC POWD 000) at 0.3 mg/kg (to correspond to the high-dose group) • Regorafenib at 0.1, 0.3, 0.5, 0.8, 1.0, 1.6 or 2.0 mg/kg
Frequency of dosing:	Once daily from post-coital (pc) Day 6 to pc day 17
Dose volume:	10 ml/kg body weight
Route of administration:	Orally by gavage
Formulation/Vehicle:	Suspension in tap water
Species/Strain:	Rat, Wistar (Hsd Cpb:WU)
Number/Sex/Group:	7 pregnant female rats/dose
Ages:	12 to 22 weeks
Weights:	195 to 250 g
Deviation from study protocol:	Initially, three dose-levels were planned (0, 0.1 and 0.3 mg/kg of regorafenib); however, based on the results at those dose levels, additional (higher) dose groups were added (i.e. without concurrent negative control). The 1.6 mg/kg group was added last.

Method notes

- Males were not dosed. Animals were individually housed from gestation day 0 onward.
- Females were dosed from pc6 to pc17, and Cesarean sections were performed on D20.

Observations and Results

Mortality

- No premature mortality of dams occurred.

Clinical Signs

- Reddish vaginal discharge was noted for one animal at 0.8 mg/kg (# 3298, on GD14 only) and three animals at 2 mg/kg (#3310, GD17-end; #3313 from GD14-end; # 3314 on GD14-15 and GD20)
- The authors conclude (report page 22) and this reviewer concurs, that the effect at 0.8 mg/kg is incidental to treatment and that the effects at 2 mg/kg are clearly treatment-related
    - The authors note that animal at 0.8 mg/kg did not experience resorptions (13 viable fetuses),
    - The three females that experienced reddish vaginal discharge at 2 mg/kg exhibited postimplantation losses (in two) or complete resorption of the litter (in one dam, #3032)

- No other treatment-related clinical signs were apparent

Body Weight

- Maternal body weight was measured on pc0 and daily from pc6 to pc20. The results are partially confounded by the variability in starting body weights, however, clear treatment-related effects remain apparent:
  - Treatment-related decreases in dam body weight gain (pc6-pc17 and pc0-20) were apparent for the 1.6 and 2.0 mg/kg groups
  - Treatment-related decreases in dam corrected body weight gain (i.e. subtracting uterine weight from the dam weight) indicating maternal toxicity were apparent for the 1.6 and 2 mg/kg groups, though at a smaller magnitude compared to total body weight; fetal toxicity occurred at the same dose levels
- From the study report (page 21):

Table 88: Regorafenib affected body weight gain in pregnant rats (report # PH-36547)

Dose → (mg/kg/day)	0	0.1	0.3	0.5	0.8	1	1.6	2
Body weight (g): pc D0	224	238	231	224	230	231	215	216
Body weight (g): pcD20	341	373	372	348	364	362	317	291**
Body weight gain (g): pc days 6-17	56.4	69.0 (+22%)	70.7 (+25%)	56.7 (+5%)	61.7 (+9%)	62.0 (+9%)	48.6 (-14%)	31.8** (-44%)
Body weight gain (g): pc days 0-20	116.9	135.1 (+56%)	140.0 (+20%)	123.6 (+6%)	134.7 (+15%)	131.0 (+12%)	102.1 (-13%)	75.6** (-35%)
Carcass weight (g; pc day 20)	268	293	285	273	287	287	255	253
Gravid uterus weight (g)	72.7	79.6	86.7	74.7	77.7	75.3	61.7	38.2**
Corrected body weight gain (g): pc days 0-20	44.1	55.6 (+26%)	53.5 (+21%)	48.9 (+11%)	57.0 (+29%)	55.7 (+26%)	40.4 (-8%)	37.4 (-15%)

Data presented as means (value in parenthesis is % control value)
** Statistically significant difference from controls, p <0.01

Feed Consumption

- No treatment-related effects on food consumption were apparent.
- Food intake was measured quantitatively (by weighing food) over 3 day intervals.
- Water intake was assessed daily, qualitatively (and reported under clinical signs)

Toxicokinetics

No toxicokinetic evaluations (in dams or fetuses) for this study

Stability and Homogeneity

The test article was designated “BAY 73-4506 GRAN 10% 010”, batch # 050413-010. The purity was reported as 9.9% (which refers to the test article being a nominal 10% suspension in tap water). Fresh formulations were prepared daily. Stability at 6 hours at room temperature was verified on July 24, 2006, and homogeneity was verified on

September 5, 2006 on representative samples (for the 0.16 and 0.2 mg/ml concentrations only).

Necropsy

- Dams were subjected to gross pathological examination on pc20.
- Authors identified no treatment-related findings outside the placenta (report page 22)
  - One female in the 0.3 mg/kg group (#3032) had no uterine implantation sites, and the uterus was filled with a yellowish fluid
  - At 1.6 mg/kg, one female (#3319) had an enlarged spleen, and another (# 3320) had a dilated renal pelvis.
- Treatment-related placenta effects noted at 1, 1.6 and 2 mg/kg

Table 89: Placental changes noted in pregnant rats receiving regorafenib (report # PH-36547)

Dose → (mg/kg/day)	Incidence	0	0.1	0.3	0.5	0.8	1	1.6	2
Total placental observations	Fetal	0	0	0	0	0	3	8*	0
	Litter						1	4	
Greyish placental border	Fetal	0	0	0	0	0	3	5	0
	Litter						1	2	
Placenta pale	Fetal	0	0	0	0	0	1	0	0
	Litter						1	0	
Double placenta	Fetal	0	0	0	0	0	0	2	0
	Litter						0	1	
Placenta engorged	Fetal	0	0	0	0	0	0	1	0
	Litter						0	1	

* Statistically significant, $p < 0.05$

Cesarean Section Data

- Cesarean sections were performed on pc20. Endpoints assessed were:
  - Number of corpora lutea
  - Number of implantations (the protocol specified that for females without visible implantation sites, the uterus would be stained with 10% ammonium sulfide as per Salewski et al. 1964)
  - Uterine weights
  - Placenta weight and appearance
  - Number of early resorptions (i.e. only implantation site visible)
  - Number of late resorptions (fetal or placental remnant visible)
  - Number of dead fetuses (fetuses without signs of life but with maceration)
  - Number of live fetuses
  - Sex of live fetuses
  - Individual weight of live fetuses
  - Fetuses: external malformations and other findings deviating from normal

- Fetuses: half were processed for visceral evaluation and half for skeletal evaluation. Note: visceral findings evaluated without knowledge of treatment group (report page 15)

- No clear effect on fertility was observed. Consistent with timing of dosing (i.e. after GD5), the authors conclude, and this reviewer concurs, that no treatment-related effect was apparent on the number of corpora lutea, preimplantation loss, or fertility rate (# of implantation/# of inseminated females).
- For the 1.6 and 2 mg/kg groups, clear effects on fetal development and viability were observed
  - Postimplantation loss (late resorptions) increased at 1.6 and 2 mg/kg, resulting in a clear decrease in the number of live fetuses
  - The authors concluded (page 23) and this reviewer concurs, that treatment did not affect the numbers of corpora lutea or preimplantation losses. This is consistent with the timing of dosing, initiating after implantation (i.e. initiating pc day 6).

Table 90: Regorafenib caused post-implantation losses in pregnant rats (report # PH-36547)

Dose → (mg/kg/day)	0	0.1	0.3	0.5	0.8	1	1.6	2
# of inseminated females	7/7	7/7	7/7	7/7	7/7	7/7	7/7	7/7
# of inseminated females with implantations	7/7	7/7	6/7 (-14%)	7/7	3/7 (-57%)	7/7	7/7	7/7
Fertility index (%)	100	100	58.7	100	42.9	100	100	100
# of females with **total** resorptions	0/7	0/7	0/7	0/7	0/7	0/7	0/7	2/7
# of females with viable fetuses on pc day 20	7/7	7/7	6/7	7/7	3/7	7/7	7/7	5/7
Mean # of corpora lutea per female [a]	15.6	15.0	16.0	14.4	14.0	15.7	15.1	14.4
Mean # of implantations per female [a]	14.1	14.6	15.5	13.6	14.0	15.0	14.0	12.6
% Preimplantation loss [a]	1.4	0.4	0.5	0.9	0	0.7	1.1	1.9
Total # of early resorptions	0	0	0	0	0	0	0	0
Total # of postimplantation losses	11	5	2	4	0	12	14	53

Dose → (mg/kg/day)	0	0.1	0.3	0.5	0.8	1	1.6	2
Mean # postimplantation loss per female [a]	1.6	0.7	0.3	0.6	0.0	1.7	2.0	5.2[a]
Total # of live fetuses on pc day 20	88	97	91	91	42	93	84	35
Mean # of live fetuses per female [b] on pc day 20	12.6	13.9	15.2	13.0	14.0	13.3	12.0	7.0**
Total # of dead fetuses on pc day 20	0	0	0	0	0	0	0	0
Mean placenta weight [b], grams	0.58	0.55	0.59	0.67**	0.59	0.59	0.53	0.48**
Mean fetal weight [b], grams	3.56	3.72	3.64	3.62	3.52	3.60	3.25**	3.27*
Mean % of litter male [b]	49.9	54.7	53.5	51.1	44.0	59.7	57.1	40.3

[a] Referring only to females with implantation sites (i.e. not the 2/7 high-dose females with no implantations) For the 2 mg/kg group, 2/7 females exhibited resorptions; the % postimplantation loss for all females in the 2 mg/kg group is 7.6%
[b] Referring only to females with viable fetuses
* Statistically significant difference from controls, $p < 0.05$
** Statistically significant difference from controls, $p < 0.01$

Offspring Data

- The authors concluded (report page 26), and this reviewer concurs, that increased incidence of common skeletal malformations were observed ≥ 1 mg/kg
  - Additional lumbar vertebrae, altered shape of sacral vertebrae, bent tail, kinked vertebral column
- The authors concluded (report page 26), and this reviewer concurs, that increased incidence of common skeletal malformations were observed at 1, 1.6 and 2 mg/kg
  - ≥ 1 mg/kg: generalized edema, increased incidence of "common skeletal malformations" (report page 26) – "(additional lumbar vertebrae, altered shape of sacral vertebra partly with pelvic shift, at higher doses further combined malformation of vertebrae partly with slightly bent tail/slightly kinked vertebral column (report page 26)
  - ≥ 1.6 mg/kg: visceral malformations, including the heart, blood vessels and diaphragm: "individually differently (hairline) ventricular septal defect of the heart, displaced heart, rightsided aortic arch, right-sided retrooesophageal ductus arteriosus, aorticopulmonary fistula, right-sided pulmonary trunk, displaced origin of subclavian and carotid arteries, double azygos vein and diaphragmatic hernia" (report page 26)
- Below, tables Table 91,Table 92, and Table 93 show selected findings, considered by this reviewer to be treatment-related

Table 91: Regorafenib exposure associated with rat fetal external anomalies (report # PH-36547)

Dose → (mg/kg/day)	Incidence	0	0.1	0.3	0.5	0.8	1	1.6	2
Fetal external observations									
# of litters evaluated		7	7	6	7	3	7	7	5
# of live fetuses evaluated		88	97	91	91	42	93	84	35
# of dead fetuses evaluated		0	0	0	0	0	0	0	0
Total # of fetuses evaluated		88	97	91	91	42	93	84	35
Eye rudiment flat (eyeball reduced in size	Fetal	0	0	0	0	0	0	0	1
	Litter	0	0	0	0	0	0	0	1
Trunk – umbilical cord shortened	Fetal	0	0	0	0	0	0	0	2
	Litter	0	0	0	0	0	0	0	2
Skin – generalized edema	Fetal	0	0	0	0	0	1	0	1
	Litter	0	0	0	0	0	1	0	1
Skin – pale	Fetal	0	0	0	0	0	0	0	1
	Litter	0	0	0	0	0	0	0	1
Skin – slightly edematous	Fetal	0	0	0	0	0	0	1	0
	Litter	0	0	0	0	0	0	1	0

Table 92: Regorafenib exposure associated with rat fetal visceral anomalies (report # PH-36547)

Dose → (mg/kg/day)	Incidence	0	0.1	0.3	0.5	0.8	1	1.6	2
Fetal visceral observations									
# of litters examined		7	7	6	7	3	7	7	4
# of fetuses examined		42	46	44	44	20	44	38	16
Thyroid – gland missing	Fetal	0	0	0	1	0	0	0	0
	Litter	0	0	0	1	0	0	0	0
Thyroid – gland reduced in size	Fetal	0	0	0	0	0	0	0	1
	Litter	0	0	0	0	0	0	0	1
Double aortic arch	Fetal	0	0	0	0	0	0	1	0
	Litter	0	0	0	0	0	0	1	0
Right-sided aortic arch	Fetal	0	0	0	0	0	0	0	1
	Litter	0	0	0	0	0	0	0	1
Malformation of (great) arteries	Fetal	0	0	0	0	0	0	2	3
	Litter	0	0	0	0	0	0	1	2
Double-sided vena azygos	Fetal	0	0	0	0	0	0	1	1
	Litter	0	0	0	0	0	0	1	1
Heart – ventricular septal defect	Fetal	0	0	0	0	0	0	1	0
	Litter	0	0	0	0	0	0	1	0
Heart – hairline ventricular septal defect	Fetal	0	0	0	0	0	0	0	1
	Litter	0	0	0	0	0	0	0	1
Heart - displaced	Fetal	0	0	0	0	0	0	0	1
	Litter	0	0	0	0	0	0	0	1
Diaphragmatic hernia	Fetal	0	0	0	0	0	0	0	2

	Litter	0	0	0	0	0	0	0	2
Kidney – dilation of renal pelvis	Fetal	0	1	0	1	0	0	2	3
	Litter	0	1	0	1	0	0	2	2
Ureter dilation	Fetal	0	0	0	0	0	0	2	0
	Litter	0	0	0	0	0	0	2	0
Uterine anomaly (not otherwise specified)	Fetal	0	0	0	0	0	0	0	1
	Litter	0	0	0	0	0	0	0	1

Table 93: Regorafenib exposure associated with rat fetal skeletal anomalies (report # PH-36547)

Dose → (mg/kg/day)	Incidence	0	0.1	0.3	0.5	0.8	1	1.6	2
Fetal visceral observations									
# of litters examined		7	7	6	7	3	7	7	5
# of fetuses examined		46	51	47	47	22	49	44	19
# of fetuses with skeletal findings	Fetal	25 (54%)	29 (57%)	30 (64%)	35 (75%)	22 (100 %)	48 (98%)	44 (100 %)	19 (100 %)
	Litter	7	7	7	7	3	7	7	5
Sternum – unossified	Fetal	0	0	0	0	1	1	3	2
	Litter	0	0	0	0	1	1	2	2
Sternum – fusion	Fetal	0	0	0	0	0	0	2	0
	Litter	0	0	0	0	0	0	1	0
Vertebral column – vertebral bodies	Fetal	16 (35%)	21 (41%)	19 (40%)	29 (62%)	22 (100 %)	46 (94%)	44 (100 %)	19 (100 %)
	Litter	7	6	5	6	3	7	7	5
Vertebral column – vertebral arches	Fetal	0	0	0	0	0	0	1	2
	Litter	0	0	0	0	0	0	1	1
Ribs – variation (14th rib)	Fetal	4 (9%)	8 (16%)	10 (21%)	5 (11%)	5 (23%)	26 (53%)	19 (43%)	13 (68%)
	Litter	4	6	5	4	3	7	6	5
Ribs – fusion (cartilaginous part)	Fetal	0	0	0	0	0	0	1	0
	Litter	0	0	0	0	0	0	1	0
Metatarsalia / carpalia less than 3	Fetal	0	0	0	0	0	0	1	0
	Litter	0	0	0	0	0	0	1	0
Cleft palate – slight	Fetal	0	0	0	0	0	0	0	1
	Litter	0	0	0	0	0	0	0	1
Skull – fontanelle enlarged	Fetal	0	0	0	0	0	1	2	1
	Litter	0	0	0	0	0	1	1	1

Note regarding report # T107639

A rabbit pilot embryofetal development study (report # T1076396) is described in report # PH-36036 (pdf pages 400-401; and also in NDA Module 2.6.6 Toxicology Written Summary). Reportedly, groups of 3 inseminated Himalayan rabbits were dosed orally

by gavage with 0, 0.5, 1, 1.6 or 2 mg/kg from post coitum (pc) days 6 to 20, with cesarean section on pc day 29. Reportedly:

- The applicant concluded that this study showed maternal toxicity at 2 mg/kg, developmental toxicity at 1.6 mg/kg, and potential teratogenicity ≥ 0.5 mg/kg
- No maternal toxicity observed ≤ 1.6 mg/kg
- At 2 mg/kg, 2/3 females exhibited total resorptions, body weight loss, moderate-to-severe reduced food intake, decreased/no feces, diarrhea, decreased water intake, decreased and discolored urination. The remaining 1 female at 2 mg/kg did not exhibit clinical signs of toxicity
- The number of viable offspring was reduced at 2 mg/kg (the one female without total resorptions had 5 living fetuses) and 1.6 mg/kg (each of the three does only had one living fetus each)
- Placental weight and fetal weights did not change with treatment
- At 2 mg/kg, four of the five fetuses had kidney malformations (e.g. kidney unilateral missing, kidney displaced with hydronephrosis)
- At 0.5 mg/kg, one fetus had a renal malformation (dilation of the renal pelvis)
- An apparent shift in fetal sex distribution (40% male at 2 mg/kg; 33.3% at 1.6 mg/kg; 31.8% at 0.5 mg/kg) was considered by the authors to be incidental, and was attributed to the low number of evaluable fetuses at these dose levels.

Study title: **BAY 73-4506 (test article: BAY 734506 GRAN 10 % 010)**
Developmental toxicity study in rabbits after oral administration

Study no:	• PH-36036 • T9077302
Study report location:	950 pages
Conducting laboratory and location:	Bayer Schering Pharma AG GDD-GED General Toxicology 42096 Wuppertal Germany
Report date:	October 16, 2009
Date of study initiation:	April 12, 2007
Experimental completion date:	October 30, 2007
GLP compliance:	Yes, signed
QA statement:	Yes, signed
Drug, lot #, and % purity:	BAY 73-4506 (as coprecipitate BAY 73-4506 GRAN 10 % 010), batch # 050817-010, active ingredient 9.9%

Key Study Findings

- Under the conditions of this study, regorafenib was fetotoxic and teratogenic
- The authors proposed that the maternal NOAEL = 0.8 mg/kg/day; however, one female was found dead at this dose (on pc day 20)
- PK data were collected (pc day 6 and pc day 20) for regorafenib and two metabolites

- As expected based on the study design (dosing initiated on gestation day 6), no treatment-related effects apparent for fertility rate, corpora lutea, preimplantation losses, or implantation sites.
- 1.6 mg/kg caused a "severe" increase in post-implantation losses. The mid-dose (0.8 mg/kg) was the NOAEL for post-implantation loss
- The results suggest male fetuses may be more sensitive to regorafenib toxicity. At the high-dose, only 35% of viable fetuses were male. The authors considered this finding incidental
- No NOAEL for teratogenicity
  - The authors consider the low-dose, 0.4 mg/kg, to be a NOAEL for developmental toxicity, but this reviewer disagrees.
  - Cardiac ventral septal defect was observed in fetuses at all regorafenib-dose levels. The authors considered the effect at 0.4 mg/kg to be incidental (based on historical control incidence and lack of a clear dose response); this reviewer disagrees (because the incidence at 0.4 mg/kg is outside the reported historical control range, and the finding was clearly confirmed at 1.6 mg/kg)
  - Increased incidence of sternebrae fusion at all dose levels
- Fetal malformations at 0.8 and/or 1.6 mg/kg:
  - "Tricuspidal atresia, cardiac ventricular septal defect, small right ventricle, large left ventricle, small pulmonary artery, with/without aorta in origin enlarged" at 0.8 and 1.6 mg/kg
  - Renal anomalies at 0.8 mg/kg (kidney and ureter missing) and 1.6 mg/kg (hydronephrosis and missing kidney/ureter, dilation of the renal pelvis, enlarged kidney)
  - Increased incidence of skull damage (parts of skull missing) and additional cervical rib (at 0.8 and 1.6 mg/kg)
  - Incomplete ossification of vertebral bodies

Methods

Doses:	0, 0.4, 0.8, or 1.6 mg/kg
Frequency of dosing:	Daily from pc day 6 to pc day 20
Dose volume:	5 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Suspensions were prepared daily using powder and tap water
Species/Strain:	Female Himalayan rabbits of the strain CHBB:HM bred by (b) (4) • Between 127 and 179 days old at initiation of mating • Weight range 2.13 to 3.06 kg at initiation of mating
Number/Sex/Group:	• Main groups: 24 female rabbits/dose • TK groups: 3 female rabbits/dose

Method notes:

- Males were used for mating only and were not treated
- The negative control group received a placebo powder (BAY 73-4506 PLAC POWD 000). The Analytical Report indicates that the placebo powder did not contain regorafenib, but did not identify the composition of the placebo powder or compare it's composition to regorafenib.
- Pregnant rabbits were dosed from pc day 6 to pc day 20, and fetuses were delivered by cesarean section on pc day 29.
- The report includes extensive historical control group data were provided in the report (report pages 401-802; the report is 951 pages total)

Observations and Results

Mortality

- One female at 0.8 mg/kg (# 677) was found dead on pc day 20. The authors consider this finding incidental to treatment.
- Based on the dramatic reduction in food consumption beginning at the start of treatment, this reviewer suspects that a gavage error (physical trauma) occurred; however, a treatment-related effect (i.e. this animal being more sensitive than the others at 0.8 or 1.6 mg/kg) cannot be ruled out
    - This one female exhibited no remarkable clinical signs from D0 to D8, then reduced water consumption (days 9-12, 15-16 and 20), reduced amount of feces (day 9-end),decreased urination (D10, D19-end), discolored urination, soft feces (day 11, 17-19), hypoactivity (on day 19), and was cold to touch (D20).
    - This animal also exhibited severely decreased food intake and severe body weight loss
    - Gross necropsy of this animal found mottled liver and lungs, large intestine with gaseous content, and thoracic cavity with fluid contents
    - (authors consider this incidential, due to a lack of an apparent dose-response)

Clinical Signs

- Clinical signs were evaluated twice daily during the week, and once daily on weekends and holidays (i.e. once on D21 and D29)
- Aside from the early decedent (# 677), no treatment-related clinical signs were apparent

Body Weight

- Body weight was measured on pc day 0 and daily from pc day 6 to pc day 29 (for main-group animals) or pc day 21 (TK animals).
- Corrected body weight gain was calculated as the D29 weight minus the uterus weight
- Body weight loss was observed in two of the 4 females that exhibited total resorptions at 1.6 mg/kg. No treatment-related weight changes were apparent in the females with viable fetuses

- The authors conclude, and this reviewer concurs, that regorafenib did not directly decrease body weight in the treated adults. with the observed total resorptions

Feed Consumption

- Animals were individually caged, and feed intake was measured by weighing the food every 3 days. Water intake was assessed qualitatively (and reported under clinical signs).
- Authors report no treatment-related effects of food consumption, water consumption; however, a treatment-related transient effect on food-intake was clearly observed. After the beginning of daily dosing on pc day 6, statistically significant increases in food consumption were observed from D6-9 (all regorafenib-dose groups, no dose-response apparent) and from D9-12 (high-dose only).
- The early decedent consumed only 39% of the food from D6-9 that it had prior to the initiation of dosing (D3-6), and this animal continued to exhibit low food consumption (no food consumed D9-12, 10% of pre-dose consumption from D12-15, 15 to 20% of pre-dose consumption from D15-D20)
- From the report (page 33):

Table 94: The initiation of regorafenib-dosing was associated with a transient increase in food consumption in pregnant rabbits (report # PH-36036)

Dose (mg/kg b.w./day)	0	0.4	0.8	1.6
mean feed intakes (g/animal/day)				
days 0 - 3 p.c.	96.4	102.3	95.6	103.9
days 3 - 6 p.c.	100.1	101.5	94.2	98.5
days 6 - 9 p.c.	91.9	107.0*	115.0**	113.3**
days 9 - 12 p.c.	85.3	97.6	98.0	103.4**
days 12 - 15 p.c.	76.3	89.0	86.5	87.9
days 15 - 18 p.c.	81.0	87.4	95.2	95.8
days 18 - 20 p.c.	91.5	90.8	93.3	90.1
days 20 - 21 p.c.	87.8	85.6	89.8	82.1
days 21 - 24 p.c.	84.2	65.9	74.6	71.0
days 24 - 27 p.c.	80.2	83.7	86.5	80.7
days 27 - 29 p.c.	88.5	90.4	92.4	83.2

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

Toxicokinetics

- Plasma concentrations of BAY 73-4506 (regorafenib), BAY 75-7495 (M-2 metabolite) and BAY 81-8752 (M-5 metabolite) were measured. Blood was collected from the adult mothers on pc days 6 and 20: pre-dose and 1, 2, 4, 7 and 24 hours post-dose.
- For regorafenib: authors considered the results consistent with a linear dose-response. Comparing pc day 6 with pc day 20, accumulation of regorafenib is apparent (~200% at the low- and mid-doses, ~125% at the high-dose)
- For BAY 75-7495: authors considered the results approximately dose-proportional. The C_{max} dropped markedly from pc day 6 to pc day 20 (from ~97% to ~70%), and accumulation was apparent (2.6-fold)
- For BAY 81-8752, the plasma concentrations were too low to evaluate a potential-dose response.

- Blood samples were analyzed for regorafenib, M-2 and M-5. Table 95 provides an 'equivalent dose' for reference (i.e. mothers were only dosed with regorafenib). From the report (pages 823-828):

Table 95: PK summary for pregnant rabbits (report # PH-36036)

		Regorafenib (BAY 73-4506)			BAY 75-7495 (M-2)			BAY 81-8752 (M-5)		
Dose of regorafenib	mg/kg	0.4	0.8	1.6	0.4	0.8	1.6	0.4	0.8	1.6
Equivalent dose of M-2	mg/kg				0.413	0.826	1.65			
Equivalent dose of M-5	mg/kg							0.402	0.803	1.61
PC day 6										
$AUC_{(0-24)}$	µg*h/L	1845	4492	14616	nc	nc	151	nc	nc	nc
$AUC_{(0-24)norm}$	kg*h/L	4.61	5.62	9.13	nc	nc	0.092	nc	nc	nc
C_{max}	µg/L	97.3	281	805	nc	nc	7.61	nc	nc	2.96
$C_{max.norm}$	kg/L	0.243	0.351	0.503	nc	nc	0.0046	nc	nc	0.002
$C(24)/C_{max}$	%	65.4	51.8	64.2	nc	nc	96.8	nc	nc	100
T_{max}	H	4	3.17	3.04	nc	nc	10.6	nc	nc	24.0
MR-1	%				nc	nc	0.917	nc	nc	0.365
MR-2	%				nc	nc	1.0	nc	nc	nc
PC day 20										
$AUC_{(0-24)}$	µg*h/L	3920	8760	18546	15.0	147	396	nc	nc	132
$AUC_{(0-24)norm}$	kg*h/L	9.80	10.9	11.6	0.0362	0.178	0.240	nc	nc	0.082
C_{max}	µg/L	212	525	986	2.74	7.89	18.6	nc	2.24	6.05
$C_{max.norm}$	kg/L	0.530	0.657	0.616	0.0066	0.0096	0.011	nc	0.0028	0.0038
$C(24)/C_{max}$	%	56.3	54.6	63.0	nc	60.3	82.1	nc	nc	92.4
T_{max}	H	4	3.1	3.17	5.81	4.82	4.82	nc	2.00	7.0
RA1	%	218	187	187	nc	nc	244	nc	nc	204
RA3	%	212	195	195	nc	nc	262	nc	nc	nc
MR-1	%				1.25	1.45	1.83	nc	0.425	0.610
MR-2	%				nc	1.63	2.07	nc	Nc	0.709

"Equivalent dose" of M-2 and M-5 based on stoichometric correction
nc = not calculated by the authors
MR-1 %: is the metabolic ratio in terms of $c_{max.norm}$ compared to regorafenib, as reported by the authors
MR-2 %: is the metabolic ratio in terms of $AUC_{0-24norm}$ compared to regorafenib, as reported by the authors
RA1: the ratio of day 20 / day 6 for $c_{max.norm}$
RA3: the ratio of day 20 / day 6 for $AUC_{0-24norm}$

Stability and Homogeneity

Analysis of the test substance was conducted; the authors report that the stability and homogeneity were confirmed in samples of 0.05, 0.1 and 1.6 mg/ml (within 96 to 102% of nominal) for two preparations (made May 7, 2007 and June 13, 2007 respectively, measured after 6 hours at room temperature to verify stability).

Necropsy

- Gross necropsy was performed on pc day 29 (main-group animals) or pc day 21 (TK animals). "Necropsy was performed without knowledge of treatment groups" for the main-group animals (report page 25).
- Aside from the placentas, no treatment-related findings were apparent. The authors considered two lesions to be incidental, based on historical control data:
  - One female at 1.6 mg/kg exhibited a spongy liver
  - One female at 0.4 mg/kg exhibited uterine cysts
- Placenta weight was higher at 1.6 mg/kg. The authors conclude, and this reviewer concurs, that the effect is secondary to the reduced litter sizes at 1.6 mg/kg
- The 1.6 mg/kg group exhibited a higher incidence of placenta changes: hardened, coarse grained placentas, engorged placenta

Table 96: Regorafenib caused adverse placental changes in pregnant rabbits (report # PH-36036)

Dose (mg/kg/day) → Endpoint ↓		0	0.4	0.8	1.6
# of litters evaluated		18	17	17	15
Parts of placenta hardened and light discolored	Litter incidence (N)	3	4	7	7
	Litter incidence (%)	16.7%	23.5%	41.2%	46.7%
	Fetal incidence (N)	5	8	10	12**
	Fetal incidence (%)	3.8%	6.5%	7.2%	20%
Placenta coarse grained	Litter incidence (N)	1	0	0	3
	Litter incidence (%)	5.6%	0	0	20%
	Fetal incidence (N)	1	0	0	3
	Fetal incidence (%)	0.8%	0	0	5%
Placenta engorged	Litter incidence (N)	0	0	0	1
	Litter incidence (%)	0	0	0	6.7%
	Fetal incidence (N)	0	0	0	1

Cesarean Section Data

- Cesarean sections were formed at necropsy (D29 for main-group animals, D21 for TK animals).
- For main-group animals (D29): evaluation of corpora lutea and implantations, uterus weight, individual weight and appearance of placenta, number of live fetuses, number of early resorption (only implantation site visible), number of late resorptions (fetal or placental remnant visible), number of dead fetuses (dead but without maceration)
- For satellite animals (D21), Cesarean sections were performed, but the only endpoint was evaluating the existence of implantations

- No treatment-related effects apparent for fertility rate, corpora lutea, preimplantation losses or implantation sites (consistent with the study design – i.e. initiating dosing after implantation)
- For the high-dose group (1.6 mg/kg), four females (4/20, 20%) exhibited total resorptions
- The authors concluded, and this reviewer concurs, that postimplantation losses were severely increased at 1.6 mg/kg
- The authors consider the increase in post-implantation loss at 0.8 mg/kg to be incidental, and this reviewer concurs
- The authors consider the decrease in males at 1.6 mg/kg to be incidental. This reviewer disagrees – the data indicate that male fetuses are more sensitive to regorafenib.

Table 97: Selected reproductive endpoints from regorafenib-treated pregnant rabbits (report # PH-36036)

Dose (mg/kg/day) → Endpoint ↓	0	0.4	0.8	1.6
General reproductive data				
# of mated females	20	20	20	20
# of mated females evaluated	20	19	18	20
# of mated females with implantations	19	17	17	19
% of mated females with implantations (i.e. fertility index)	95%	89.5%	94.4%	95%
Mean # of corporal lutea (per female with implantation sites)	8.7	8.6	10.1	9.1
Mean # of preimplanatation loss (per female with implantation sites)	1.0	0.5	0.8	0.4
Mean # of implantations (per female with implantation sites)	7.7	8.1	9.2	8.6
Mean # of implantations (per female with viable fetuses)	7.9	8.1	9.2	8.5
Gestation rate data				
# of females with viable	18	17	17	15

fetuses on D29				
% of females with implantations that also bore viable fetuses on D29 (i.e. gestation index)	94.7%	100%	100%	78.9%
# of abortions	0	0	0	0
# of females with total resorptions	1	0	0	4
Parameters of intrauterine development				
Mean placental weight (g)	4.58	4.78	4.30	5.14*
# of live fetuses per group	131	123	139	60
Mean # of fetuses per female	7.3	7.2	8.2	4.0**
Mean % of implantations per group that resulted in live fetuses (counting only females with implantations)	91.6%	89.8%	88.5%	46.9% **
Mean % of implantations per female that resulted in live fetuses (counting only females with implantations)	91.1%	90.8%	88.9%	47.8% **
# of dead fetuses	0	0	0	0
Total # of postimplantation losses per group[a,b]	15 (12)	14 (14)	18 (18)	104** (68**)
Mean # of post-implantation loss per female[a]	0.8 (0.7)	0.8 (0.8)	1.1 (1.1)	5.5 (4.5)**
% male of viable fetuses per female	49.3%	48.9%	46.5%	35.0%
Mean fetal weight per female (g)	37.15	38.81	38.12	39.39

[a]The first number is the # of post-implantation losses including females with implantations; the number in parenthesis is the # of losses only for females with viable fetuses.
[b]Early resorptions were only observed in 1 litter of the control group (3 losses) of females with implantations; therefore the incidence of late resorption is essentially similar to the incidence of post-implantation loss
* Statistically significant, $p < 0.05$
** Statistically significant, $p < 0.01$

Offspring Data

- For the main-group animals, on pc day 29, fetal endpoints measured include: sex of live fetuses; individual weight of live fetuses; external findings; findings in the abdominal, pelvic, and thoracic organs and the brain; findings in the skeletal system.
- Skeletal findings were assessed without knowledge of treatment groups
- As noted in Table 97 above, fetal weights were higher at 1.6 mg/kg. The authors concluded, and this reviewer concurs, that the effect is secondary to smaller litter size.

- Treatment-related malformations were observed at 0.8 and 1.6 mg/kg.
- The authors consider 0.4 mg/kg to be a NOAEL for fetal malformations, and consider the malformations observed at this dose-level to be incidental, because they are within the historical control range reported in this study report. This reviewer disagrees – the incidence of heart malformation at 0.4 mg/kg appears treatment related.
    - Note: the historical control data reports observing cardiac septal defect in 0 to 1.4% of control rabbits (report pages 524 – 538)
- From report pages 45-46:
    - At 0.4 mg/kg: "malposition of forelimb(s) , cardiac ventricular septal defect, and of the axial skeleton (sternebrae, caudal vertebral bodies, and ribs)"
    - At 0.8 mg/kg: "increased incidences of malposition of forelimb(s) or hind limb(s), malformation of the heart and major vessels, urinary system (missing kidney and ureters), and skeleton (skull bones, caudal vertebral bodies"
        - The authors consider the incidence of asymmetrical position of the caudal vertebral bodies to be incidental, and this reviewer concurs
        - The authors consider the other findings to be treatment-related
    - At 1.6 mg/kg: malformations "of the urinary system (hydronephrosis, missing kidney and ureter, and small, deformed, and malpositioned kidney), of the heart (cardiac ventricular septal defects), and of the axial skeleton (sternebrae, vertebrae, and ribs)"

Table 98: Selected malformation data (external and visceral malformations) from the rabbit embryofetal study (report # PH-36036)

Dose (mg/kg/day) → Endpoint ↓		0	0.4	0.8	1.6
# of litters evaluated	N	18	17	17	15
# of fetuses evaluated	N	131	123	139	60
# of live fetuses evaluated	N	131	123	139	60
# of dead fetuses evaluated	N	0	0	0	0
Total malformations	Fetal incidence	17 (13.0%)	13 (10.6%)	26 (18.7%)	29** (48.3%)
	Litter incidence	13 (72.2%)	8 (47.1%)	10 (58.8%)	13 (86.7%)
Forelimb malposition (ventral flexure at the region of wrist)	Fetal incidence	4 (3.1%)	5 (4.1%)	8 (5.8%)	1 (1.7%)
	Litter incidence	3 (16.7%)	4 (23.5%)	4 (23.5%)	1 (6.7%)
Hindlimb malposition	Fetal incidence	0	0	1 (0.7%)	0
	Litter incidence	0	0	1 (5.9%)	0
Cardiac ventral septal defect	Fetal incidence	0	4 (3.3%)	1 (0.7%)	4* (6.7%)
	Litter incidence	0	3 (17.6%)	1 (5.9%)	4 (26.7%)
Tricuspidal atresia, cardiac	Fetal incidence	0	0	3	1

Dose (mg/kg/day) → Endpoint ↓		0	0.4	0.8	1.6
ventricular septal defect, small right ventricle, large left ventricle, small pulmonary artery, with/without aorta in origin enlarged	Litter incidence	0	0	3	1
Hydronephrosis	Fetal incidence	0	0	0	11** (18.3%)
	Litter incidence	0	0	0	7** (46.7%)
Kidney and ureter are missing	Fetal incidence	0	0	2 (1.4%)	11** (18.3%)
	Litter incidence	0	0	2 (11.8%)	8** (53.3%)
Kidney small, deformed and malpositioned (beside urinary bladder)	Fetal incidence	0	0	0	7** (11.7%)
	Litter incidence	0	0	0	6** (40.0%)
Dilation of the renal pelvis	Fetal incidence	0	0	0	5** (8.3%)
	Litter incidence	0	0	0	2 (13.3%)
Kidney enlarged	Fetal incidence	0	0	0	1 (1.7%)
	Litter incidence	0	0	0	1 (6.7%)
Liver – whitish discoloration	Fetal incidence	0	0	0	1 (1.7%)
	Litter incidence	0	0	0	1 (6.7%)
Liver spongy	Fetal incidence	0	1 (0.8%	1 (0.7%)	0
	Litter incidence	0	1 (5.9%)	1 (5.9%)	0

** statistically significant, $p < 0.01$

Table 99: Selected fetal skeletal observations from the rabbit embryofetal study (report # PH-36036)

Dose (mg/kg/day) → Endpoint ↓	0	0.4	0.8	1.6
# of fetuses evaluated	131	123	139	60
Sternebrae – fusion	4 (3.1%)	8 (6.5%)	31** (22.3%)	14** (23.3%)
Cervical ribs- right 7th rib present	2 (1.5%)	0	6 (4.3%)	8** (13.3%)
Cervical ribs- left 7th rib present	1 (0.8%)	1 (0.8%)	3 (2.2%)	4 (6.7%)
13th rib – right – comma shaped (either attached or detached)	0	2 (1.6%)	2 (1.4%)	0

Dose (mg/kg/day) → Endpoint ↓	0	0.4	0.8	1.6
# of fetuses evaluated	131	123	139	60
Cervical vertebral body – 3rd incompletely ossified	0	0	0	4** (6.7%)
Cervical vertebral body – 4th incompletely ossified	0	0	0	5** (8.3%)
Thoracic vertebral bodies – 6th dumbbell shaped	0	0	0	1 (1.7%)
Presacral vertebrae- one supernumerary	0	0	0	3 (5%)
Caudal vertebrae – 8th right and left arches present	112 (85.5%)	108 (87.8%)	113 (81.3%)	46 (76.7%)
Caudal vertebrae – 14th body present	131 (100%)	122 (99.2%)	133 (95.7%)	56* (93.3%)
Parts of skull missing[a]	1	0	2	1

[a]Parts of skull missing was observed with multiple other malformations (not selected for tabulation)
* statistically significant, $p < 0.05$
** statistically significant, $p < 0.01$

Prenatal and Postnatal Development

No pre- and post-natal development studies were submitted to NDA 203085.

10 Special Toxicology Studies

The Applicant submitted the following two studies to evaluate the potential for regorafenib-mediated phototoxicity:

PH-35625	BAY 81-8752: Study of Photoreactive Potential in Mice (LLNA/IMDS). Study No. T 6079361
PH-35785	BAY 75-7495, BAY 81-8752: *In Vitro* 3T3 NRU (neutral red uptake) Phototoxicity Assay. Study No. T 8079020

These studies are reviewed briefly.

The *In vitro* 3T3 NRU (neutral red uptake) phototoxicity assay was conducted by Bayer Healthcare AG, BSP-GDD-GED-General Toxicology, Wuppertal, Germany. Mouse fibroblasts (Balb/c 3T3.A31) were treated for 1 hr with test compound (regorafenib) at concentrations ranging from 0.01 to 50 µg/mL or with chlorpromazine (positive control). After treatment, the test plates but not the control plates were treated with UVA-irradiation with an intensity of 5 J/cm^2 for 50 minutes. Cell viability (EC_{50}) was quantified by neutral red uptake after 24 hours of treatment (Speilman et al 1998, Toxicology *in*

vitro 12: 305-327). In this *in vitro* assay chlorpromazine hydrochloride (positive control) demonstrated clear phototoxicity with a photo-irritation factor PIF of 16, BAY 73-4506 had a (PIF) of 3. Under the conditions of the assay a PIF >2 and <5 predicts probable phototoxicity.

Further evaluation of the phototoxic potential of BAY 73-4506 carried out in an in vivo study in mice (Bayer Healthcare AG, BSP-GDD-GED-General Toxicology, Wuppertal, Germany). NMRI mice (25-32 g body weight, approximately 7 weeks old) were treated orally with 0, 1, 5, 20 mg/kg BAY 73-4506 and then exposed to UVA irradiation for 30 minutes at 20J UV-A/cm^2. One group was treated with 20 mg/kg BAY 73-4506 without UVA irradiation. Two groups were treated with 200 mg/kg of a positive control (BAY V 1749, Sparfloxacin), ±UVA irradiation. Animals were treated for three consecutive days and euthanized on day 4 (one day after the last treatment). The appropriate organs (ears and auricular lymph nodes) were removed.

BAY 73-4506 did not affect ear weights and ear swelling compared to vehicle treated and UVA irradiated animals. BAY 73-4506 did not show photoreactive potential regarding the weight or cell counts of the draining lymph nodes in mice after oral administration and UVA irradiation. The positive control treated animals demonstrated the sensitivity of the test method.

11 Integrated Summary and Safety Evaluation

Regorafenib is a kinase inhibitor with multiple cellular receptor and intracellular targets. In order to support the clinical use of regorafenib for the treatment of metastatic colorectal cancer, the Applicant submitted *in vitro* and *in vivo* studies investigating the pharmacology, pharmacokinetics, safety pharmacology, and toxicology of both regorafenib and two major active human metabolites, M-2 and M-5. In patients both M-2 and M-5 were present at high levels following treatment with regorafenib; however, neither metabolite was present at high levels in rats or dogs, the major species used for toxicological analysis during regorafenib development. Thus, M-2 and M-5 were examined independently in a number of pharmacology and toxicology studies to further understand the potential for regorafenib-mediated toxicity in patients.

Pharmacology:

The pharmacology and mechanism of action for regorafenib were studied in a series of non-GLP *in vitro* and *in vivo* assays. Regorafenib and the M-2 and M-5 metabolites were tested in a biochemical screening assay against 175 kinases at a concentration of 1 µM. Following the initial screening, IC_{50} values were determined for kinases with decreased activity following incubation with regorafenib at the 1 µM concentration. A large number of kinase proteins were inhibited by regorafenib at concentrations below the clinical C_{max} concentration of regorafenib associated with the 160 mg dose. Kinases inhibited at the lowest concentrations of regorafenib in either biochemical assays or cellular assays examining the phosphorylation of downstream

targets included RET and several RET variants, PTK5, VEGFR-1,-2, and -3, FGFR-1 and -2, DDR2, SAPK2, Lyn, Tie2, Abl, TrkA, EphA2, KIT and several Kit variants, c-RAF, BRAF, and $BRAF^{V600E}$. With each of these proteins, both the M-2 and M-5 metabolites showed inhibitory activity that was similar to and occasionally higher than the regorafenib parent compound. Regorafenib and/or the M-2 and M-5 metabolites had IC_{50}s of <100 nM against kinase activity for each of the proteins in one or more *in vitro* assays. At the Cmax observed in patients at steady state of 3.9 µg/mL, regorafenib concentration is approximately 807 nM, though the drug is highly protein bound, thus, inhibition constants of ≤ 100 nM are of potential clinical relevance. These pharmacology studies demonstrate that regorafenib is a kinase inhibitor with multiple targets. The targets of regorafenib as defined in these assays are well established as important for normal physiology and cell activity; some, such as the vascular endothelial growth factor receptor (VEGFR) family, also have well established roles in pathogenic processes such as tumor angiogenesis.

The potential of regorafenib to inhibit proliferation and mitosis and to induce apoptosis was evaluated *in vitro* in a panel of 25 human colorectal cancer cell lines and seven pancreatic cancer cell lines. Under the conditions of this multiplex assay, effects of regorafenib on proliferation were unclear; regorafenib was able to induce apoptosis in four of the cell lines. In an *in vivo* study examining the effects of regorafenib exposure on angiogenesis, female Fisher 344 rats were intramuscularly implanted with a rat brain glioblastoma cell line. Effects on angiogenesis were determined using a magnetic resonance imaging (MRI) technique in which the MRI signal versus time curve for 360 seconds was measured. This signal was dependent on tumor blood vessel volume and total permeability surface of the tumor blood vessels, thus, inhibition of angiogenesis following test-article administration was expected to result in decreased signal compared to control article. Administration of regorafenib or the M-2 metabolite either by intravascular or oral routes led to similar decreases in MRI signal. When rats were administered regorafenib or M-2 for 4 consecutive days, the decreased MRI signal persisted up to 2 days following the final dose. An *in vivo* experiment specifically examining anti-VEGF activity was also conducted with regorafenib and the M-2 and M-5 metabolites. Groups of 6 catheterized male Wistar rats were administered iv with control vehicle, 0.1, or 1 mg/kg of regorafenib, 1 mg/kg of M-2, or 1 mg/kg of M-5, followed by 9 µg/kg of recombinant human VEGF 10 minutes later. Blood pressure (systolic and diastolic) was then measured for 25 minutes. VEGF induced an immediate reduction of blood pressure (25 to 40 mm Hg) in control rats; however, previous administration of 1 mg/kg of regorafenib or either metabolite prevented this decrease in blood pressure demonstrating a clear effect of regorafenib on inhibition of VEGF activity.

The activities of regorafenib, M-2, and M-5 were also examined xenograft experiments using nude mice implanted with human tumor cell lines including several colorectal carcinoma (CRC) lines and a breast carcinoma line. In both the HT-29 CRC model and the MDA-MB-231 breast cancer model, treatment of implanted mice with regorafenib, M-2, or M-5 resulted in dose-dependent inhibition of tumor growth compared to negative control treated animals. Treatment of these animals by oral gavage daily for 27 days at dose levels of 3 or 10 mg/kg did not cause significant weight loss and there were no clear differences in anti-tumor activity apparent among

regorafenib, M-2, and M-5. In three out of four additional CRC models, regorafenib treatment at the 10 mg/kg dose level resulted in slower tumor growth than in control treated animals. Additional studies were conducted using Balb/c mice implanted with syngeneic mouse hepatoma cells or 4T1 breast cancer cells. When hepatoma bearing mice were administered regorafenib at a dose level of 10 mg/kg daily, there was increased survival compared to either untreated or vehicle treated controls. In 4T1 breast cancer implanted Balb/c mice, daily administration of 10 mg/kg regorafenib slowed tumor growth and decreased the number of tumor metastases compared to control-treated animals. These studies demonstrate *in vivo* anti-tumor activity of regorafenib in these models.

Safety Pharmacology:

Sixteen safety pharmacology studies examining regorafenib and its metabolites were submitted to NDA 203085 to support safety. Cardiovascular safety was assessed in a series of *in vitro* and *in vivo* investigations. Regorafenib, M-2, and M-5 were each examined in the *in vitro* hERG assay. Human embryonic kidney cells (HEK 293 cells) were transfected to express the human ether-a-go-go-related gene (hERG) potassium (K^+) channel and then incubated with regorafenib, M-2, or M-5 to assess the effects of the test article on the ability of the channel to conduct a signal. Regorafenib itself demonstrated weak potential for inducing QTc prolongation either in the hERG assay where incubation with the drug resulted in potassium channel inhibition at an IC_{50} of 27 µM or in a subsequent Purkinje fiber assay where no prolongation of action potential duration was observed. Both the M-2 and M-5 metabolites, however, were potent hERG inhibitors with IC_{50}s of 1.1 µM and 1.8 µM, respectively. ECG endpoints were included in 1-month, 13-week, and 52-week general toxicology studies performed in Beagle dogs administered daily oral doses of regorafenib. *In vivo* cardiovascular safety studies were also performed in a series of single dose investigations in anesthetized dogs. While a trend was detected towards a decreased heart rate at the high dose level of 16 mg/kg (320 mg/ m^2) in dogs at the 51 week time-point, no significant effects were apparent on ECG parameters including QTc, respiratory parameters, or hemodynamic parameters including body temperature or blood pressure in any of these ECG studies. In an intraduodenal dosing study, dogs were treated with regorafenib at dose levels of 10, 30, and 100 mg/kg (200, 600, and 2000 mg/m^2). No clear drug-related effects on hemodynamics, ECG, or respiration endpoints were observed, though as several other drugs were used to facilitate drug delivery by the intraduodenal route in this study, subtle changes would have escaped detection. At doses ≥ 600 mg/ m^2, a small increase was observed in plasma sodium levels in these animals that may reflect the chemical/physical properties of regorafenib on electrolyte and water movement from the gut into systemic circulation. In a theoretically related finding, a single dose study examining the effect of orally-administered regorafenib administration on gastrointestinal motility in rats, regorafenib inhibited gastrointestinal motility with clear dose-dependence, though no treatment-related effects were detected on ileum contractility in a separate *ex vivo* study. Intravenous infusion studies to investigate cardiovascular safety of regorafenib, M-2, and M-5 were also conducted. In each of these studies dogs were treated with 3 infusions of 30 minutes in duration at increasing

dose levels of 0.25, 0.75, and 2.25 mg/kg with no washout period (total dose of 3.25 mg/kg over 90 minutes). There were no clearly adverse effects noted for regorafenib or either of its metabolites in these studies.

The effects of regorafenib and its major metabolites on neurobehavioral endpoints were examined in several dedicated studies. When regorafenib was administered to male rats as a single dose at the 2, 10 or 50 mg/kg (12, 60, or 300 mg/ m^2) dose level, there were no symptoms or behavioral changes noted for any animal and no changes in open-field behavior or body temperature; however as observations were only continued for 2 hours after dosing, a time before T_{max} for drug exposure, the study would have underestimated any effect of regorafenib on behavior. In similar studies examining the effects of M-2 and M-5, 6 male rats/group were administered a single dose of 1, 5, or 20 mg/kg (6, 30, or 180 mg/m^2)of either individual metabolite. Rats were observed for up to 4 hours following dose administration. While there were no findings in rats after treatment with M-5, mild effects following treatment with M-2 at the high dose of 180 mg/ m^2 occurred. At this dose, one rat displayed prone position and stereotypic licking in the open-field box, 30 minutes after dosing. Rats receiving 180 mg/m^2 of M-2 exhibited attenuation of field box habituation at the 4 hour time-point post dose administration based on distance traveled and a mild but sustained increase in body temperature compared to control rats. In a separate study, 7 male rats/ group were treated with a single dose of 2, 10, or 50 mg/kg (12, 60, or 300 mg/ m^2) regorafenib followed thirty minutes later by pentylenetratrazole to induce convulsions. Thirty minutes after the convulsion test, rats were exposed to heat and observed for hindpaw licking as a distinct pain-induced reaction followed 15 minutes later by hexobarbital injection to induce sleep. No treatment related effects of regorafenib were observed for any of these endpoints, although rats were only observed during the first 2 hours following dose administration. Overall, the effects of regorafenib and its metabolites on neurobehavioral endpoints suggest that the drug has low potential for significant effects on the central nervous system.

Single dose studies were conducted in groups of 10 male rats administered regorafenib at dose levels of 0, 2, 10, or 50 mg/kg (12, 60, or 300 mg/ m^2) to examine the effects of the drug on renal function, blood pharmacology, lipid metabolism, and glucose levels. Urine samples were collected for 2 hours following dosing with regorafenib. At the 2 hour time-point, animals were also sacrificed and blood was collected for measurement of hematology, coagulation, triglyceride and cholesterol endpoints. No treatment-related changes in any of the endpoints captured following a single dose of regorafenib, though the 2 hour time-point is shorter than the T_{max} for the drug. In the glucose study, rats were either fed ad libitum or fasted overnight preceding administration of regorafenib. Blood glucose concentration was measured at the 30 minute, and 1, 2, and 3 hour time-points for each dose group. No treatment-related effects were noted in non-fasted rats. In fasted rats, control animals showed an increase in glucose levels over time, which appeared attenuated in the treated rats, suggesting a possible effect on liver function.

Pharmacokinetics (ADME)

The metabolic profile of regorafenib in humans was compared to its profile in the Rhesus monkey, Beagle dog, Himalaya rabbit, Wistar rat, CD-1 mouse, and NMRI mouse by incubating radiolabelled regorafenib, [^{14}C]BAY 73-4506, with liver microsomes from each species for 60 or 180 minutes. Samples were then analyzed by LC-^{14}C and LC-^{14}C-UV-MS/MS. Mono-hydroxylation of the methyl group, leading to the metabolite M-3 predominated in rat and dog liver microsomal incubations. N-oxidation of the pyridine moiety, leading to the metabolite M-2 was the preferred biotransformation reaction in man, monkey, mouse, and rabbit microsome preparations.

During an *in vitro* investigation of plasma protein binding, BAY 73-4506 was stable for at least 2 hours at 37 °C in the plasma of the mouse, rat, rabbit, dog, monkey, and man. The extent of protein binding was high in all species investigated. The fraction of unbound regorafenib in plasma from 5 human volunteers ranged from 0.3 to 0.5 %. The main binding protein in human plasma was serum albumin with an unbound fraction of 2.21 %. The free fraction of BAY 73-4506 was independent of pH. The partition coefficient between erythrocytes and plasma (PE/P) ranged from 0.285 to 0.627 in dog blood and from 0.163 to 0.252 in rat blood. In human blood the partition coefficient ranged from 0.157 to 0.265.

The absorption and excretion of radioactive 2 mg/kg [^{14}C]BAY 73-4506 were investigated in intact and bile duct-cannulated (b.d.c.) male Wistar rats after single dose oral or intravenous administration. Only 0.02 % of the radioactive dose was expired as $^{14}CO_2$ within 24 h of oral administration of [^{14}C]BAY 73-4506 to rats. Thus, the radiolabel was shown to be metabolically stable. The liquid scintillation counting method used in this study did not distinguish between unchanged compound and radioactive metabolites. The radioactivity was mainly excreted via the biliary/fecal route. Seven days after intravenous administration to intact rats, 86% of the radioactivity was found in feces and 6% was excreted via urine. Similarly, seven days after oral administration to intact rats 88% of the radioactivity was found in feces and 6% in urine. Oral administration to b.d.c. rats resulted in 1.9 % of the dose excreted in urine, 34 % in bile, and 10% in feces with 27% in the gastrointestinal tract within 24 h of regorafenib administration. After intravenous administration to b.d.c. rats, fecal excretion amounted to 8.2 % of the total dose; urinary and biliary excretion amounted to 1.4 % and 43.4 %, respectively, of the administered dose in the same interval.

Whole body autoradiography was performed after administering [^{14}C]BAY 73-4506 to male albino rats (Wistar) orally and intravenously at 3 mg/kg. [^{14}C]BAY 73-4506 was also administered orally to female albino rats (Wistar) and one male pigmented rat (Long Evans). The distribution patterns of radioactivity were determined up to two hours post-dose following intravenous administration and up to seven days post-dose following oral administration. The estimation of radioactivity concentration was performed qualitatively, i.e. by visual ranking of radiographic intensities. The qualitative distribution patterns were apparently identical in both sexes by either route of administration. Radioactivity was thoroughly distributed to almost all organs and tissues. A heterogeneous pattern of radioactivity distribution was observed in some organs, e.g., adrenal gland (cortex > medulla), testes and epididymides, kidneys (cortex and outer medulla > inner medulla), skin, and liver. Blood-brain barrier penetration was low. In the pigmented Long Evans rat, there was no significant evidence of specific affinity of substance-associated radioactivity for melanin-bearing tissues. Probably, there was no

evidence of irreversible binding or retention of [^{14}C]BAY-73-4506 and or its labeled metabolites in organs and tissues of rats.

A separate distribution study was performed in pregnant rats; in these animals, at t_{max} (8-hours post-dose), the organs with the highest regorafenib exposure were the maternal liver and adrenal glands. The amniotic fluid and maternal brain showed regorafenib exposures of approximately 10% of those observed in maternal blood. Exposure in the fetal adrenal glands exceeded the maternal blood concentration. Fetal brain concentration exceeded the maternal brain concentration by 2-fold indicating significantly higher penetration of the blood/brain barrier in the fetus compared to the dam. In terms of AUC_{0-24}, the highest exposure was observed for the maternal liver and adrenal glands (cortex>medulla), kidneys (cortex, outer medulla>inner medulla), ovaries, brown adipose tissue, and amnion. Exposures in each of these tissues were between 2- and -7 fold higher than the exposure in the maternal blood. In the mammary gland exposure was approximately 2-fold higher than that in maternal blood supporting the finding of high levels of regorafenib or labeled metabolite secretion in milk observed in a dedicated excretion study. In this excretion study, milk and plasma samples were collected up to 48 h after dosing of pregnant rats with radiolabelled regorafenib. Approximately 50% of [^{14}C]BAY 73-4506 radioactivity (parent drug and radioactive metabolite) was secreted in milk within 48h after administration indicating that the administered radioactive dose was subject to mammary secretion. The half-lives of the total radioactivity in milk and plasma were 10.3 h and 9.25 h, respectively. The resulting milk/plasma ratio for AUC was 6.8 (AUC_{milk} 128000 µg-eq.h/L; AUC_{plasma}: 18800 µg-eq.h/L). These studies demonstrate that regorafenib and its metabolites are excreted at high levels in milk in rats, suggesting a high risk for neonatal exposure to regorafenib in breast milk from women taking Stivarga.

General Toxicology:

In order to better understand the safety of regorafenib, the sponsor performed chronic toxicity studies in mice (4 and 5 weeks), rats (2, 4, 13, and 26 weeks) and dogs (4, 13, and 52 weeks).

In a study reviewed in the initial IND submission, male and female **rats** were dosed orally at 1, 4 or 16 mg/kg/day (6, 24, 96 mg/m^2/day) for 4 weeks with a subsequent recovery period of 4 weeks. Administration of regorafenib (BAY 73-4506) did not result in mortality at any dose level during the dosing period but did result in the early sacrifice of 4/5 high dose group males and 1/5 high dose group females during the recovery period (Days 45-56). Drug related toxicities were observed in the GI tract, hematopoietic/lymphocytic system, heart, liver, kidneys, adrenal glands, pancreas, thyroid/parathyroid, and male and female reproductive systems. At the high dose level, females in this study presented with increased necrotic corpus lutea and atrophy in the ovaries and uterus. Males in the same dose group had increases in histopathological findings of mononuclear infiltration and cellular debris as well as decreased weight of the testes, prostate, and seminal vesicles compared to control animals. Findings in the epididymides had not resolved by the end of the recovery period. There were also findings of tubular atrophy and degeneration in the testes and atrophy of the seminal vesicles noted in high dose group recovery males. Increases in TSH along with decreases in T4 levels were observed at the high dose demonstrating significant thyroid

toxicity in these animals. There were also significant increases in AST/ALT, cholesterol and bilirubin along with hematopoietic findings including bone marrow hypocellularity, thymic atrophy and single cell necrosis, and atrophy of the spleen and lymph nodes in animals at the high dose level of 96 mg/m^2/day. Toxicokinetic samples were collected during this study. There were no apparent differences in the toxicokinetics between male and female rats. At the high dose level of 96 mg/m^2, exposure to regorafenib measured by AUC was 65 µg*h/mL on Day 1 and 81.1 µg*h/mL on day 20. These values were similar to the AUC observed in humans at steady state of 58.3 µg*h/mL.

In a GLP-compliant chronic toxicology study, Wistar rats were administered regorafenib (BAY73-4506) daily by oral gavage at dose levels of 0.1, 0.5, or 2 mg/kg (0.6, 3, or 12 mg/m^2). Doses of up to 12 mg/m^2/day for 26 weeks did not cause any mortality. There were no remarkable changes in body weight, food consumption, or ophthalmological findings. The concentrations of serum sodium, potassium, calcium, chloride, and phosphate did not change in males or females. Hematological findings included increases in erythrocytes and hemoglobin concentration in high dose animals. There was an increase in plasma AST and ALT levels in animals treated at the 12 mg/m^2 dose level. Histopathological changes considered to be treatment-related were seen in the kidneys (glomerulopathy, tubular degeneration), liver (periportal cytoplasmic basophilia), spleen (extramedullary hematopoiesis), heart (thickening of the atrioventricular valve), and thyroid gland (flattened follicular epithelium). There was no sex-related difference in exposure. Slightly more than dose-proportional increases in AUC $_{0\text{-}24}$ and C_{max} were observed between the 0.6 and 3 mg/m^2 dose levels; however, increases were dose-proportional between the 3 and 12 mg/m^2 dose levels. The exposure levels of the metabolites BAY-7495 (M-2 metabolite of BAY 73-4506) and BAY 81-8752 (M-5 metabolite of BAY 73-4506) compared to overall exposure to regorafenib were very low (less than 0.7% in terms of C_{max} and less than 1% in term of AUC $_{(0\text{-}24)}$ for each metabolite). The NOAEL value in rats determined in this study was 0.6 mg/m^2 based on the findings in the kidneys and liver at higher doses.

A 4-week repeat dose GLP-compliant toxicology study in Beagle dogs was reviewed at the time of the original IND submission. Dogs were given regorafenib daily by oral gavage at dose levels of 0, 5, 20, or 80 mg/kg (0, 100, 400, or 1600 mg/m^2). There were no drug-related early deaths during this study either during the dosing period or in the 4 week recovery period. Major target organs of drug related toxicities noted in the review of this study included the GI tract, pancreas, heart, liver, hematopoietic/lymphocytic system, kidneys, bone, and teeth. In a longer term repeat dose study of 13 weeks in Beagle dogs at the same dose levels used in the 4-week study, oral administration of BAY 73-4506 still did not result in early drug-related mortality in **Beagle dogs** (4/sex/group). Dosing in this study was, however, interrupted due to morbidity in males at the mid and high dose levels for several days during Weeks 9 to 12. Clinical observations included findings of liquid feces/blood in the feces, vomiting of whitish/yellowish mucus/foam/watery liquid/food mash, and alopecia. Swelling of eyelids and gum bleeding/anemic gum were observed in animals at the 400 and 1600 mg/m^2 dose levels. Food intake and body weight gain were also reduced in mid and high dose group animals. Electrocardiograms did not show any drug-related changes in any of the treated animals. There were no treatment dependent changes in hematology. Organ weight changes (thymus, kidneys, ovary, and testes) correlated with

histopathological findings. Histopathologically, the most prominent findings occurred in the kidneys (nephropathy, glomerulopathy) and in the lymphatic system with pronounced lesions in the spleen (increased hematopoiesis, deposition of a proteinaceous material in the white pulp), the thymus (atrophy) and other lymphatic tissue (atrophy/degeneration of the tonsils, the lymphoid follicles of the larynx, stomach, and gall bladder, the mesenteric and mandibular lymph node). Distinct degenerative lesions were also observed in the liver. Furthermore, the liver of mid and high dose group dogs showed a centrilobular hypertrophy and cytoplasmic change. In the bone marrow of the sternum and the femur an increase in fatty replacement was noticed. Toxicologically relevant findings were also seen in the digestive system (degeneration and lymphangiectasia in the small intestine as well as increased cellular infiltration in the cecum and colon) of mid and high dose group animals. The male and female reproductive systems were also targets of regorafenib-mediated toxicity. Males at dose levels ≥ 400 mg/m^2 had histopathological findings of retarded maturation of the testes along with aspermia/oligospermia in the epididymides. In females, findings of reduced follicular development and increased follicular degeneration were noted at the same dose levels. Treatment with BAY 73-4506 also affected the skeletal system with dentin alterations in the teeth at doses ≥ 400 mg/m^2 In addition at the high dose level there were changes in cartilage and increased findings of persistent femoral epiphyseal growth plate compared to control or lower dose group animals. In some animals the growth plate was also thickened. Theses alterations are known to occur in growing dogs treated with VEGF inhibitors. There was no evidence of sex-related differences in exposure in this study. Exposure to BAY 73-4506 measured by AUC $_{0\text{-}24}$ and C_{max} increased dose-dependently but less than dose proportionally. After repeated dosing, considerable decreases in AUC and C_{max} were observed on week 13 compared to the values obtained on Day 1. At the Day 1 time-point doses of 400 and 1600 mg/m^2 resulted in regorafenib exposures in dogs of approximately 71 and 130%, respectively, of the human exposure measured by AUC at steady state; by the Week 13 time-point administration at the same dose levels resulted in exposures of only 43 and 83%, respectively, of the human exposure. The concentration of BAY 75-7495 (M-2 metabolite of BAY 73-4506) compared to overall exposure was very low (less than 2%).

A systemic chronic toxicity study in **Beagle dogs** was also carried out by intragastric administration of BAY 73-4506 at doses of 1.0, 4.0, and 16.0 mg/kg (20, 80, and 320 mg/m^2) daily for 52 weeks. All animals survived until scheduled termination of the study. Clinical signs included dose- and duration-dependent increases in hair loss, skin/mucosa reddening, diarrhea, discolored feces, halitosis, vomiting, hyperactivity, head shaking, tremor, and irregular respiration. High dose animals showed body weight loss over the whole treatment period. The test compound did not affect eyes, heart rate, body temperature or nervous system functions in a dose or time dependent manner. Compound related increases in glucose, ALP, and AST were observed. BAY 73-4506 did not affect triiodothyronine (T3) and thyroxine (T4) but a time-dependent increase (up to 7-fold) in thyroid stimulating hormone (TSH) was observed in high dose animals from week 6 onwards. Compound related gross findings were observed in the gallbladder (jelly like), kidney (enlarged pale), skeletal muscle (atrophy), and skin (alopecia). At the end of the treatment period compound-related decreases in thymus and ovary weights, along with increases in kidney weight were observed. These

findings in thymus and ovary correlated microscopically with atrophy. Histopathological findings were noted in the kidney (tubular degeneration/regeneration, glomerulosclerosis), liver (mononuclear cell infiltration), gallbladder (lymphoid follicles), skin (pigment clumping), pituitary gland (perivascular mononuclear cell infiltration), lung (alveolar macrophages), thyroid gland (mineralization), male and female reproductive tract (degeneration in ovaries and epididymides), bone marrow (granulopoiesis, erythropoiesis), and thymus (atrophy). Exposure to BAY 73-4506 increased dose-dependently but the observed increases in $AUC_{(0-24)}$ and C_{max} were less than dose-proportional comparing low, medium, and high doses. The exposure of the M-2 and M-5 (active metabolites of BAY 73-4506) at the low, medium and high dose were regarded as minor (below 2.5%).

BAY 75-7495 and BAY 81-8752 are the M-2 and M-5 metabolites of BAY 73-4506 (regorafenib). Because these major active metabolites were each present in humans at levels as high as 33% of the total exposure of regorafenib, but made only minor contributions to total exposure in rats and dogs, the major species used for toxicological assessment during the development of regorafenib, To characterize the toxicity of these active metabolites, M-2 and M-5 were administered orally at dose levels of 1, 5 or 20 mg/kg/day (3, 15, or 60 mg/m^2/day) for 4 weeks in separate experiments to male and female **CD-1 mice**. Satellite groups were treated for 3 weeks for toxicokinetics. In the M-5 study, one of 19 toxicokinetic group males at the 15 mg/m^2 dose level was found dead on day 14. One male and one female in the 60 mg/m^2 satellite groups were found dead on days 14 and 21, respectively. The cause of death for these animals was not investigated. Clinical signs, body weight gain and food consumption were not affected up to 60 mg/m^2/day. Plasma bilirubin was increased in both sexes at 60 mg/m^2. Histopathology revealed slightly dilated bone marrow sinuses and a slight hypocellularity (femur and sternum) in two high dose males. There was an increase in dentin alteration in 60 mg/m^2 females. These finding were seen in the upper and lower jaws. There are similar findings with other VEGF inhibitors. On day 21, AUC_{0-24} and C_{max} values increased dose-dependently with no evidence of sex-related differences in exposure. An increase in administered doses was accompanied by a more than dose-proportional increase in both AUC_{0-24} and C_{max}.

In the M-2 study, there were no treatment-related effects on mortality, clinical signs, or body weights. Food consumption at 15 and 60 mg/m^2 was decreased in both sexes compared to control animals. Hematological investigation revealed a statistically significant increase in reticulocytes in males at 60 mg/m^2. Aspartate aminotransferase and alanine aminotransferase values were mildly increased (~20%) in high dose males only and bilirubin was increased in both sexes at 60 mg/m^2. There were increases in spleen weight, both absolute (20-31%) and relative to body weight (17-33%), in males at doses ≥ 15 mg/m^2. An increase in the weight of ovaries relative to body weight was observed at 3 mg/m^2 but there was no dose-response associated with this finding making its toxicological relevance is questionable. At 60 mg/m^2 both males (9/10) and females (10/10) there were findings of alterations in dentin in the incisor teeth of the upper and lower jaw. Increased extramedullary hematopoiesis was seen in the liver of high dose females. Lymphoid depletion of the spleen was observed in one high dose male. There were no sex-related differences in exposure. The C_{max} and AUC_{0-24} values

were decreased on Day 30 compared to Day 1. Microscopic evaluation of slides prepared for cytogenetics evaluation was not performed.

The Applicant also conducted *in vivo* and *in vitro* studies to assess the phototoxic potential of regorafenib. While an *in vitro* neutral red uptake assay suggested that regorafenib is a probable phototoxic agent, an *in vivo* assay in regorafenib-treated mice showed no affect on skin irritation or swelling after UV exposure and no immune reaction in draining lymph nodes from the UV exposed sites.

Genotoxicity:

The genotoxic potential of regorafenib was assessed in a large number of *in vitro* and *in vivo* assays. This set of experiments examined the genotoxic activity of not only the regorafenib parent compound, but also the M-2 and M-5 metabolites and a number of impurities associated with the total drug product. BAY 73-4506 (regorafenib) at concentrations of up to 3 mg/plate with or without metabolic activation did not show any mutagenic activity in TA 1535, TA 100, TA 1537, TA 98, and TA102 strains (Ames test) when compared with negative controls. The positive controls sodium azide, nitrofurantoin, 4-nitro-l ,2-phenylene diamine, mitomycin C, cumene hydroperoxide and 2-aminoanthracene all demonstrated marked mutagenic effects, measured by biologically relevant increases in mutant colonies. BAY 73-4506 at concentrations up to 90 µg/mL did not show any clastogenic potential in a chromosome aberration test in Chinese hamster V79 cells in either the absence or presence of metabolic activation. There was no indication of a clastogenic effect of intraperitoneally administered BAY 73-4506 GRAN 10% 010 at doses up to 2 g/kg (6 g/m^2) in the *in vivo* micronucleus test in the male mice. BAY 81-8752 (human metabolite of BAY 73-4506, M-5) at concentrations of up to 5000 µg/plate was also non-mutagenic with and without metabolic activation in the bacterial reverse mutation test and did not cause any biologically relevant or statistically significant increases in the numbers of aberrant metaphases in Chinese hamster V79 cells. BAY 75-7495 (human metabolite M-2 of BAY 73-4506), on the other hand, while non-mutagenic in the presence or absence of metabolic activation in the Salmonella/microsome test did induce biologically relevant increases in number of aberrations in Chinese hamster V79 cells at 24 µg/ml in the presence of metabolic activation. As the M-2 metabolite represents a high percentage of the overall regorafenib in humans, this finding is relevant to the safety of the drug.

Five impurities were analyzed for genotoxicity: (b) (4) at concentrations of up to 5 mg/plate in the presence or absence of metabolic activation (S9 mix) did not show mutagenic potential in the bacterial reverse mutation test (Ames test). (b) (4) showed bacteriotoxic effects but did not induce mutagenic effects at 5000 µg per plate in the same assay. Rats treated with (b) (4) did not show biologically relevant or statistically significant increases in micronucleated polychromatic erythrocyte (PCE) or normochromatic erythrocyte (NCE) cell counts as compared to vehicle control treated animals. A comet assay performed using liver tissue from the same animals used for the micronucleus analysis of (b) (4)

showed a statistically significant increase in DNA damage at all doses tested (750, 1500, or 3000 mg/m^2/day for three consecutive days). The maximum fold increase was 1.6-fold (males) and 1.4-fold (females) compared to the vehicle control group. This impurity was not present in the drug product at levels that exceeded the ICH limit. (b) (4) was positive for mutagenic potential in the Ames test in the presence of metabolic activation at concentrations of 50 µg per plate for typhimurium TA100 and 300 µg per plate for TA 98. Though at the proposed specification level of (b) (4) exposure to this genotoxic impurity will be higher than the traditional threshold of toxicological concern for genotoxic impurites of 1.5 µg/day, as discussed in ICH S9, this threshold is not appropriate for the treatment of patients with cancer, and from a pharmacology/toxicology perspective, the specification of (b) (4) is acceptable, resulting in a daily exposure of no more than (b) (4) (b) (4) at 1600 µg/plate in the presence of S9 mix showed mutagenic activity in the Ames test. Incubation of Chinese Hamster V79 Cells with (b) (4) in the presence and absence of metabolic activation resulted in biologically relevant and statistically significant increases in the numbers of cells with aberrant metaphases; thus, (b) (4) is considered to be clastogenic for mammalian cells *in vitro*. This impurity is, however, controlled to levels below the traditional threshold for toxicological concern for genotoxic impurities, below 1.5 µg/day.

Reproductive and developmental toxicology:

The applicant submitted two embryofetal development studies, a pilot study in rats (not GLP) and a GLP-compliant study in rabbits. Additionally, the applicant provided summaries of two range-finding studies (one in rats, the other in rabbits), but did not provide reports for either of these two studies to the NDA. Based on the results from the submitted studies, neither the submission of the two range-finding embryofetal studies nor repetition of the non-GLP rat study (i.e. repeated under GLP) would be warranted to inform patient safety. The pilot embryofetal toxicity study in rats included cesarean section endpoints for dams and fetuses. No toxicokinetic data was obtained. Comparisons of the nonclinical doses tested to clinical exposure are based on toxicokinetic data obtained in 1-month repeat-dose rat toxicology study. In the rat embryofetal study 7 dams per group were administered regorafenib daily on Post-coital Days 6-17 at dose levels of 0, 0.1, 0.3, 0.5, 0.8, 1, 1.6, or 2 mg/kg (0, 0.6, 1.8, 3, 4.8, 6, 9.6, or 12 mg/m^2). Maternal toxicity evidenced by body weight loss and placental findings of pale placenta, grayish placental border, and double or engorged placental was apparent at doses ≥ 6mg/ m^2/day. At doses ≥ 6 mg/ m^2, occasional findings of total resorption of litters were noted; at doses ≥ 5 mg/kg (30 mg/m^2) total resorption was observed in all animals. At the highest dose of 12 mg/m^2 used in the submitted rat embryofetal study, dams showed a clear increase in post-implantation loss. Skeletal variations were observed in the fetuses that survived maternal doses ≥ 4.8 mg/ m^2. At doses of ≥ 9.6 mg/ m^2, fetal evaluation detected decreases in fetal weight and increases in cardiac malformations including ventricular septal defect, malformation of the great vessels as well as increases in other visceral findings such as diaphragmatic hernia and urinary system findings of dilation of the renal pelvis and ureter dilation.

Embryofetal studies were also conducted in Himalayan rabbits. At doses ≥1.6 mg/kg (19.2 mg/m^2, approximately 25% of the human exposure at the recommended daily dose of 160 mg) there were increases in post-implantation loss in two studies. In the submitted GLP-compliant rabbit embryofetal study, pregnant rabbits were administered regorafenib daily on Post-coital Days 6-20 at dose levels of 0, 0.4, 0.8, or 1.6 mg/kg (0, 4.8, 9.6, or 19.2 mg/m^2). Even at the lowest dose tested of 0.4 mg/kg (approximately 3% of the human exposure at the recommended daily dose of 160 mg), cardiac ventral septal defect was observed. Additional defects were observed at doses ≥ 9.6 mg/ m^2 including tricupsidal atresia; findings of dilation of the renal pelvis and hydronephrosis; and small, deformed, malpositioned, or missing kidney. Similar to the treatment-related changes observed in the rat embryofetal study, doses > 9.6 mg/m^2 were associated with increases in skeletal variations and malformations. The similarity of the findings in two species at dose levels lower than those resulting in maternal toxicity and resulting in exposures significantly below the exposures in humans at the recommended daily dose, in the context of the mechanism of action of drug as a kinase inhibitor targeting multiple pathways critical during development, support a recommendation for Pregnancy Category D in the label for regorafenib.

12 Appendix/Attachments

Appendix 1 is an excerpt from the original IND review for regorafenib by Dr. Haleh Saber and includes the 4-week rat and dog general toxicology sections.

Drug-related toxicities were observed in the: GI tract, hematopoietic/lymphocytic system, heart, liver, kidneys, adrenal glands, pancreas, thyroid/parathyroid, ♂ and ♀ reproductive system.

Study no.: T1074622
Report #: PH-34206
Volume #, and page #: Module 4 (electronic submission)
Conducting laboratory and location: Bayer HealthCare AG
PH-R8cD Toxicology
D-42096 Wuppertal
Date of study initiation: Aug 2, 2004
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: BAY 73-4506, Batch # 040607-010 (content: 10%)
Test article was administered as a co-precipitate with BAY 73-4506 PLAC POWD 000 in tap water. The animals of the control, low and mid dose groups were administered with the same amount of BAY 73-4506 PLAC POWD 000 as those of the high dose groups.

BAY 73-4506 PLAC POWD 000 appears is a placebo powder with 0% test article

Methods
Doses: daily x 28 days (followed by 4 weeks of recovery period)

	Dose (mg/kg)	Dose (mg/m^2)	Main		Recovery		TK satellite	
Control	0	0	10 ♂	10 ♀	5 ♂	5 ♀	3 ♂	3 ♀
LD	1	6	10 ♂	10 ♀	—	—	9 ♂	9♀
MD	4	24	10 ♂	10 ♀	—	—	9 ♂	9♀
HD	16	96	10 ♂	10 ♀	5 ♂	5 ♀	9 ♂	9♀

BAY 73-4506 was administered as a 10% co-precipitate, suspended in water.

Species/strain: Wistar rats
Number/sex/group or time point (main study): 10/sex/group
Route, formulation, volume, and infusion rate: oral gavage, 10 mL/kg
Satellite groups used for toxicokinetics or recovery: see Table above
Age: 6 weeks at initiation
Weight: ♂s: ~170 g and ♀s: ~130 g

Unique study design or methodology: liver samples were collected at necropsy for measurement of activities of metabolizing enzymes and concentrations of cytochrome P450 and triglycerides.

Observation and Times:
Mortality: twice daily
Clinical signs: general exam: daily (main and recovery)
Detailed exam: weekly (main and recovery)
Body weights: daily (weekly, during recovery period)

Food consumption: weekly
Water consumption: weekly
Ophthalmoscopy: not done
EKG: not done
Hematology: week 4/5 (main)
Clinical chemistry: week 4/5 (main)
Urinalysis: week 4 (main)
Gross pathology: at necropsy
Week 5 (main)
Week 9 (recovery)
Organ weights: brain, heart, liver, spleen, kidneys, thymus, adrenal glands, epididymides and testes, prostate, seminal vesicle and uterus.
Histopathology:
Adequate Battery: yes (X), no ( )
Peer review: yes (X), no ( )

Organ	Fixative	Groups evaluated histopathologically
Abnormalities	F	01 - 06
Adrenal glands	F	01 - 06
Aorta	F	01, 04, 05, 06
Brain (cerebrum, cerebellum, brain stem)	F	01, 04, 05, 06
Epididymides	F	01 - 06
Esophagus	F	01 - 06
Eyes	D	01, 04, 05, 06
Eyelids	F	not done
Exorbital lacrimal glands	F	01, 04, 05, 06
Femur (with joint)	F	01 - 06
Harderian glands	F	01, 04, 05, 06
Head (with skull cap)	F	
- Nasal Cavity with teeth		01 - 06
Heart	F	01 - 06

Organ	Fixative	Groups evaluated histopathologically
Intestine		
- Peyer's patches	F	01 - 06
- Duodenum	F	01 - 06
- Jejunum	F	01 - 06
- Ileum	F	01 - 06
- Cecum	F	01 - 06
- Colon	F	01 - 06
- Rectum	F	01 - 06
Kidneys	D	01 - 06
Larynx	F	01, 04, 05, 06
Liver	F	01 - 06
Lungs	F	01 - 06
Lymph nodes, mandibular	F	m: 01, 04, 05, 06 f: 01 - 06
Lymph nodes, mesenteric	F	01 - 06
Optic nerves*	D/F	01, 04, 05, 06
Ovaries	F	01 - 06
Oviducts	F	01 - 06
Pancreas	F	01 - 06
Pharynx	F	not done
Pituitary gland	F	01 - 06
Prostate	F	01 - 06
Salivary glands (parotid, submandibular, sublingual)	F	m: 01, 04, 05, 06 f: 01 - 06
Sciatic nerve	F	01, 04, 05, 06
Seminal vesicles (incl. coagulating glands)	F	01 - 06
Skeletal muscle (thigh)	F	01, 04, 05, 06
Skin (mammary region)	F	01, 04, 05, 06
Spinal cord (cervical, thoracic, lumbar)	F	01, 04, 05, 06

Organ	Fixative	Groups evaluated histopathologically
Spleen	F	m: 01, 04, 05, 06 f: 01 - 06
Sternum with Bone Marrow	F	01 - 06
Stomach	F	01 - 06
Testes	D	01 - 06
Thymus	F	m: 01, 04, 05, 06 f: 01 - 06
Thyroid glands (with parathyroids)	F	01 - 06
Tongue	F	01 - 06
Trachea	F	01 - 06
Ureters	F	01, 04, 05, 06
Urethra	F	not done
Urinary bladder	F	01, 04, 05, 06
Uterus (with cervix)	F	01 - 06
Vagina	F	01 - 06
Zymbal's glands	F	not done
Physical identifier	F	not done

F = 10 % neutral buffered formalin, D = Davidson's solution
* Brain parts fixed in formalin, eye parts fixed in Davidson's
m: males; f: females

Key to "groups evaluated":

- Group 1: control; main
- Group 2: LD; main
- Group 3: MD; main
- Group 4: HD; main
- Group 5: control; recovery
- Group 6: HD; recovery

Additional assessments:

Tissue Investigations:	
Tissue Samples Taken at Necropsies:	
Determination of Liver Enzymes	liver sampling during necropsies of main groups (n=10)
Measurements of Phase I and II Enzymes	liver sampling during necropsies of main groups (n=5)
Immunotoxicology	Samples of spleen and blood were taken at necropsy of main groups (n=5)

At necropsy from the first 5 living animals of the main groups blood was collected during exsanguination and half of the spleen were taken and immediately placed in chilled test tubes containing Hank's BSS. The following parameters were evaluated: Determination of the cell counts in the spleen. FACScan analyses, pooled per group, to determine subpopulations of the spleen cells.

Determination, on a per-animal basis, of the antibody (IgG, IgM and IgA) titers in the sera using the Sandwich ELISA method.

Toxicokinetics:

Toxicokinetics	
Days of Blood Sampling:	days 1 and 29
Time Points per Day:	
- treated animals	5 (1, 2, 4, 7 and 24 hours after administration)
- control animals	1 (2 hours after administration)
Number of Animals per Time Point:	3

Results:

Mortality: drug-related mortality occurred at HD, during recovery period. Animals were sacrificed moribund.

- 4/5 HD ♂s and 1/5 HD ♀s (days 45-56)
- 1/10 MD ♀ died due to aspiration pneumonia

Clinical signs:
Main animals:

- Ungroomed coat (MD and HD), gait (MD), pallor (HD)

Recovery animals (HD):

- Poor health, apathy, ungroomed coat, emaciation, squatting position, gait, white or missing tooth, ↑growth of tooth, discolored feces

Body weights: ↓BW gain was observed for the duration of dosing at HD. ↓BW was observed during the recovery period.

Main and recovery groups

	Main D1-D29 weight gain (g)		**Recovery D28-D57 weight gain (g)**	
	♂	♀	♂	♀
Control	122	44	70	34
LD	108	54	—	—
MD	106	45	—	—
HD	72*	29*	-1	-3

* Statistically significant difference from control.
— No recovery data available.

Food consumption: ↓food intake was observed at HD during the treatment and the recovery period.

Water intake: ↓intake at HD

Ophthalmoscopy: not done

EKG: not done

Hematology:
Since half of the recovery animals were sacrificed prescheduled in moribund state within the recovery period, clinical pathology (hematology, clinical chemistry, urinalysis) and enzymes in homogenized liver tissue were not assessed at the end of the recovery period.

Toxicologically significant hematology changes included:

HD

- ↓Platelets: 20% in ♂s and ♀s
- ↑WBC: 30% in ♂s and 16% in ♀s
- ↑Neutrophils: 1.9-fold in ♂s and 2.2-fold in ♀s
- ↑Monocytes:↑1.8-fold in ♂s and 20% in ♀s
- ↑Atypical leukocytes: 65% in ♂s and 75% in ♀s

MD:

- ↑WBC, neutrophils, and monocytes in ♂s

Dose mg/kg	ERY 10E12/l	HB g/l	HCT l/l	MCV fl	MCH pg	MCHC g/l ERY	RETI o/oo	THRO 10E9/l	HQUICK sec
m	Day 28/ 29								
0	7.81	150	0.460	59.0	19.2	325	25	980	28.2
1	8.17 +	155 ++	0.469	57.4	19.0	331	23	907	28.1
4	8.29 ++	162 ++	0.489 ++	59.0	19.5	331	23	910	27.5
16	8.30 ++	166 ++	0.497 ++	59.9	20.0 ++	334 ++	13 ++	777 +	22.9 ++
f	Day 28/ 29								
0	8.18	152	0.458	56.1	18.6	332	23	1095	25.7
1	8.26	156	0.466	56.4	18.9	334	19	945	25.7
4	8.31	161	0.477	57.5	19.3	336	16	857	25.8
16	6.78 ++	132 ++	0.387 ++	57.8	19.5 +	338	40	850	21.8 ++

Dose mg/kg	LEUCO 10E9/l	NEUTRO 10E9/l	LYM 10E9/l	MONO 10E9/l	EOS 10E9/l	BASO 10E9/l	ATYP 10E9/l
m	Day 28/ 29						
0	10.44	0.69	9.39	0.13	0.11	0.03	0.09
1	12.37 +	0.79	11.10	0.22 +	0.13	0.03	0.10
4	12.07 +	1.10 ++	10.41	0.28 ++	0.12	0.04	0.14
16	13.80 +	2.02 ++	11.08	0.36 ++	0.16	0.04	0.15 +
f	Day 28/ 29						
0	9.60	0.60	8.60	0.19	0.10	0.03	0.08
1	10.80	0.80	9.56	0.18	0.12	0.03	0.11
4	9.86	0.78	8.68	0.20	0.09	0.02	0.09
16	11.38	1.95 ++	8.99	0.23	0.04 ++	0.03	0.14 +

Tables provided by the sponsor.

Clinical chemistry: no recovery data is available

		AST	ALT	Cholesterol	Triglycer	t-Bilirub	Albumin
MD	♂	↑33%*	↑80%*	—	—	↑40%*	—
	♀	↑33%	↑90%*	—	—	↑15%*	—
HD	♂	↑33%*	↑125%*	↑115%*	↑28%	↑40%	↓18%*
	♀	↑110%*	↑250%*	↑190%*	↑44%	↑42%*	↓27%*

		BUN	T4	TSH
MD	♂	—	—	—
	♀	—	—	—
HD	♂	—	↓47%*	↑2-fold*
	♀	↑20%	↓50%*	↑2.5-fold*

* Statistically significant

Urinalysis: No recovery data available

- ↑protein excretion at HD: ↑5-fold in ♂s and ↑30-fold in ♀s
- ↓urinary volume at HD is likely due to the ↓water consumption

Dose mg/kg	VOL ml	Density g/l	pH	PROT* VOL mg	UREA* VOL mmol	CREA* VOL mcmol
m	Day 24/ 25					
0	15.8	1014	7.7	8.5	4.2	62
1	13.8	1016	7.4	11.3	4.4	59
4	17.7	1010	7.3 +	10.7	3.9	49
16	7.1 +	1029 +	7.1 +	50.0 +	3.4	45
f	Day 24/ 25					
0	8.7	1017	7.1	1.2	3.2	34
1	8.2	1023	6.8	1.7	3.4	34
4	6.5	1022	7.1	1.6	3.3	35
16	4.4	1030 +	7.4	33.1 ++	2.2	27

Gross pathology: findings were seen at HD. Renal findings were also seen at MD.
Main:

- Discolorations in: adrenal glands, teeth and kidneys
- Thickened duodenum

Recovery:

- Teeth: discoloration, thickening, fractures
- Bones: discoloration

Organ weights:
Since half of the recovery animals were sacrifice moribund, organ weights were not evaluated at the end of the recovery period.

Relative (organ:BW) at HD; end of treatment

	Adrenal	**Thymus**	**Heart**	**Kidney**	**Prostate**	**Uterus**
♂	↑24%	↓20%	↓15%	↑27%	↓30%	NA
♀	↑30%	↓30%	—	↑40%	NA	↓48%

NA: not applicable

Main groups; absolute organ weights

Dose mg/kg	Body W. G	Brain mg	Adrenals mg	Thymus mg	Heart mg	Liver mg	Spleen mg
m	Terminal Sacrifice						
0	294	1910	49	576	1103	11547	569
1	278	1905	48	571	1007	11154	616
4	279	1864	45	509	981 +	10553	629
16	242 ++	1852	51	393 ++	762 ++	8899 ++	520
f	Terminal Sacrifice						
0	180	1691	59	372	720	7445	459
1	186	1721	63	417	716	7719	487
4	179	1742	58	383	688	6905	469
16	162 +	1715	69 +	234 ++	644	6335 ++	407

Table provided by the sponsor.

Dose mg/kg	Body W. G	Kidneys mg	Testes mg	Epididym. mg	Prostate mg	Seminal vesicle mg	Uterus mg
m	Terminal Sacrifice						
0	294	2079	3322	1058	820	1022	
1	278	1875	3122	1105	713	1185	
4	279	1903	3021 +	1080	731	1124	
16	242 ++	2158	2808 ++	1003	594 ++	971	
f	Terminal Sacrifice						
0	180	1274					765
1	186	1369					1079
4	179	1279					621
16	162 +	1605 ++					356 +

Table provided by the sponsor.

Histopathology:

Major histopathology findings for main sacrifice groups

	Control	LD	MD	HD
Heart				
Perivascular/interst edema	—	—	—	7/10 ♂s (grade 1) 9/10 ♀ (grade 1-2)
Pericarditis—	—	—	1/10 ♂ (grade 3)	—
Infiltration/mononuclear	—	—	3/10 ♀ (grade 1)	1/10 ♀ (grade 2)
Tongue				
↓mast cells	—	—	—	8/10 ♂s (grades 1-4) 10/10 ♀ (grade 3-4)
Intracellular vacuolation	—	—	—	4/10 ♂s (grade 1-2) 10/10 ♀ (grade 1-2)
↑interstitial edema	—	—	—	3/10 ♂ 5/10 ♀ (grade 2)
Stomach				
hyperkeratosis	—	—	—	6/10 ♂s (grade 1) 5/10 ♀ (grade 1)
inflammation	—	—	—	2/10 ♂ (grade 1)
Cellul. Hypertrophy/pyl.	—	—	—	10/10 ♀ (grade 1-2)
Duodenum				
Hypertrophy/mucosa	—	—	—	5/10 ♂ (grade 2-4) 9/10 ♀ (grade 3-4)
Hypertrophy/muscul.	—	—	—	3/10 ♂ (grade 1-3) 8/10 ♀ (grade 1-3)
Degeneration/regenerat.	—	—	—	5/10 ♂ (grade 1-4) 10/10 ♀ (grade 2-5)
Inflammatory infiltration	—	—	2/10 ♂ (grade 1)	1/10 ♂ (grade 1-3) 10/10 ♀ (grade 2-4)
Peyer's patch				
↑single cell necrosis	—	—	—	3/9 ♀ (grade 1)
Cecum				
Edema/submucosal	1/10 ♀ (grade 1)	1/10 ♀ (grade 1)	1/10 ♂ (grade 1) 1/10 ♀ (grade 1)	2/10 ♂ (grade 1-2) 5/10 ♀ (grade 1-2)
Liver				
Bile duct proliferation	—	—	—	1/10 ♂ (grade 2) 2/10 ♀ (grade 1)
Kupffer cell activation	—	—	1/10 ♂ (grade 1) 2/10 ♀ (grade 2)	6/10 ♂ (grade 1) 9/10 ♀ (grade 1-2)
Pancreas				
↑apoptotic cells	—	—	—	4/10 ♂ (grade 1) 8/10 ♀ (grade 1-3)
Atrophy/degeneration	—	—	—	1/10 ♂ (grade 3)

				9/10 ♀ (grade 2-4)
Inflam. Interst. Edema	—	—	—	2/10 ♂ (grade 1-3) 5/10 ♀ (grade 1-3)
↑mitosis	—	—	—	6/10 ♀ (grade 1-2)
Kidneys				
Tubular degener/regener	—	4/10 ♂ (grade 1-2)	8/10 ♂ (grade 1-2)	10/10 ♂ (grade 2-4) 10/10 ♀ (grade 2-4)
Glomerulopathy	—	—	7/10 ♂ (grade 1-2) 3/10 ♀ (grade 1)	10/10 ♂ (grade 1-3) 10/10 ♀ (grade 1-4)
Tubular dialation	3/10 ♂ (grade 1-2)	1/10 ♂ (grade 1)	6/10 ♂ (grade 1-2)	5/10 (grade 1-3) 8/10 ♀ (grade 2-4)
Fibrosis/(sub-) capsular	—	—	—	3/10 ♂ (grade 1-2)
Epididymides				
Infiltration/mononuclera	5/10 ♂ (grade 1-2)	4/10 ♂ (grade 1)	6/10 ♂ (grade 1)	8/10 ♂ (grade 1)
Sperm granuloma	1/10 ♂ (grade 2)	1/10 ♂ (grade 3)	1/10 ♂ (grade 3)	2/10 ♂ (grade 3-4)
Cellular debris	2/10 ♂ (grade 1)	4/10 ♂ (grade 1-2)	2/10 ♂ (grade 1)	8/10 ♂ (grade 1-2)
Ovaries				
↑necrotic corpus lutea	—	—	—	8/10 ♀ (grade 1-3)
atrophy	—	—	—	10/10 ♀ (grade 1-4)
Uterus and Cervix				
Atrophy	—	—	1/10 ♀	6/10 ♀
Pituitary				
↑pale cells	—	—	—	6/10 ♂ (grade 1-3) 6/10 ♀ (grade 1)
Thyroid gland				
Flattened follicular epith	—	—	1/10 ♂ (grade 1)	8/10 ♂ (grade 1-3) 10/10 ♀ (grade 1-3)
Parathyroid				
↑interstitial fibrosis	—	—	—	1/10 ♀ (grade 2)
Adrenal glands				
Cort. Cell hypertrophy	—	—	—	1/10 ♂ (grade 2)
Peliosis	—	—	—	1/10 ♂ (grade 1) 10/10 ♀ (grade 1-4)
Necrosis	—	—	—	2/10 ♂ (grade 3) 4/10 ♀ (grade 2-4)
Intracyt. vacuolation	—	—	—	10/10 ♀ (grade 1-4)
Thymus				
↑single cell necrosis	—	—	3/10 ♀ (grade 1-3)	6/10 ♀ (grade 1-2)
Inflammation	—	—	1/10 ♂ (grade 1)	—
Mesenteric lymph node				
Atrophy	—	—	—	2/10 ♂ (grade 1-2) 9/10 ♀ (grade 1-3)
Mandibular lymph node				
Erythrophagocytosis	5/10 ♂ (grade 1-3)	♂: ND 2/10 ♀ (grade 1)	♂: ND 1/10 ♀ (grade 1)	7/10 ♂ (grade 1-3) 2/10 ♀ (grade 1)
inflammation	—	ND	ND	1/10 (grade 2)
Femur				
Hypocell./growth plate	—	—	2/10 ♂ (grade 1)	9/10 ♂ (grade 2-3) 10/10 ♀ (grade 2-4)
Growth plate; thick	—	—	4/10 ♂ (grade 1-2)	10/10 ♂ (grade 3-5) 10/10 ♀ (grade 5)
Chondrodystrphy/ joint	—	—	—	10/10 ♂ (grade 2) 10/10 ♀ (grade 2-3)
Sternum				
Chondrodystrohy	—	—	—	10/10 ♂ (grade 1-3) 10/10 ♀ (grade 3)
Myelofibrosis	—	—	3/10 ♂ (grade 1)	4/10 ♂ (grade 1-2) 2/10 ♀ (grade 1)
Teeth/incisors				

Dentin alteration	3/10 ♂ (grade 1-2)	2/10 ♂ (grade1)	5/10 ♂ (grade 1-2) 9/10 ♀ (grade 1-4)	10/10 ♂ (grade 2-4) 10/10 ♀ (grade 2-4)
Angiectasis/periodontal ligament	—	—	2/10 ♀ (grade 3-4)	10/10 ♂ (grade 1-4) 10/10 ♀ (grade 3-4)

ND: not done

Some findings were reversible, some were persistent and some progressed (or new findings occurred) through the recovery period. Histopathology report of ♂ recovery group is listed below for the purpose of comparison to the main terminal findings. Comparison of the incidence for main and recovery groups was made by the reviewer for ♂ animals only (see description in parenthesis, in bullet points)

Effects in the following organs/tissues seen at the end of recovery period included:

- Tongue: ↓mast cells (same incidence/persistent), interstitial edema (↓incidence)
- Esophagus: hyperkeratosis (new event)
- Stomach: hyperkeratosis (↑incidence), submucosal edema
- Peyer's patch: single cell necrosis (↑incidence)
- Pancreas: atrophy/degeneration (↑incidence)
- Kidneys: tubular degeneration (same/persistent), glomerulopathy (↓incidence), mononuclear cell infiltration, tubular dilation, interstitial fibrosis (new event), fibrosis, acute hemorrhage (new event)
- Thyroid: flattened follicular epithelium (↑incidence)

Male: recovery

TONGUE :	5	5
- Decr.Mast Cells :	-	4
Grade 1:	-	1
Grade 3:	-	-
Grade 4:	-	-
Grade 5:	-	3
- Atrophy/Ser.Gl. :	-	2
Grade 2:	-	1
Grade 4:	-	1
- Incr.Interst.Edema :	-	1
Grade 1:	-	1
ESOPHAGUS :	5	5
- Hyperkeratosis :	-	2
Grade 1:	-	1
Grade 2:	-	1
STOMACH :	5	5
- Glandular Dilation :	-	1
Grade 1:	-	1
- Hyperkeratosis/Fore.:	-	4
Grade 1:	-	3
Grade 2:	-	1
- Submucosal Edema :	-	3
Grade 2:	-	3
Grade 3:	-	-
PEYER'S PATCHES :	5	5
- Incr.Single C.Necr. :	-	1
Grade 2:	-	1

LIVER :	5	5
- Infiltr. Mononuc./PP:	-	1
Grade 1:	-	1
Grade 2:	-	-
Grade 3:	-	-
- Kupffer C. Accumul. :	2	2
Grade 1:	1	2
Grade 2:	1	-
- Decreased Glycogen :	-	4
Grade 3:	-	-
Grade 4:	-	3
Grade 5:	-	1
- Increased Glycogen :	-	1
Grade 3:	-	1
PANCREAS :	5	5
- Atrophy/Degeneration:	-	1
Grade 1:	-	-
Grade 3:	-	1
- Hypertrophy/Compens.:	-	2
Grade 1:	-	1
Grade 2:	-	1
Grade 3:	-	-
KIDNEYS :	5	5
- Basophilic Tubules :	4	-
Grade 1:	2	-
Grade 2:	2	-
- Tub.Degen./Regen. :	-	5
Grade 1:	-	-
Grade 2:	-	3
Grade 3:	-	1
Grade 4:	-	1
Grade 5:	-	-

- Glomerulopathy	:	-	4
Grade 1:		-	2
Grade 2:		-	1
Grade 3:		-	1
Grade 4:		-	-
- Atrophy/Adjacent Fat:		-	5
Grade 2:		-	2
Grade 3:		-	-
Grade 4:		-	2
Grade 5:		-	1
- Infiltr. Mononuclear:		-	2
Grade 1:		-	1
Grade 3:		-	1
- Tubular Dilation	:	1	3
Grade 1:		1	-
Grade 2:		-	-
Grade 3:		-	3
Grade 4:		-	-
- Interstit.Fibrosis	:	-	3
Grade 1:		-	2
Grade 2:		-	1
Grade 3:		-	-
- Transit.C.Hyperpl.	:	1	-
Grade 1:		1	-
- Fibrosis/(Sub-)Caps.:		-	1
Grade 1:		-	1
- Acute Hemorrhage	:	-	1
Grade 4:		-	1
TESTES	:	5	5
- Tub.Atrophy/Degen.	:	-	3
Grade 1:		-	1
Grade 2:		-	1
Grade 3:		-	1
EPIDIDYMIDES	:	5	5
- Infiltr. Mononuclear:		1	-
Grade 1:		1	-
- Sperm Granuloma	:	-	1
Grade 3:		-	1
- Cellular Debris	:	-	3
Grade 2:		-	1
Grade 3:		-	1
Grade 4:		-	1
- Oligospermia	:	-	2
Grade 2:		-	1
Grade 3:		-	1

PROSTATE :	5	5
- Fluid Alteration :	-	4
Grade 2:	-	1
Grade 3:	-	3
COAGULATING GLANDS :	5	5
- Atrophy :	-	4
Grade 2:	-	1
Grade 3:	-	1
Grade 4:	-	2
SEMINAL VESICLES :	5	5
- Atrophy :	-	4
Grade 2:	-	1
Grade 3:	-	1
Grade 4:	-	2

Thyroid gland

- Foll.C.Hypertrophy :	1	-
Grade 1:	1	-
- Flattened Foll.Epit.:	-	5
Grade 1:	-	1
Grade 3:	-	1
Grade 4:	-	3
ADRENAL GLANDS :	5	5
- Intracyt.Vac./ZF :	3	-
Grade 1:	2	-
Grade 2:	1	-
- Cort.Cell Hypertr. :	-	2
Grade 1:	-	1
Grade 2:	-	1
- Atrophy/Adjacent Fat:	-	5
Grade 2:	-	1
Grade 3:	-	1
Grade 4:	-	3
- Single Cell Necroses:	-	1
Grade 1:	-	1
- Pigmentl.Macrophages:	-	1
Grade 3:	-	1
Grade 4:	-	-
- Fibrotic Tissue :	-	1
Grade 2:	-	-
Grade 3:	-	-
Grade 4:	-	1
SPLEEN :	5	5
- Atrophy/White Pulp :	-	2
Grade 1:	-	1
Grade 4:	-	1
THYMUS :	5	4
- Atrophy :	-	3
Grade 3:	-	-
Grade 4:	-	1
Grade 5:	-	2

MANDIB.LYMPH NODES :	5	5
- Plasma C.Hyperplasia:	2	5
Grade 1:	1	-
Grade 2:	1	-
Grade 3:	-	5
- Single Cell Necroses:	-	5
Grade 2:	-	5
PAROTID GLANDS :	5	5
- Acinar Atrophy :	-	3
Grade 2:	-	1
Grade 3:	-	1
Grade 4:	-	1
SUBMANDIBULAR GLANDS :	5	5
- Acinar Atrophy :	-	2
Grade 2:	-	1
Grade 3:	-	1
FEMUR/GROWTH PL./BM :	5	5
- Fat Replacement/BM :	5	4
Grade 2:	1	1
Grade 3:	4	2
Grade 4:	-	1
- Hypocell./Grow.Plate:	-	4
Grade 2:	-	1
Grade 3:	-	2
Grade 4:	-	1
- Growth Plate Thick. :	-	3
Grade 1:	-	-
Grade 2:	-	1
Grade 3:	-	1
Grade 4:	-	1
- Hyperostosis/Gr.Pl. :	-	4
Grade 1:	-	2
Grade 2:	-	2
- Chondrod./Joint Car.:	-	3
Grade 1:	-	2
Grade 2:	-	1
- Incr.Blood Content :	-	2
Grade 2:	-	1
Grade 3:	-	-
Grade 4:	-	1

STERNUM/BONE MARROW :	5	5
- Chondrodys./Symphys.:	-	5
Grade 1:	-	4
Grade 2:	-	1
- Fat Replacement/BM :	5	4
Grade 1:	2	1
Grade 2:	3	1
Grade 3:	-	2
Grade 4:	-	-
- Hypocellularity :	-	3
Grade 1:	-	1
Grade 2:	-	1
Grade 4:	-	1
- Incr.Blood Content :	-	1
Grade 2:	-	-
Grade 3:	-	1
- Incr.Granulocytes :	-	1
Grade 3:	-	1
TEETH/INCISOR/UP.J. :	5	5
- Dentin Alteration :	1	5
Grade 1:	1	-
Grade 4:	-	5
- Hemorrhage/Per.Lig. :	-	1
Grade 4:	-	1
- Edema/Per.Lig. :	-	4
Grade 2:	-	1
Grade 3:	-	3
- Ameloblast Degener. :	-	3
Grade 4:	-	3
TEETH/INCISOR/LO.J. :	4	5
- Dentin Alteration :	-	5
Grade 3:	-	-
Grade 4:	-	5
- Angiec./Per.Lig. :	-	5
Grade 2:	-	-
Grade 3:	-	2
Grade 4:	-	3
- Edema/Per.Lig. :	-	5
Grade 2:	-	1
Grade 3:	-	4
- Fracture :	-	4
- Osteodystrophy :	-	4
Grade 3:	-	1
Grade 4:	-	3

Enzyme activity in homogenized liver tissue:
Investigations in homogenized liver samples at the end of the treatment period revealed changed O-demethylase activity and cytochrome P450 concentrations in HD ♂s (O-DEM) and HD ♀s (P 450). Since all individual values were within 2x range of historical data, no toxicologica1 relevance were attributed to these changes.

The activities of the cytochrome P450-dependent monooxygenases [ECOD (CYP 1A1, 2B 1, 2D1, 2E1), EROD (CYP 1A1), ALD (CYP 2B1, 3A1, 3A4, 2C11)], epoxide

hydrolase (EH), and the conjugation enzymes (GS-T, GLU-T) were evaluated. No clear treatment-related effect was found.

Immunotoxicity:

Data were not reviewed. The following has been excerpted from the submission.

FACS data:

In males a pronounced decrease was observed for CD4(total), CD45(high) and CD8(total) positive cells in the spleens at HD. Furthermore, the marker for antigen presenting cells (I-a) showed a clear increase on positive cells after treatment with the highest dosage of the test item. Finally, a pronounced increase of PanB(total) positive cells (B cells) was detected at MD and HD.

In accordance with the male animals, the female rats showed also a pronounced decrease of the CD4(total) and the CD45(high) positive cells at HD. Furthermore, the CD8(total) positive cells were clearly decreased at MD and HD, more pronounced at HD. In addition, there was a clear increase of PanB(total) and the I-a(total) positive cells at MD and HD. Finally, there was a decrease concerning the CD4(total) positive cells at the mid dose. However, this change may be judged as an isolated effect because it was not corroborated by other findings, e.g. by analysis of CD4(total) positive cells of the CD4/CD45 double-labeling. The reason for this change is not known.

Antibody titers:

The IgM titer were statistically significantly increased at HD in both sexes. On the other hand, the IgG titer of these HD groups were statistically decreased (statistically significant in ♀s). This could point to an increase of more non-mature B cells or to an inhibition of normal class switching from IgM subclass to IgG.

Toxicokinetics:

Since differences in the ♂ and ♀ exposures were slight, data were combined.

Day 1 TK data:

Dose: BAY 73-4506 [mg/kg]		1.00	4.00	16.0
AUC(0-24)	[mg·h/L]	4.18	14.7	65.0
$AUC(0\text{-}24)_{norm}$	[kg·h/L]	4.18	3.68	4.06
C_{max}	[mg/L]	0.405	1.43	5.56
$C_{max,norm}$	[kg/L]	0.405	0.357	0.348
$C(24)/C_{max}$	[%]	6.91	7.82	10.6
t_{max}	[h]	4.00	2.00	4.00

Day 29 TK data:

Dose: BAY 73-4506 [mg/kg]		1.00	4.00	16.0
AUC(0-24)	[mg·h/L]	5.75	20.4	81.1
$AUC(0\text{-}24)_{norm}$	[kg·h/L]	5.75	5.10	5.07
C_{max}	[mg/L]	0.490	1.70	5.05
$C_{max,norm}$	[kg/L]	0.490	0.426	0.316
$C(24)/C_{max}$	[%]	10.4	15.3	38.2
t_{max}	[h]	2.00	2.00	2.00
R_{A1}	[%]	121	119	90.8
R_{A3}	[%]	138	139	125

R_{A1} = C_{max}_Day 29 / C_{max}_Day 1 in [%]
R_{A3} = AUC_Day 29 / AUC_Day 1 in [%]

Table provided by the sponsor.

Summary of the study

SD rats were dosed with BAY 73-4506 for 28 days at 6, 24, and 96 mg/m2. The HD resulted in 4/5 unscheduled sacrifices in ♂s and 1/5 in ♀s, during the recovery period (Days 45-56). Deaths occurred after 2 weeks of recovery period (recovery days 17-28). No clinical pathology or organ weight data was provided for recovery animals, due to high mortality at HD. However, histopathology data indicated that some events persisted or progressed through recovery period. In addition, new events (of low incidence) were reported during the recovery period.

Drug-related events included:

- Coagulation: thrombocytopenia
- Hematopoietic/lymphocytic system: ↑WBC and differentials (mainly neutrophils and monocytes), atrophy and/or inflammation of lymph nodes, inflammation and single cell necrosis in thymus, single cell necrosis of Peyer's patch, thymic and spleen atrophy
- Hepato-biliary toxicities: ↑ALT, AST, total bilirubin, cholesterol and triglycerides, ↓albumin, bile duct proliferation, mononuclear cell infiltration (recovery)
- Thyroid/pathyroid: hypothyroidism (↓T4 and ↑TSH), ↑incidence of flattened follicular epithelium in thyroid especially during the recovery period, interstitial fibrosis in parathyroid
- Renal toxicity (↑protein excretion, ↑weight of kidneys), tubular degeneration, glomerulopathy, subcapsular fibrosis, tubular dilation, interstitial fibrosis (recovery), hemorrhage (recovery)
- Adrenal toxicity: ↑weight of adrenal glands, single cell necrosis, cortical cell hypertrophy (recovery), fibrotic tissue (recovery)
- Pancreas: atrophy/ degeneration
- Cardiac toxicity (cardiac perivascular/interstitial edema, pericarditis, mononuclear cell infilatration),
- GI tract (discolored feces, inflammatory infiltration, hyperkeratosis, submucosal dema, degeneration/regeneration, single cell necrosis in peyer's patch

- Reproductive system (♂ and ♀)
- Bone: hypocellularity, myelofibrosis, thickening of growth plate, chondrodystrophy
- Teeth: dentin alteration, angiectasis, ameloblast degeneration (recovery), edema of periodontal ligament (recovery), osteodystrophy (recovery)

Toxicokinetics:

- Tmax was reported at 2-4 hrs post-dose
- Increase in AUC0-24 was approximately dose-proportional for all doses tested
- Day 29 exposures (AUCs) were ~30% higher than Day 1, suggesting slight drug accumulation upon repeated dosing

The HD exceeded STD10. The MD was well tolerated; however, no recovery data is available for MD to assess the potential for death during the drug-free period.

Of note, drug-related toxicities are similar to what was reported for sorafenib.

Study title: Subacute Oral Toxicity Study in Beagle Dogs (4 Week Gavage Study with 4 Week Recovery Period)

Key study findings: Drug-related events were seen in the GI tract, pancreas, heart, liver, hematopoietic/lymphocytic system, kidney, bone, and teeth

Study no.: T 2074704
Report#: PH-34182
Volume #, and page #: module 4 of electronic submission
Conducting laboratory and location: Bayer HealthCare AG
PH-R&D; Toxicology
Date of study initiation: October 4, 2004
GLP compliance: yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Test substance: BAY 73-4506 GRAN 10% 010
Batch No.: 040816-010
Content of active ingredient: 9.8 %

Test article was administered as a co-precipitate with BAY 73-4506 PLAC POWD 000 in tap water. BAY 73-4506 PLAC POWD 000 is a placebo powder to solubilize BAY 73-4506

Methods

Doses: daily x 28 days (followed by 4 weeks of recovery)

Group	Dose		# of animals			
			Main		Recovery	
	mg/kg	mg/m2	♂	♀	♂	♀
Control	0	0	3	3	2	2
LD	5	100	3	3	—	—
MD	20	400	3	3	—	—
HD	80	1600	3	3	2	2

Control group received placebo in tap water.
The test substance (as coprecipitate) was formulated in tap water.

Species/strain: Beagle dogs
Number/sex/group (main study): see Table above
Route, formulation, volume, and infusion rate: oral gavage, 10 mL/kg
Satellite groups used for recovery: see Table above
Age: 23-27 weeks on Week -1
Weight: 7.4-10.7 kg on Week -1

Observation and Times:
Clinical signs: twice daily
Detailed exam (e.g. reflexes) was done prior to initiating the study, during Weeks 4 (main) and 8 (recovery)

Body weights: weekly

Food consumption: daily

Ophthalmoscopy: before the start of the study (week -2) and in week 4 and 8 (recovery groups only) of the study. In the ophthalmoscopic investigations the external sections of the eye (conjunctivae, lids, sclera, cornea, lacrimal apparatus) were first examined by adspection. The transparent media (cornea, anterior chamber, iris, lens, vitreous body) and the fundus were then evaluated with the aid of the ophthalmoscope system.

EKG and blood pressure: once before the start of the study (week -2), and before and 2h after administration in week 1 and 4 of the study and once in week 8 (recovery groups only).

Blood pressure was measured invasively in the femoral artery with the animal in a lying position. Systolic and diastolic blood pressures, mean arterial blood pressure and heart rate.

EKG: the lead electrodes were fixed subcutaneously on the outer surface of the four extremities. The standard leads I, II, III, aVR, aVL, and aVF were recorded. Heart rates were calculated automatically from the ECG-measurements.

Hematology: before the start of the study (week-2) and in week 2, 4 and 8 (recovery groups). Blood was obtained from the jugular vein.

Clinical chemistry: before the start of the study (week -2) and in week 2, 4 and 8 (recovery groups). Blood was obtained from the jugular vein.

Urinalysis: before the start of the study (week -2) and in week 2, 4 and 8 (recovery groups). 6-hr collection.

Gross pathology: at necropsy (Week 5)

Organ weights: heart, lung, liver, kidneys, spleen, testes, prostate, ovaries, thyroid with parathyroids, adrenals, thymus, brain, pituitary, pancreas, empty gall bladder, epididymides, and uterus/oviduct

Histopathology:

Adequate Battery: yes (x), no ( )
Peer reviewed: yes

Table: Organs fixed and evaluated histopathologically.

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

* fixation in Davidson's solution
$ additional specimen fixed in 10 % neutral buffered formalin
# no histopathology performed

Toxicokinetics:
Plasma samples for TK were collected on day 1 and during week 4 of the study. Samples were collected from LD, MD, and HD main animals before and 1, 2, 4, 7 and 24 h after administration (controls and HD recovery group: only 2 h after administration).

Other: liver samples were taken at necropsy for the determination of phase I and – II enzymes.

Results:

Mortality: none
(One control animal was sacrificed due to intubation problems)

Clinical signs:
- ♂s: no signs
- ♀s: liquid feces at HD, one HD ♀ presented with red diarrhea, gait, bleeding gum at the end of the dosing period.

Body weights: no toxicologically significant changes

Food consumption: slightly reduced in HD ♀s towards the end of the treatment period

Ophthalmoscopy: no drug-related changes

EKG: no effect on body temperature, blood pressure, heart rate, or EKG parameters

Hematology: findings were reversible
- HD ♂s (Week 4): ↑Monocytes: ↑60% in
- MD ♀s (Week 4): ↑ESR-1 (6-fold), ↑ESR-2 (8-fold), ↑WBC (50%), ↑neutrophils (60%), ↑monocytes (100%), ↑atypical leukocytes (1.5-fold)
- HD ♀s (Week 2): ↑ESR-1 (3-fold), ↑ESR-2 (4-fold)
- HD ♀ (Week 4): ↑ESR-1 (12-fold), ↑ESR-2 (12-fold), ↑WBC (35%), ↑monocytes (85%), ↑atypical leukocytes (90%), ↓erythrocytes (15%), ↓hemoglobin (↓14%)

Clinical chemistry: Effects were not toxicologically significant at MD. Findings were reversible.
- HD ♂s (Week 4): ↑ALP (50%), ↑ALT (100%), ↑AST (100%), ↑CK (3-fold), GGT (↑100%)
- HD ♀s (Week 4): ↑ALP (30%-60%), ↑ALT (1-5 fold), ↑AST (50%-100%), ↑GGT (100%)

Urinalysis: no relevant changes were detected

Gross pathology: no drug-related finding

Organ weights: No relevant finding

A dose dependent increase in relative weight of adrenals was seen in ♂s (15%, 30%, and 33% at LD, MD, and HD, respectively)

Histopathology:
Findings were predominantly at MD and HD. Effects in teeth were seen at LD as well.

Main sacrifice

- *Kidney*: slight to moderate nephropathy in 1/3 HD ♀
- *Liver:* centrilobular hypertrophy at MD and HD (♂s and ♀s)
- *Spleen*: ↑hematopoiesis and ↑iron storage at MD and HD
- *Lymphatic tissue*: Slight effects consisting of cortical atrophy and/or ↑single cell necroses in the thymus (HD), follicular necrosis in the germinal center of the lymph follicles of the tonsils (MD and HD) and of the larynx (HD ♂).
- *Digestive system*: acinar atrophy in the pancreas (HD), ↑ number of apoptotic bodies in HD ♂s and hypertrophy of zymogenic cells in the stomach (HD)
- *Bone*: thickening of the epiphyseal growth plate with hypocellularity of the adjacent bone marrow, and chondrodystrophy of the sternal symphyses (MD and HD)
- *Tooth*: dentin alterations

Recovery sacrifice

- *Hepatobiliary*: minimal to slight bile duct proliferation
- *Tooth*: minimal dentin alterations
- *Bone*: slight thickening of the epiphyseal growth plate (1 ♀)

Toxicokinetics:

Day 1 TK data (n=3)

Dose: BAY 73-4506 [mg/kg]		5.00		20.0		80.0	
		Mean geom.	S.D. geom.	Mean geom.	S.D. geom.	Mean geom.	S.D. geom.
AUC(0-24)	[mg·h/L]	13.3	1.12	35.6	1.21	93.9	1.14
$AUC(0\text{-}24)_{norm}$	[kg·h/L]	2.67	1.12	1.78	1.21	1.17	1.14
C_{max}	[mg/L]	2.78	1.10	5.54	1.10	13.3	1.23
$C_{max,norm}$	[kg/L]	0.555	1.10	0.277	1.10	0.167	1.23
$C(24)/C_{max}$	[%]	1.81	1.53	6.31	1.23	8.18	2.22
t_{max}	[h]	1.26	1.43	1.41	1.46	1.26	1.43

Day 26 TK data (n=3)

Dose: BAY 73-4506 [mg/kg]		5.00		20.0		80.0	
		Mean geom.	S.D. geom.	Mean geom.	S.D. geom.	Mean geom.	S.D. geom.
AUC(0-24)	[mg·h/L]	9.32	1.24	26.0	1.64	44.0	1.73
$AUC(0\text{-}24)_{norm}$	[kg·h/L]	1.86	1.24	1.30	1.64	0.550	1.73
C_{max}	[mg/L]	1.62	1.31	4.21	2.00	7.20	1.89
$C_{max,norm}$	[kg/L]	0.324	1.31	0.210	2.00	0.0900	1.89
$C(24)/C_{max}$	[%]	2.39	1.97	5.20	2.40	4.77	1.58
t_{max}	[h]	1.41	1.46	1.26	1.43	1.32	1.46
R_{A1}	[%]	58.3	1.29	76.0	2.05	54.0	2.11
R_{A3}	[%]	69.9	1.19	73.1	1.86	46.9	1.78

$R_{A1} = C_{max\_Day\ 26} / C_{max\_Day\ 1}$ in [%]
$R_{A3} = AUC_{\_Day\ 26} / AUC_{\_Day\ 1}$ in [%]

Summary of the study

Beagle dogs were treated with BAY 73-4506 for 28 days at 100, 400, or 1600 mg/m2. All doses were well tolerated.

Drug-related events included the following:

- GI tract: red liquid feces, apoptosis and hypertrophy of zymogenic cells in the stomach
- Pancreas: acinar atrophy
- Coagulation: bleeding (GI, gum)
- Heart: ↑CK
- Hepatobiliary: ↑ALT, ALP, AST, GGT, centrilobular hypertrophy, bile duct proliferation (recovery)
- Hematopoietic/lymphocytic: ↑neutrophils, monocytes, and atypical leukocytes, ↑erythroid sedimentation rate, ↑hematopoiesis and iron storage in spleen, cortical atrophy and/or ↑single cell necroses in the thymus, follicular necrosis in the germinal center of the lymph follicles of the tonsils and of the larynx
- Kidney: nephropathy
- Bone: thickening of the epiphyseal growth plate with hypocellularity of the adjacent bone marrow, and chondrodystrophy of the sternal symphyses (MD and HD)
- Tooth: dentin alterations

Toxicokinetics:

- Absorption was relatively fast, with a tmax of approx. 1.5 hr
- Increased in AUCs were less than dose proportional at MD and HD on both days 1 and 26. In addition, AUCs were lower on Day 26 than Day 1 for all doses. This may suggest saturation of absorption.

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/s/

M A GOHEER
09/07/2012

ANDREW J MCDOUGAL
09/10/2012

WHITNEY S HELMS
09/10/2012

MEMORANDUM

Date: September 7, 2012
From: Whitney S. Helms, Ph.D.
Pharmacology Supervisor
Division of Hematology Oncology Toxicology
For the Division of Oncology Products 2
To: File for NDA #203085
Regorafenib (STIVARGA)
Re: Approvability of Pharmacology and Toxicology

In NDA 203085, Bayer has submitted clinical trial data to support the use of regorafenib for the treatment of patients with metastatic colorectal cancer. Non-clinical studies examining the pharmacology and toxicology of regorafenib provided to support this indication were reviewed in detail by M. Anwar Goheer, Ph.D. and Andrew J. McDougal, Ph.D., D.A.B.T. The application included studies of orally administrated regorafenib and two major active human metabolites, M-2 and M-5, in rats, dogs, mice, and rabbits that investigated the drug's pharmacology, pharmacokinetics, safety pharmacology, general toxicology, genetic toxicity (*in vivo* and *in vitro*), and reproductive toxicity.

The pharmacology studies submitted to this NDA demonostrate that regorafenib is a kinase inhibitor. "Kinase inhibitor" is an existing established pharmacological class and this designation is reflected in the indication statement of the label for regorafenib. Like other approved kinase inhibitors, regorafenib targets a number of enzymes at concentrations that could be achieved clinically (based on Cmax at the recommended human dose of 160 mg) including RET and several RET variants, PTK5, VEGFR-1,-2, and -3, FGFR-1 and -2, DDR2, SAPK2, Lyn, Tie2, Abl, TrkA, EphA2, KIT and several Kit variants, c-RAF, BRAF, and $BRAF^{V600E}$. During the development of regorafenib, the Applicant discovered that two of the major metabolites present in human serum at levels ≥33% of the total regorafenib exposure, M-2 and M-5, were present in the serum of rats and dogs, the primary species used to study the safety of the drug, only at minor levels of ≤ 3%. In *in vitro* biochemical and cellular assays, both of the metabolites inhibited kinase activity at levels similar to or exceeding the regorafenib parent compound, showing that these were active metabolites. M-2 and M-5 also inhibited VEGF-mediated decreases in blood pressure with no clear differences compared to regorafenib effects in an *in vivo* study conducted in Wistar rats. To address the safety issue arising from the low exposure to these metabolites of the major species used for toxicological evaluation, the Applicant conducted additional genetic toxicology, safety pharmacology, and 4-week general toxicology studies using both M-2 and M-5 as individual agents in order to determine a more accurate safety profile of the drug. The submitted metabolite studies are sufficient to support the safety of clinical exposure of the metabolites following regorafenib administration in the intended patient population.

Target organs for regorafenib-mediated toxicity identified in toxicology studies conducted using both rats and dogs included the liver, kidney, adrenal gland, thyroid, pancreas, gastrointestinal tract, and hematopoietic/lymphoid system. Skin toxicity was also observed in dogs. Signs of

toxicity in all of these organs have also been observed clinically. In addition, the reproductive system and skeletal system were identified as targets of regorafenib-mediated toxicity in animal studies. In general toxicology studies conducted in rats and dogs, findings in both males and females suggest that impairment of fertility in humans is likely, though there is insufficient data to predict possible long term effects on fertility after discontinuing regorafenib. Findings of changes in the epiphyseal growth plate and alterations in dentin suggest increased toxicity in developing organs and have been observed with other compounds that inhibit VEGFR signaling. These findings may be more relevant to a pediatric patient population. No unique toxicities were identified in 1-month repeat dose toxicology studies conducted in mice administered the M-2 or M-5 metabolites. While studies of animals administered regorafenib itself included findings of significant renal toxicity, in studies examining only the metabolites significant renal toxicity was not noted. This difference may account for higher levels of renal toxicity seen in animals compared to humans at the time of NDA submission.

Dedicated studies exploring the cardiovascular safety of regorafenib were conducted both *in vitro* and *in vivo*. In *in vitro* experiments, while regorafenib itself showed low potential for QTc prolongation, the M-2 and M-5 metabolites each showed higher potential, though neither regorafenib nor the metabolites had clearly adverse effects on cardiovascular or hemodynamic endpoints in single dose *in vivo* studies. Cardiovascular endpoints were also included in repeat-dose toxicology studies. In rats, histopathological findings in the heart included perivascular/interstial edema and pericarditis in a 4-week study and thickening of the atrioventricular valve in a 26-week study. In dogs none of the studies revealed significant changes in ECG paratmeters or significant histopathological changes. Hypertension and cardiac ischemia and infarction are included as Warnings for regorafenib. A QT study of regorafenib in patients is ongoing.

Though dedicated studies to examine the effects of regorafenib on fertility and pre- and post-natal development were not conducted to support the use of the drug in patients with advanced cancer, embryofetal development studies were conducted in 2 species—Wistar rats and Himalayan rabbits. When administered regorafenib during the period of organogenisis, pregnant animals of both species showed increases in post-implantation loss at dose levels resulting in exposures significantly lower than those achieved in humans at the clinically recommended dose. Teratogenic effects observed in litters from these animals included skeletal and cardiovascular malformations and renal findings of dilation of the renal pelvis or hydronephrosis. Pregnancy Category D is recommended. In distribution and excretion studies performed in pregnant rats, high levels of regorafenib (measured by levels of radioactivity following administration of radiolabeled drug) were present in the mammary glands and in milk. The milk-plasma ratio for regorafenib exposure based on AUC was 6.8. These studies demonstrate that regorafenib is excreted in milk in rats and suggest a high risk of neonatal exposure to regorafenib in breast milk from women taking Stivarga.

Regorafenib was negative for genotoxic potential in both *in vitro* and *in vivo* assays; however, the M-2 metabolite was positive for clastogenicity. In addition, two impurities were clearly identified as genotoxic in the Ames assay. For one of these 2 impurities, the Applicant has proposed a specification of (b) (4) At this level, the highest dose of the impurity delivered to patients through regorafenib would be (b) (4) Although (b) (4) exceeds the theoretical

threshold of toxicology concern of 1.5 µg/day for genotoxic impurities, this threshold is based on a lifetime risk of carcinogenic potential for a compound and, thus, as discussed in ICH S9 does not adequately reflect the risk/benefit consideration for a patient population with advanced cancer. With the risk/benefit consideration of the metastatic colorectal cancer patient population in mind, the (b) (4) dose level at the proposed specification was considered acceptable for this impurity. A second, non-genotoxic, impurity was identified during the course of the review as being above the level for qualification. The specification for this impurity is (b) (4) and it was qualified in the 13-week dog study.

Recommendations: I concur with the conclusion of Drs. Goheer and McDougal that the pharmacology and toxicology data support the approval of NDA 203085 for STIVARGA. There are no outstanding nonclinical issues related to the approval of STIVARGA for the treatment of patients with metastatic colorectal carcinoma.

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/s/

WHITNEY S HELMS
09/10/2012

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA

NDA Number: 203-085 **Applicant:** Bayer Healthcare **Stamp Date:** April 27, 2012

Drug Name: Regorafenib (Stivarga®) **NDA Type:** new molecular entity (NME)

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	√		CTD format
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	√		Electronic submission
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	√		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	√		Carcinogenicity – not done/not required Mutagenicity – done Teratogenicity – done Fertility – not required Juvenile studies – not done/not required Acute and repeat dose toxicity – done (6-month in rats and 52-week in dogs) ADME – done Safety pharmacology – done (cardio, neuro, renal, gastrointestinal, pulmonary, local tolerance)
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	√		Similar formulations were used in pivotal preclinical and clinical studies
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	√		Same route of administration
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	√		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA

	Content Parameter	Yes	No	Comment
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	√		
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	√		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	√		Appears sufficient for filing. This issue is under review.
11	Has the applicant addressed any abuse potential issues in the submission?		√	Does not constitute a filing issue
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable. This NDA is NME

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? _YES__

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant. **N/A**

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter. **None**

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/s/

M A GOHEER
05/25/2012

ANDREW J MCDOUGAL
05/25/2012