List of Abbreviation

In addition to the legally regulated abbreviations of the basic SI units and the prefixes for decimal multiples and fractions of units, the following abbreviations are used:

a.m.	Ante meridiem (= before noon)
APh	Alkaline phosphatase
AUC	Area under the concentration-time curve
CHOL	Cholestrol
C_{max}	Maximum of concentration-time curve
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic acid
ERY	Erythrocytes
F	Female
FIBR	Fibrinogen
HB	Hemoglobin
HCT	Hematocrit
HDO	High definition oscillometry
M	Male
Med.	Median
Min.	Minimum
Max.	Maximum
MONO	Monocytes
N/A	Not applicable
NOAEL	No-Observed-Adverse-Effect-Level
PEG	Polyethylene glycol
PTT	(Activated) Partial Thromboplastin Time
p.m.	Post meridiem (= afternoon)
QT	QT-interval
RA	Ratio of accumulation
rel.	Relative
S.D.	Standard deviation
THRO	Thrombocytes
TT	Thrombin time
T3	Liothyronine (L-Triiodothyronine)
T4	L-Thyroxine (L-Tetraiodothyronine)
%	Percent
-	Not statistically significantly different from controls (used only in the Annex)
+/*	Difference compared to controls statistically significant with $p \leq 0.05$
++/**	Difference compared to controls statistically significant with $p \leq 0.01$

For further abbreviations see respective Appendices.

1. Summary

1.1 Study Objectives

BAY 123 is a potent, What-Ever-Inhibitor in development for the treatment and prophylaxis of something.

In order to investigate the toxicological profile of BAY 123, a systemic toxicity study was performed in Beagle dogs with once daily oral (gavage) administration over a period of approximately 4 weeks. Recovery of potentially treatment-related effects was evaluated in a 4 weeks post-treatment period.

1.2 Description of Study

3 male and 3 female Beagle dogs per group were administered with a solution of BAY 123 in PEG400 as vehicle by daily oral (gavage) administration at doses of 0, 2, 6 and 20 mg/kg over a period of approximately 4 weeks (28-31 administrations) with an application volume of 2.0 mL/kg undergoing necropsy one day after the last treatment.

A control group of 3 males and 3 females was treated with an equivalent volume of the vehicle in a similar manner. Further 2 males and 2 females of control and high dose group were treated likewise and were used as recovery groups undergoing necropsy 4 weeks after end of treatment.

Possible effects of BAY 123 were evaluated using clinical parameters (mortality, general observation, food intake, body weight, ophthalmoscopy, body temperature, nervous function tests, ECG, heart rate and blood pressure), clinical pathology (hematology and hemostasis, clinical chemistry, thyroid hormones, urinalysis) and full post mortem examination including necropsy, organ weight analysis and microscopic examination, as well as determination of liver enzyme activity.

For toxicokinetic evaluation, the concentrations of BAY 123 were determined in plasma samples taken on days 1/2 and days 28/29 at 1, 2, 4, 7, and 24 h after administration, respectively. In week 5, additional samples were taken at 48 and 96 hours after the last administration from recovery animals.

1.3 Summary of Results

The results of clinical, clinical pathology as well as post mortem examinations are summarized in Table 1–1.

Table 1–1: Noteworthy and Potentially Test Item-related Findings including Lowest Dose of Occurrence

Endpoint	Findings	Sex	Dose [mg/kg]
Mortality	Premature sacrifice 1 animal on dosing day 17	F	20
Clinical findings	Changed feces consistency (pasty, increased incidence)	M+F	20
	Increased incidence of salivation and vomiting after administration	M+F	20
	Reduced motoric activity/apathy (sporadically)	M+F	20
Body weight	Decrease in body weight	M+F	20
Food intake	Reduced food intake in individual animals beginning in week 1	M+F	20
Hematology	Decreases in ERY, HB, HCT and THRO	M+F	20
	Increase in MONO	M+F	20
Hemostasis	Prolongation of PTT starting from day 2	M+F	6
	Increase in FIBR starting from day 9	M+F	20
	Prolongation of TT starting from day 9	M+F	20
Clinical Chemistry	Increase in APh	M/F	20/6
	Increase in CHOL	M+F	20
	Decrease in albumin, relative albumin and albumin/globulin ratio	M+F	20
	Increases in alpha, beta and gamma globulins	M+F	20
Necropsy	Blood clot in the cervical spinal cord	F	20
Histopathology	Infiltrates of the granulocytic cell lineage in the liver	M+F	2
	Infiltrates of the granulocytic cell lineage in the choroid plexus	M+F	2
	Subarachnoidal hemorrhage in the spinal cord	M+F	2
	Increased granulopoiesis in bone marrow of sternum and femur	M+F	6
	Hypercellularity in the bone marrow	M+F	20
	Single cell necrosis in the liver	M+F	20
	Increased erythro- and megakaryocytopoiesis in the spleen	M+F	20
	Tubular degeneration and Sertoli cell vacuolation in the testes	M	20

M = male F = Female
M+F = in males and females at the same dose level
M/F = in males and females at different dose levels

At the end of the four weeks treatment-free observation period, all effects showed a tendency towards reversibility or were no longer apparent.

Toxicokinetic evaluation revealed no sex-related differences in exposure, the increase in exposure was more than dose-proportionate in terms of AUC(0-24) as well as in terms of C_{max} with individual t_{max} found to be between 1 and 7 h for all dose groups and trough concentrations ranging from 6.8 to 27.7% of the respective C_{max}. No significant change of exposure was observed after repeated dosing.

Table 1–2: Systemic Exposure Summary (Day 28)

Dose	[mg/kg]	2		6		20	
Parameter	Unit	**gMean**	**gSD**	**gMean**	**gSD**	**gMean**	**gSD**
AUC(0-24)	µg·h/L	11700	1.67	49300	1.23	260000	1.45
$AUC(0\text{-}24)_{norm}$	kg·h/L	5.86	1.67	8.21	1.23	13.0	1.45
C_{max}	µg/L	1180	1.51	4470	1.14	17000	1.35
$C_{max,norm}$	kg/L	0.591	1.51	0.746	1.14	0.850	1.35
t_{max}	h	2.83	1.46	2.52	1.76	3.65	1.68
$C(24)/C_{max}$	%	6.8	2.31	11.0	1.78	27.7	1.69
RA-AUC(0-24)	%	99.1	1.54	121.4	1.25	151.9	1.49
RA-C_{max}	%	108.9	1.53	125.4	1.18	154.3	1.41

1.4 Conclusion

The daily oral administration of BAY 123 at doses of 0, 2, 6 and 20 mg/kg to Beagle dogs over a period of 4 weeks revealed test item-related findings beginning at the low dose of 2 mg/kg. Liver, brain, spleen, bone marrow and testes were identified as target organs of toxicity. Findings in liver and choroid plexus started at 2 mg/kg, in the bone marrow at 6 mg/kg and in all other affected organs at the high dose of 20 mg/kg.
At the end of the four week recovery period, all effects were reversible or showed a tendency towards normalization.

A no-observed-adverse-effect-level (NOAEL) could not be established. At the high dose of 20 mg/kg an AUC of 260 mg·h/L and a C_{max} of 17 mg/L were determined.

2. Introduction

BAY 123 is a potent, What-Ever-Inhibitor in development for the treatment and prophylaxis of something.

For risk assessment of first use in humans and in order to support treatment up to 4 weeks , a 4-week toxicity study was performed to evaluate the systemic tolerability of the test item after repeated oral administration in Beagle dogs. This included the identification of possible target organs of toxicity, the dose-response-relationship and the evaluation of the no-observed-adverse-effect-level (NOAEL).

3. General Information

3.1 Test Facility and Test Site(s)

The in-life part of the study, clinical pathology and pathology were conducted at Bayer AG, GLP test facility Nonclinical Drug Safety (NDS), 13353 Berlin, Germany.

The following examinations were performed at other test sites:

The test item analytics in the formulation, the determination of thyroid hormones and liver enzyme activity as well as the toxicokinetics investigation were performed at Bayer AG, GLP test facility Early Development Bayer, 42096 Wuppertal, Germany.

3.2 Responsibilities

Table 3–1 gives an overview of responsibilities of persons involved in the study.

Table 3–1: Responsibilities

Responsibility	Name	Affiliation/Address
Study Director:		NDS, Berlin
Study Director from 08 to 11 Apr 2017		NDS, Berlin
Head of Experimental Toxicology:		NDS, Berlin
Head of Test Facility:		NDS, Berlin
Animal experimentation, preparation of Formulations, clinical examinations:		NDS, Berlin
Head of Investigational Toxicology:		
Clinical Pathology:		NDS, Berlin
Head of Pathology:		NDS, Berlin
Pathology:		NDS, Berlin
Histopathology cross check:		NDS, Berlin
Electrocardiography and Blood Pressure Investigation:		NDS, Berlin
Toxicokinetic Evaluation:		EDB, Wuppertal
Analysis of Test Formulation:		EDB, Wuppertal
Clinical Pathology (Thyroid Hormones)		EDB, Wuppertal
Determination of Liver Enzyme Activity		EDB, Wuppertal
Head of Archiving:		NDS, Berlin
Head of Quality Assurance:		NDS, Berlin

3.3 Key Study Data

Table 3–2 gives an overview on key study data.

Table 3–2: Key Study Data

Study Identification	
Study ID	T100
Pathology reference number	100
Animals	
Species	Dog
Strain	Beagle
Delivery of animals	16 Mar 2017
Animal age at start of treatment	10.5-11 months
Body weight at start of treatment	
Males	6.5 - 11.5 kg
Females	5.8 - 7.6 kg
Study Dates	
Study initiation date	23 Mar 2017
Experimental starting date	11 Apr 2017
Total duration of study	8 weeks
End of in-life-phase (= last day of last necropsy)	07 Jun 2017
Duration of treatment - Main groups	4 weeks
Duration of treatment - Recovery groups	4 weeks
Duration of recovery period	4 weeks
Necropsy of main groups	09 to 12 May 2017
Necropsy of recovery groups	06 to 07 Jun 2017
Experimental completion date	18 Aug 2017
Study completion date	see Signature Date of Statement of Compliance

3.4 Archiving

The study protocol, raw data, specimens and final report are retained in the archive of the GLP test facility Nonclinical Drug Safety, Bayer AG, 13353 Berlin, Germany .

Raw data of the study phases toxicokinetics, formulation analysis, thyroid hormone and liver enzyme activity determination as well as a retention sample of the test item are stored in the archive of the GLP test facility Early Development Bayer, Bayer AG, 42096 Wuppertal, Germany.

4. Materials and Methods

4.1 Guidelines

This study was conducted in compliance with:

Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 (Official Journal of the European Communities L 311/67 of 28 November, 2001) in its current version as amended in Commission Directive 2003/63/EC of 25 June, 2003 (Official Journal of the European Communities L 159/46 of 27 June 2003).

Note for Guidance on Repeated Dose Toxicity (CMP/SWP/1042/99 corr.), 27 July 27 2000 and 18 March 2010.

Note for Guidance on Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals (CPMP/ICH/286/92), June 2009

ICH Topic S 3 A: Toxicokinetics: A Guidance for Assessing Systemic Exposure in Toxicology Studies (CPMP/ICH/384/95); 1 June 1995.

4.2 Test Item and Formulation

The following tables give an overview of key study data concerning the test item and formulations (Table 4–1) and control article (Table 4–2).

Table 4–1: Test Item and Formulation

Test article/item	
Code number/test item	BAY 123
Batch number	BTR7ND9
Purity/Concentration	94.8 %
Expiry date	23 Jul 2017 dated
Certificate of analysis	13 Mar 2017 solid,
Appearance	yellow room
Storage	temperature
Manufacturer	Bayer AG
Formulations	
Type of formulation	Solution
Batch numbers and Amount	32433-A01 bis 04: 10 mg/mL
of Test Item per Unit	23433-B01 bis 04: 3 mg/mL
	23433-C01 bis 04: 1 mg/mL
Stability	15 days
Storage of formulations	Room temperature, protected from light
Vehicle composition	PEG400 (100%)
Manufacturer	Bayer AG

Table 4–2: Control Article and Formulation

Control Article	
Vehicle Composition	PEG400
Batch number	BFBS1795
Stability	May 2018
Manufacturer	Sigma-Aldrich, Saint Louis, USA
Control Formulation	
Type of formulation	Solution
Batch numbers	32433-P01 bis 04
Stability	15 days
Storage of formulation(s)	Room temperature
Manufacturer	Bayer AG

4.2.1 Preparation of Formulations

The formulations were prepared taking into account the analytically determined stability of 15 days.

4.2.2 Analytics Prior to Start of Treatment

Prior to the start of treatment, the test formulation was checked with regard to accuracy of concentration and stability of dosage forms prepared in the same way as it was done in the study. Analyses were carried out before the start of the study under T.

4.2.3 Analytics During the In-life Phase

During the in-life phase of the study, the test formulation was checked with regard to accuracy of concentration and homogeneity as well as content of test item in the test formulation for the formulations prepared on day -1 and day 23.

4.3 Study Design

4.3.1 Rationale for Selection of Species, Route of Administration and Dose Levels

The dog was selected since it is one of the internationally recommended non-rodent species for toxicological characterization of new drugs. Furthermore, a vast amount of background data is available for this species. Furthermore, the dog is responsive to the pharmacological mode of action of BAY 123 and, based on in vitro data, is human like in terms of metabolism of BAY 123.

The oral route of administration corresponds to the intended clinical route in humans.

In a preceding pilot study (T) in dogs, BAY 123 was administered at doses up to 30 mg/kg over a period of 2 weeks. Up to the mid dose of 10 mg/kg BAY 123 was generally well tolerated. With increasing dose, an increased incidence of vomiting was noted, at 30 mg/kg also reduced food intake and body weight loss was seen. In addition, liver, thymus, thyroid and testes were identified as potential target organs of toxicity. Based on these results, the high dose of 20 mg/kg was chosen. This dose was estimated to result in 100-fold of expected human therapeutic exposure in terms of $AUC_{unbound}$. The low dose of

2 mg/kg was estimated to represents about 2 times the expected human exposure, the mid dose of 6 mg/kg was selected to allow for characterizing a dose-response relationship.

4.3.2 Dosing Schedule

The test item was administered once daily by oral (gavage) administration to the animals from the first day of treatment until scheduled end of treatment or prescheduled sacrifice.

The dosing schedule and the distribution of the animals to the groups are given in Table 4–3.

At all dose levels, 3 animals per dose and sex were used as main groups treated once daily for 28 to 31 days until one day prior to scheduled sacrifice.

In addition, 2 animals per dose level and sex at the control and the high dose level only were used as post treatment observation collectives treated for a period of 28 days and then observed for further 29 to 30 days until scheduled sacrifice.

Control and test item treated animals were handled each in the same manner.

Table 4–3: Dosing Schedule

Group	Dose [mg/kg]	Subgroup	Subgroup	ARMCD	No of Animals	Gender	Animal Numbers
1	0	1	Main	G1A1	3	M	0101-0103
1	0	2	Recovery	G1A2	2	M	0104-0105
2	2	1	Main	G2A1	3	M	0106-0108
3	6	1	Main	G3A1	3	M	0109-0111
4	20	1	Main	G4A1	3	M	0112-0114
4	20	2	Recovery	G4A2	2	M	0115-0116
1	0	1	Main	G1A1	3	F	0117-0119
1	0	2	Recovery	G1A2	2	F	0120-0121
2	2	1	Main	G2A1	3	F	0122-0124
3	6	1	Main	G3A1	3	F	0125-0127
4	20	1	Main	G4A1	3	F	0128-0130
4	20	2	Recovery	G4A2	2	F	0131-0132

M = male; F = female
ARMCD = Arm-Code
G = Gruppe

The administration volume of 2.0 mL/kg was adjusted to actual body weight on day 1 and 7 in week 1 and twice weekly from in week 2 onwards. The animals of group 1 (control group) received the vehicle only.

4.4 Randomization of Animals to Study Groups

Randomization of the dogs to study groups was done online using a randomization procedure within the Pristima® System taking the body weights into account.

4.5 Experimental Animals and Housing Conditions

Data on experimental animals used and housing conditions are shown in the following Table 4–4.

Table 4–4: Experimental Animals and Housing Conditions

Species	Dog
Strain	Beagle
Breeder	Marshall Farms, USA
Husbandry	Under conventional conditions
Location	In-life phase in S108, room -1.502 / -1.503
Acclimatization	14 days
Animal identification	Tattoo number, Chip number (Transponder)
Cage type and number of animals per cage	one animal per cage after dosing and during feeding time, otherwise cages connected for group housing (up to 3 animals per group)
Enrichment	Denta Fun Rugbyball and other dog enrichtment articles (Fa. Trixie Heimtierbedarf GmbH & Co. KG, Industriestr. 32, 24963 Trier)
Food	Ssniff® HD-H (ssniff Spezialdiaeten GmbH, 59494 Soest, Germany)
Water	Tap water ad libitum, Specification of contaminants: According to actual German drinking water standards
Room Temperature	20.0 - 26.1°C
Relative Humidity	54 - 63 %
Air Change	> 10 per hour
Light/Dark Regimen	12h/12h (artificial light: 06:00 a.m. to 06:00 p.m.) Radio program playing during the light period.

4.6 Schedule of Investigation

An overview of scheduled activities is given in Table 4–5.

Table 4–5: Scheduled Activities

Clinical Observation	Pre-doing: once daily Dosing: twice daily (a.m./p.m.) incl. inspection for morbidity and mortality Recovery: week 1: twice daily, from week 2 onwards: once daily
Body Weight	Pre-Random: once Pre-Dosing: once Dosing: week 1: day 1 and 7, from week 2 onwards: twice weekly Recovery: once weekly
Food Intake	Daily Addition of wet food in individual animals
Ophthalmology	Pre-Dosing: once Dosing: week 3 Recovery: week 4
Neurological Examination	Pre-Dosing: once Dosing: week 3, 2-3 hours after administration Recovery: week 4
Body Temperature	Pre-dosing: once Dosing: week 3, 2-3 hours after administration Recovery: week 4
ECG, Heart rate	Pre-dosing: once Dosing: week 1 and 4, before and 2-3 hours after administration Recovery: week 4
Blood pressure	Pre-dosing: once Dosing: week 1 and 4, before and 2-3 hours after administration Recovery: week 4
Hematology	Pre-Dosing: once Dosing: days 9, 17 (animal 131) and 25 Recovery: week 4
Hemostasis	Pre-Dosing: once Dosing: days 2, 9, 17 (animal 131) and 25 Recovery: week 4
Clinical Chemistry	Pre-Dosing: once Dosing: days 2, 17 (animal 131) and 25 Recovery: week 4
Thyroid Hormones	Pre-Dosing: once Dosing: day 25 Recovery: week 4
Urinalysis	Pre-Dosing: once Dosing: week 4 Recovery: week 4
Toxicokinetics	Dosing Day 1/2, 16 (only animal No 131) and 28/29 at 1, 2, 4, 7 and 24 h after administration as well as 48 and 96 h after the last administration in recovery animals
Necropsy	Dosing Day 29 to 32 (main groups) Recovery Day 29 to 30 (recovery groups)

4.7 Clinical Examinations

4.7.1 Clinical Observations

On working days the experimental animals were inspected twice daily for morbidity and mortality.

Detailed clinical observations or in-cage observations were performed once daily in the pre-dosing period and twice daily (once in the morning prior to, during and immediately after administration, once in the afternoon) in the dosing period. In the recovery period, clinical observation was performed twice daily in recovery week 1 and once daily from recovery week 2 onwards. Any clinical findings and abnormalities were recorded. Body surfaces and orifices, posture, general behavior, breathing and excretory products were assessed, findings and abnormalities were recorded.

On weekends, the last check was carried out just before the technicians left the laboratory in the late morning. The check consisted of an evaluation of the general condition of each individual animal. All alterations from baseline condition of the animals and time of scheduled sacrifice were recorded.

4.7.2 Body Weights

The body weights of the animals were determined once in the pre-random period to support body weight based randomization, once in the predosing period, twice weekly in the dosing period and once weekly in the recovery period. Cumulative body weight gain was calculated at all measurement time points.

Furthermore, body weights were recorded immediately before scheduled necropsy from exsanguinated animals for calculation of organ weights in % of body weights.

4.7.3 Food Intake

Food intake was recorded daily per animal during the pre-dosing period (pre-dosing phase day 5 to day 11), during the dosing period daily for all animals from day 1 to day 28 and during the recovery period daily from day 1 to 29 of post-dosing phase.

On the basis of these data the following parameters were calculated:

- For each feeding interval: Mean daily food intake per group.

In addition, in the afternoon canned food was offered to individual animals with significantly reduced or absence of intake of dry food. The intake of canned food was documented separately.

The statistical calculations were performed for each measurement period. Statistical significant differences on individual days were only discussed if considered indicative for test-item related changes.

4.7.4 Neurological Examinations

The following neurological examinations were performed in week 1/2 of the pre-dosing phase (pre-value), in week 3 of the dosing period 2-3 hours after treatment and in week 4 of the recovery period:

Brain reflexes:	pupillary reflex, corneal reflex
Spinal nerve reflexes:	patellar reflex
Attitudinal reactions:	extensor, startle and flexor reflex

A detailed description of the tests is given in Appendix - In-Life Data - Material and Methods.

4.7.5 Body Temperature

The rectal body temperature was determined using an electronic thermometer once in the pre-dosing period (pre-value), in week 3 of the dosing period 2-3 hours after administration and in week 4 of the recovery period. A detailed description of the test is given in Appendix - In-Life Data - Material and Methods.

4.7.6 Ophthalmology

Ophthalmologic examinations were carried out in all animals in the pre-dosing period, in weeks 3 of the dosing period and in week 4 of the recovery period.

The pupillary reflex of both eyes was first tested in a darkened room and the anterior regions of the eye were inspected. After dilating the pupils with Mydriaticum Stulln® drops, the refractive elements of the eye as well as iris and fundus were examined using an fundus camera. In addition, the optical media were examined with a slit lamp microscope.

4.7.7 Electrocardiography and Blood Pressure

Electrocardiography (ECG) and blood pressure measurements were conducted once pre-dosing, and once in week 1 and week 4 (before and 2-3 h after administration) of the dosing period, and in Week 4 of the recovery period (see Appendix - In-Life Data Individual Data and Appendix - In-Life Data Summary Data).

4.7.7.1 ECG

ECG measurements were performed using the Ponemah data acquisition system (Version 5.1). During the procedure the dogs were fixed in lateral position and subcutaneous ECG needle electrodes were positioned in standard limbs configuration I, II, III, aVR, aVL, and aVF. The data were collected of a period of at least 30 sec and stored on the hard disk of the Ponemah system.

4.7.7.2 Blood Pressure

Blood pressure was measured indirectly using the high definition oscillometry (HDO) method. For this purpose the dogs were fixed in lateral position and equipped with a tail cuff connected to the HDO system. The measured blood pressure values (systolic and diastolic) of at least 2 (up to 6) measurements were averaged and documented manually.

4.8 Clinical Pathology

Blood samples for clinical pathology were collected according to the schedule given in Table 4–5 by puncture of the vena jugularis.

The methods used are listed in the App – In Life Data Materials and Methods.

4.8.1 Hematology and Hemostasis

The samples for the hematological determinations and for thrombocyte count were collected in tubes coated with EDTA (anticoagulant) and the samples for the determinations of the hemostasis parameters were collected in tubes with sodium-citrate.

The following parameters were determined in peripheral blood of 3 to 5 males and 3 to 5 females per group (pre-random and dosing) and of 2 males and 2 females of groups 1 and 4 (recovery):

Table 4–6: List of Parameters for Hematology and Hemostasis

Hematology		**Hemostasis**
Erythrocyte count	Leucocyte count	Partial thromboplastin time
Hemoglobin concentration	Differential blood count:	Fibrinogen
Hematocrit	Neutrophils	Prothrombin time
Mean corpuscular hemoglobin	Lymphocytes	Thrombin time
Mean corpuscular hemoglobin concentration	Eosinophils	Thrombocyte count
Mean corpuscular volume	Basophils	
Reticulocyte count	Monocytes	
	Large unstained cells/ atypical lymphocytes	
	Lobularity index	

4.8.2 Clinical Chemistry

The samples for the determinations of the clinical chemistry parameters were collected in tubes with gel/clot activator.

The following parameters were determined in the serum of 3 to 5 males and 3 to 5 females per group (pre-random and dosing) and of 2 males and 2 females of groups 1 and 4 (recovery):

Table 4–7: List of Parameters for Clinical Chemistry

Enzymes	**Substrates**	**Electrolytes**
Alanine aminotransferase[a]	Glucose	Chloride
Aspartate aminotransferase[a]	Cholesterol	Calcium
Alkaline phosphatase[a]	Triglycerides	Potassium
Gamma glutamyl transferase[a]	Creatinine	Sodium
Glutamate dehydrogenase[a]	Urea nitrogen	Inorganic phosphate
Creatine kinase[a]	Total bilirubin	Magnesium
Lactatdehydrogenase[a]	Total protein	
	Albumin	
	Protein electrophoresis (incl. albumin/globulin ratio)	

[a] These parameters composed the limited panel determined on day 2

4.8.3 Urinalysis

The following parameters were determined in the urine of 3 to 5 males and 3 to 5 females per group (pre-random and dosing) and of 2 males and 2 females of groups 1 and 4 (recovery):

Table 4–8: List of Parameters for Urinalysis

Quantitatively	Semiquantitatively
Osmolality	pH
	Glucose
	Protein
	Blood
	Bilirubin
	Ketone bodies
	Urobilinogen

4.9 Determination of Thyroid Hormones

Serum samples were analyzed for L-thyroxine (T4), L-triiodothyronine (T3) and thyroid stimulating hormone (TSH). Hormone concentrations were measured using appropriate methods (for details see Appendix - Clinical Pathology (2)).

4.10 Pathology

4.10.1 Necropsy and Determination of Organ Weights

All animals alive on the date of their scheduled necropsy and all animals to be sacrificed prematurely were sacrificed by exsanguination under Pentobarbital-injection intravenously to complete euthanasia. On all animals a full necropsy was performed. The necropsy was a systematic gross examination of each animal's general physical condition, body orifices, external and internal organs and tissues.

The body weights were recorded immediately before necropsy from exsanguinated animals and the weight of the organs listed in Table 4–9 were determined and used for calculation of organ weights in % of body weights.

4.10.2 Histopathology

Organs and tissues or representative pieces of them were processed for histopathology as listed below in Table 4–9. Testes, epididymides and eyes were fixed in Davidson's fixative, the pituitary gland in Bouin’s solution, all other organs in 10 % neutral buffered formalin.

Prior to immersion fixation, the left caudal lobe of the lungs was instilled with 10 % neutral buffered formalin and then postfixed in 10 % neutral buffered formalin.

Osseous tissues (femur, sternum) were first decalcified and then like all other organs, embedded in Paraplast®.

Sections were prepared from the organs assigned to histopathological examination. Slides were stained with hematoxylin and eosin (H&E).

For technical reasons other organs may have been cut and stained, however they were not evaluated.

Bone marrow smears were prepared and stained according to the method of May-Gruenwald-Giemsa but not evaluated histologically.

All tissues were examined by a veterinary pathologist with experience in evaluating laboratory animal tissues.

In addition, a peer review was performed comprising the target organs (liver, kidneys, spleen, testes, brain, femur) with identification of the no-observed-adverse-effect-level (NOAEL) as well as all slides of 1 animal per sex of dose group 4 for terminal sacrifice, 1 male of dose group 4 sacrificed at the end of the recovery period and 1 female animals sacrificed prematurely on day 17 of the dosing period. The findings reported are those agreed-upon between the cross-checking and the study pathologist.

The following organs were evaluated histopathologically.

Table 4–9: Organs Fixed, Selected for Weighing and for Histological Examination (continued)

Organs	Organ weights	Fixative	Histopathological evaluation
Abnormalities		Fo	X
Adrenal glands	X	Fo	X
Aorta		Fo	X
Brain (7 regions)	X	Fo	X
Epididymides	X	D	X
Esophagus		Fo	X
Eyes (with optic nerve)		D	X
Femur (with bone marrow/ joint)		Fo	X
Gallbladder		Fo	X
Heart	X	Fo	X
Intestines		Fo	
- Duodenum			X
- Jejunum			X
- Ileum			X
- Cecum			X
- Colon			X
- Rectum			X
Kidneys	X	Fo	X
Lacrimal glands		Fo	
Larynx		Fo	X
Liver	X	Fo	X
Lungs		Fo[a]	X
Lymph node, mandibular		Fo	X
Lymph node, mesenteric		Fo	X
Lymph nodes, iliac		Fo	X
Mammary gland		Fo	X
Nose		Fo	
Ovaries	X	Fo	X
Oviducts		Fo	X

Table 4–9: Organs Fixed, Selected for Weighing and for Histological Examination (continued)

Organs	Organ weights	Fixative	Histopathological evaluation
Pancreas		Fo	X
Pituitary gland		Bo	X
Prostate gland		Fo	X
Salivary glands		Fo	
- Sublingual gland			X
- Submandibular gland			X
- Parotid gland			X
Sciatic nerve		Fo	X
Skeletal muscle (M. sartorius)		Fo	X
Skin (back - lumbar region)		Fo	X
Spinal cord		Fo	
- cervical			X
- thoracic			X
- lumbar			X
Spleen	X	Fo	X
Sternum (with bone marrow)		Fo	X
Stomach		Fo	X
Testes	X	D	X
Thymus	X	Fo	X
Thyroid / Parathyroid glands		Fo	X
Tongue		Fo	X
Tonsils		Fo	
Trachea		Fo	X
Ureters		Fo	X
Urinary bladder		Fo	X
Uterus	X	Fo	
- Horns			X
- Cervix			X
- Corpus			X
Vagina		Fo	X
Vein (Vena cava caudalis)		Fo	

X	Conducted
D	Davidson's fixative
Fo	neutral buffered Formalin
Bo	Bouin‘s solution
a	Parts fixed by instillation

The tissue slides were examined by a veterinary pathologist. In addition, a formal cross-check was performed. The findings reported are those agreed-upon between the cross-checking and the study pathologist. For details see Appendix - Pathology.

4.11 Activity of Metabolizing Enzymes in the Liver

For determination of the activity of a panel of enzyme activities, liver specimen were taken at necropsy and frozen on dry ice and kept at ≤ -15 °C until investigation.

Enzyme activities were measured in appropriate tissue preparations using biochemical and chemical-analytical methods.

The activity of the enzymes listed in Table 4–10 were determined in homogenized liver:

Table 4–10: Enzymes Investigated in Liver Tissue

7-Ethoxycoumarin deethylase (ECOD)
7-Ethoxyresorufin deethylase (EROD)
Epoxide hydrolase (EH)
Glutathione-S-transferase (GS-T)
UDP-glucuronyltransferase (GLU-T)

4.12 Toxicokinetics

Plasma concentrations of BAY 123 were determined by a validated LC-MS/MS method in Beagle dogs. Starting on dosing Day 1 and on dosing Day 28 blood samples were collected at 1, 2, 4, 7 and 24 h after once daily oral administration of BAY 123. In the recovery groups, additional blood samples were collected 48 and 96 h after end of dosing on Day 28. Due to premature dissection, plasma samples of one animal were collected on dosing Day 16 instead of dosing Day 28.

Table 4–11: Frequency and Dates of Toxicokinetic Investigation

Days of Blood Sampling:	day 1/2 and 28/29
Time Points per Day:	5 (1, 2, 4, 7 and 24 h after administration)
Number of Animals per Time Point:	group 1+4: 5M+5F group 2+3: 3M+3F

The analytical methods used in this investigation as well as the results are presented in the Toxicokinetics Report (see Appendix - Toxicokinetics).

4.13 Statistical Evaluation

The procedure of collection, processing and evaluation of data is described in Appendix - In-Life Data Materials and Methods.

Statistical tests of clinical and clinical pathology parameter were performed using standard procedures within the Pristima System®. Statistical tests on body weights and weight gain as well as on absolute organ weights were done using the Dunnett Exact Homogeneous Test. For relative organ weights the Dunnett Exact Homogeneous Test after log. Transformation was used. The weights were entered on-line into the PathData® computer program. If appropriate, detailed statistical calculations were made using DUNNETT's test procedures available within the PathData® program.

The data on cardiovascular safety (blood pressure, heart rate, ECG) were transferred offline after termination of the respective investigation, into the Pristima System®. Descriptive statistics were provided per sex, dose group and time point for all parameters that were recorded with a specified unit. This included measures of general tendency (mean and median) and general variability (standard deviation, minimum and maximum) as appropriate. All statistical tests were performed using Dunnett Exact Homogeneous Test within the

Pristima system for comparison of treatment groups with the vehicle group. Due to the small number of animals no statistical test was performed in the recovery groups.

The statistical evaluation of T3, T4 and TSH in serum was carried out using Bonferroni/Mann-Whitney U-Test (addressed as “U-Test”) (Pristima® Suite).

With respect to toxicokinetic data the Toxkin program, Version 4.0.7.1.1 or higher (Entimo AG) was used for calculation of descriptive statistics by calculation of geometric means and standard deviations.

Statistical tests were not performed for groups, which were smaller than 3.

4.14 Deviations from Study Protocol

No relevant deviations from study protocol occurred.

The result of the investigation of the corneal reflex in animal No 115 on dosing day 16 session 2 was not documented in Pristima®. Since the corneal reflex was present in all other animals including high dose group, the deviation from the study plan has no impact on the outcome of the study.

5. Results

5.1 Mortality

One female animal (No. 0131) was sacrificed prematurely on day 17 of the dosing period for animal welfare reasons since a significantly impaired general condition was observed indicated by reduced food intake, body weight loss and the clinical findings apathy/reduced motility, lacrimation of the eyes, ungroomed coat and swelling around the muzzle prior to sacrifice.

In the post-mortem examinations hematoma and subacute to chronic inflammation in the subcutis around the snout and inflammation and edema in the conchae of the nose were the most probable causes for the clinical symptoms. No relation to the test item is postulated.

5.2 Clinical Observations

Changed feces consistency (pasty) was noted in all dose groups, but with significantly higher incidence at the high dose of 20 mg/kg.

A significantly increased incidence of vomiting was observed at the high dose of 20 mg/kg. Furthermore, increased salivation was noted sporadically.

Reduced motoric activity / apathy was observed sporadically in individual animals of the high dose group.

During the administration procedure vocalization, resistance to administration and aggression were noted temporarily in the female animal sacrificed prematurely on day 17 (No 131F). A relation to treatment is not considered and the observation is most likely related to the inflammation around the snout.

Further clinical findings such as swelling of the vulva or mammary gland, discharge from the vagina and sporadic lameness in one animal were not considered test item-related since they are signs of the hormonal cycle in females dogs or were noted only sporadically in individual animals.

The results of the clinical observations are presented in the form of cumulative group incidences in Appendix - In-Life Data - Summary Data and as individual findings per animal in Appendix - In-Life Data - Individual Data.

5.3 Body Weights

A decrease in body weight was noted in males and females at the high dose of 20 mg/kg.

In one female animal at the low dose of 2 mg/kg a body weight loss of 600g over the whole study period was noted despite of normal food intake. Besides sporadic diarrhea also observed in other animals no findings explaining this decrease were noted.

Body weights at the end of the dosing and the post treatment observation periods, are shown in Table 5–1.

Table 5–1: Overview on Development of Mean Body Weights (Control Data in kg, Treatment Groups Shown as Percent Change Compared to Control)

Sex	Males				Females			
Dose [mg/kg]	0	2	6	20	0	2	6	20
Main Group								
Day 29	9.08	+5	-3	-14	7.04	-4	-8	-10
Recovery Group								
Day 29	8.5	-	-	-7	6,95	-	-	-6

Group means with statistical data are shown in Appendix - In-Life Data - Summary Data. In addition, individual data for all animals are shown in Appendix - In-Life Data - Individual Data.

5.4 Food Intake

At the high dose of 20 mg/kg significantly reduced food intake in individual animals was reported beginning in week 1 of the study.
In these animals (No 115M, 116M, 131F and 132F), canned food was offered after regular feeding time in the afternoon which was taken up almost completely by these animals. In week 2, during a period of 3 days (dosing day 11 to 13) without canned food, in these animals only marginal intake of dry food was noted. Therefore canned food was offered again beginning on day 14 until the end of the dosing period (except animals 116 only until day 24) or premature sacrifice of animal No 131F.

Food intake during dosing and the recovery periods, are shown in Table 5–2.

Table 5–2: Overview on Development of Mean Food Intake (Control Data in g, Treatment Groups Shown as Percent Change Compared to Control)

Sex	Males				Females			
Dose [mg/kg]	0	2	6	20	0	2	6	20
Main Group								
Day 28	337	-1	-9	-54 *	250	-14	+7	-22
Recovery Group								
Day 28	176	-	-	+29	264	-	-	-26

* = $p \leq 0.05$
PTO Post treatment observation

Individual food intake data per animal per day including intake of canned food are presented in Appendix - In-Life Data - Individual Data. Mean values with statistical data are given in Appendix - In-Life Data - Summary Data.

5.5 Neurological Examinations

No test item-related findings were observed.

The summary tables are shown in Appendix - In-Life Data - Summary Data and the individual data are shown in Appendix - In-Life Data - Individual Data.

5.6 Body Temperature

No test item-related findings were observed.

Individual data per animal per day are presented in Appendix - In-Life Data - Individual Data. Mean values with statistical data are given in Appendix - In-Life Data - Summary Data.

5.7 Ophthalmology

No test item-related findings were observed.

Individual data are presented in Appendix - In-Life Data - Individual Data, summary tables are given in Appendix - In-Life Data - Summary Data.

5.8 Electrocardiography and Blood Pressure

5.8.1 ECG and Heart Rate

Compared to controls, no treatment related effects on heart rate and ECG intervals were found. Statistically significant change of the heart rate and RR interval found in males 2 - 3h after administration of 20 mg/kg in Week 1 is considered a chance finding since it was not found in Week 4 or in females of the 20 mg/kg group. A statistically significant prolongation of the QTcf interval (males, 20 mg/kg, Week 4, 2-3h) by 5% compared to QTcf before administration is regarded to be without biological relevance.

Incomplete bundle branch block was found in all ECG recordings (including pre-dosing) of 1 female dog (No. 0126) of the 6 mg/kg group. One female dog (No. 0129) of the 20 mg/kg group showed isolated 2nd degree AV-block (2:1, Mobitz type) in Week 4 (before and 2-3h after administration). Since occurrence of isolated 2nd degree AV-block is a background finding in Beagle dogs and found only in 1 out of 10 dogs treated with the high dose, this finding is regarded not to be test item-related.

5.8.2 Blood Pressure

Compared to controls and pretreatment data, no statistically significant changes in arterial blood pressure occurred under treatment with BAY 123.

Details on the results of ECG and blood pressure measurements are described in Appendix - Cardiovascular Assessment.

5.9 Clinical Pathology

5.9.1 Hematology and Hemostasis

At the low dose of 2 mg/kg hematology and hemostasis revealed no evidence for test item-related effects on red or white blood cell parameters or on blood coagulation.

Test item-related effects observed at the mid dose of 6 mg/kg and higher are summarized in Table 5–3. These include pharmacodynamic effects on hemostasis indicated by a prolongation of activated partial thromboplastin time starting at the mid dose of 6 mg/kg and a slight prolongation of thrombin time and a transient decrease in thrombocytes at the high dose of 20 mg/kg.
The decreases in ERY, HB and HCT at the high dose of 20 mg/kg point to an anemic status.

Table 5–3: Test Item-related Findings in Hematology and Hemostasis

	Findings	Sex	Dose [mg/kg]
Hematology	Decreases in ERY, HB and HCT in males on days 9 and 25 and in females on days 9 and 17 (No 131)	M+F	20
	Decrease in THRO in males on day 9 and in females on days 9 and 17 (No 131)	M+F	20
	Increase in MONO on day 25	M+F	20
Hemostasis	Prolongation of PTT beginning on day 2	M+F	6
	Increase in FIBR beginning on day 9	M+F	20
	Prolongation of TT beginning on day 9	M+F	20

M = male, F = Female
M+F = in males and females at the same dose level

Further parameters showed statistically significant differences to controls, however, as the changes were either without exposure dependence, too small to be biologically relevant or all values were within the known physiological range, they were not considered as test item-related.

The data for group means including statistics are shown in
Appendix - In-Life Data - Summary Data and the individual animal data can be found in
Appendix - In-Life Data - Individual Data.

5.9.2 Clinical Chemistry

At the low dose of 2 mg/kg clinical chemistry revealed no evidence for test item-related effects.

Test item-related effects observed at the mid dose of 6 mg/kg and higher are summarized in Table 5–4.

Table 5–4: Test Item-related Findings in Clinical Chemistry

Findings	Sex	Dose [mg/kg]
Increase in APh on day 25 in males and individual females starting from day 2	M/F	20/6
Increase in CHOL in males on day 25 and female animal (No 131) on day 17	M+F	20
Decrease in albumin in males on day 25 and females on day 17 (No 131) and day 25	M+F	20
Decreases in relative albumin and albumin/globulin quotient in males on day 25 and females on day 17 (No 131) and day 25	M+F	20
Increases in alpha, beta and gamma globulins in males on day 25 and females on day 17 (animal 131) and day 25	M+F	20

M = male F = Female
M+F = in males and females at the same dose level
M/F = in males and females at different dose levels

The changes in clinical chemistry parameters, such as the decrease in albumin in combination with the increases in alpha-, beta- and gamma-globulins as well as the increases in fibrinogen and monocytes noted at 20 mg/kg are indicative for an inflammatory response. Furthermore increases in total cholesterol and in alkaline phosphatase were seen at 20 mg/kg.

The data for group means including statistics are shown in Appendix - In-Life Data - Summary Data and the individual animal data can be found in Appendix - In-Life Data - Individual Data.

5.9.3 Urinalysis

Urinalysis did not reveal any test item-related effects up to the high dose of 20 mg/kg.

Individual urine data can be found in Appendix - In-Life Data - Individual Data and group means including statistics are shown in the Appendix - In-Life Data - Summary Data.

5.10 Determination of Thyroid Hormones

Measurement of T3, T4 and TSH did not provide evidence for a test item-effects.

Results are summarized in Table 5–5.

Table 5–5: Group Means of T3, T4 and TSH Serum Levels

Dose mg/kg	T3 nmol/l	T4 nmol/l	TSH µg/l
Males		**Day 1 - Pre-dosing**	
0	1.134	22.36	0.112
2	1.190	17.67	0.120
6	1.353	22.33	0.127
20	1.266	25.04	0.178
Males		**Day 25 - Dosing**	
0	1.248	19.68	0.126
2	1.343	18.60	0.107
6	1.323	17.00	0.203
20	1.384	12.84	0.242
Males		**Day 24 - Recovery**	
0	1.065	17.90	0.180
20	1.200	17.95	0.120
Females		**Day 1 – Pre-dosing**	
0	1.010	21.60	0.152
2	0.993	21.90	0.313
6	1.233	26.90	0.120
20	1.154	24.62	0.158
Females		**Day 25 - Dosing**	
0	1.060	20.92	0.200
2	0.993	18.80	0.133
6	1.090	20.87	0.163
20	1.223	16.13	0.215
Females		**Day 24 - Recovery**	
0	1.055	19.30	0.100
20	1.075	21.55	0.185

Further details on the results of the thyroid hormone determination can be found in Appendix - Clinical Pathology (2).

5.11 Activity of Metabolizing Enzymes in the Liver

Following administration of BAY 123 for four weeks a trend to increased ECOD activity in both sexes at ≥6 mg/kg and a trend to decreased EROD, EH and GLU-T activities mainly at 20 mg/kg were observed. These observations should not be considered to be of biological relevance. Importantly, in the absence of any increase of further enzyme activities, the modest increase of ECOD activity in both sexes should not be considered as an indication of a beginning induction of drug-metabolizing liver enzymes.

At the end of the recovery period trends to moderate decreases of EROD activity in both sexes and of GS-T activity in females was observed. The magnitude of these decreases was

small and these observations should not be considered to be of biological relevance. Furthermore, decreases of EROD activity are not considered to be of toxicological relevance.

On the whole, the observations made in this study do not suggest relevant treatment-related effects on liver drug metabolizing enzyme activities.

Details on the results can be found in Appendix - Biochemical Analytics Liver. This includes the individual animal data and group means including statistics of data.

5.12 Pathology

5.12.1 Necropsy

All gross findings recorded at necropsy are listed in tabulated form in the respective incidence tables in the Appendix - Pathology Data.

Necropsy did not reveal any test item related findings up to the dose of 6 mg/kg.

At the dose of 20 mg/kg, a blood clot in the cervical spinal cord in the prematurely sacrificed female dog was observed. This finding corresponds to acute subarachnoidal hemorrhage observed histologically.

Ventricular dilatation in the brain in male and female animals was observed across all groups. In the control group, one female animal at terminal sacrifice and two male animals after the post treatment period showed ventricular dilatation, whereas two male and one female animal at the mid dose and three male and two female animals at the high dose at terminal sacrifice were affected. A histologic correlation could not be observed, thus, due to the number of animals also affected in the control group, no relation to treatment is postulated.

5.12.2 Organ Weights

All organ weights are listed in tabular form in the Appendix - Pathology Data.

No test item-related effects on absolute or relative organ weight were found up to the high dose of 20 mg/kg.

The decrease in relative organ weight of the heart (- 13%) at the mid dose of 6 mg/kg BAY 123 is considered incidental as it is slight, not dose-related, observed in only one sex and unaccompanied by correlated morphological findings.

At the high dose, only the male dose group could be compared statistically to mean vehicle-control values, as one female animal was euthanized prematurely and the remaining number of animals was too small for statistical evaluations. Comparing individual values in female animals at the high dose to the weight range observed in vehicle-control animals, no test item-related changes at the high dose were detected.

The comparison of absolute and relative organ weights at the high dose post treatment group compared to the post treatment vehicle-control values did likewise not reveal any clear-cut test-item related changes.

5.12.3 Histopathology

All histopathological findings recorded are listed in tabular form in the respective incidence tables in the Appendix - Pathology Data. Noteworthy histopathological findings are presented in a separate incidence table in the Appendix - Pathology Data.

Test item related findings were observed at the low dose of 2 mg/kg and higher.

In the **liver**, cellular infiltrates of the granulocytic cell lineage were seen with up to moderate severity at terminal sacrifice. They were observed primarily as perivascular cuffs of granulopoietic cells, varying in maturity from immature to differentiated. In some areas, the cells appeared as vacuolated perivascular cuffs. At higher doses, unclassifiable cells and granulopoietic cells were additionally diffusely distributed in the sinus. At the high dose, some of these infiltrating cells and single hepatocytes underwent single cell necrosis. The observed glycogen depletion in several animals with up to moderate severity was observed without clear dose relation or sex prevalence. The female high dose animal which was in poor general condition and showed body weight loss before sacrifice showed marked glycogen depletion.

In the **choroid plexus** of the brain, cellular infiltrates of the granulocytic cell lineage similar to those seen in the liver were observed. They were minimal in one male low dose and one female mid dose animal and minimal to moderate at the high dose, where five of six animals were affected.

In the **bone marrow**, increased granulopoiesis (dysplasia of the bone marrow) was observed. First signs were seen in the sternal bone marrow in one male (slight) and one female (minimal) animal at the mid dose. At the high dose, all animals showed increased granulopoiesis in sternal and/or femoral bone marrow. The differentiation was mainly myeloblastic, however areas with differentiated granulocytes could be distinguished. At the high dose, up to moderate hypercellularity accompanied the dysplasia.

The **spleen** showed moderate erythro- and megakaryocytopoiesis in two male and two female animals at the high dose.

In the **testes**, tubular degeneration associated with Sertoli cell vacuolation was observed. Due to the age of the animals, all groups showed single animals with up to slight tubular degeneration and Sertoli cell vacuolation and multinucleated spermatogenic giant cells reflecting puberty and young adulthood of the animals. In the high dose however, moderate degeneration and Sertoli cell vacuolation was observed in two animals. In the affected tubuli, late stages of spermatogenesis were missing.

In the **spinal cord**, acute subarachnoidal hemorrhage in the cervical part of the spinal cord was observed at terminal sacrifice in one male animal at the low dose (slight), one female animal at the mid dose (minimal) and one male (slight) and two female (minimal, slight) animals at the high dose which in most cases coincided with infiltrates in the choroid plexus.

In the **kidneys**, all female animals at terminal sacrifice including the control animals showed tubular vacuolation with a slight increase in severity in treated animals, however, as it was also noted in all control females, it is not considered adverse.

All other microscopic findings were incidental and spontaneous in nature and could not be attributed to the test item.

The observed tubular dilation in the kidneys in the prematurely sacrificed animal is considered to be due to the poor general condition of the animal. One male mid dose animal showed arteritis of a large coronary vessel with subacute transmural and adventitial inflammatory cell infiltration, subendothelial inflammatory cells and apoptosis as seen in Beagle Pain Syndrome. As this is a single event and no other location was seen within the animal, no exposure-relationship and no further affected animals were observed, this finding is considered to be within the range of incidental findings and of the type routinely observed in dogs of this age.

Post-Treatment Phase

After a four weeks post treatment period, all changes showed a tendency towards reversibility or were no longer apparent. In the liver, minimal infiltration and single cell necrosis remained in some animals and one male post treatment high dose dog still showed minimal infiltration in the choroid plexus. In the femur, minimal hypercellularity of the bone marrow persisted in both male animals, minimal dysplasia in one male animal. Minimal subarachnoidal hemorrhage in the lumbar part of the spinal cord was found in the same male animal which showed also minimal infiltration in the choroid plexus in the brain.

5.13 Results of Analytics of the Formulations

The analytical results confirmed proper preparation and that defined acceptance criteria with regard to content were fulfilled. Analysis of vehicle samples (0 mg/mL) revealed no measurable traces of test item.

The detailed results of these investigations are given in Appendix - Formulation Analytics.

5.14 Toxicokinetics

The systemic exposure on Day 28 (geometric means and geometric SD, usually n = 6 (n = 3 per gender)) was as follows:

Table 5–6: Systemic Exposure Summary

Dose	[mg/kg]	2		6		20	
Parameter	Unit	gMean	gSD	gMean	gSD	gMean	gSD
AUC(0-24)	µg·h/L	11700	1.67	49300	1.23	260000	1.45
$AUC(0\text{-}24)_{norm}$	kg·h/L	5.86	1.67	8.21	1.23	13.0	1.45
C_{max}	µg/L	1180	1.51	4470	1.14	17000	1.35
$C_{max,norm}$	kg/L	0.591	1.51	0.746	1.14	0.850	1.35
t_{max}	h	2.83	1.46	2.52	1.76	3.65	1.68
$C(24)/C_{max}$	%	6.8	2.31	11.0	1.78	27.7	1.69
RA-AUC(0-24)	%	99.1	1.54	121.4	1.25	151.9	1.49
RA-C_{max}	%	108.9	1.53	125.4	1.18	154.3	1.41

AUC = Area under the concentration-time curve
C_{max} = Maximum of concentration-time curve
RA = Ratio of accumulation

Dependence on Sex:

There was no evidence of sex-related differences in exposure. Therefore, in the result tables and the evaluation only the combined mean parameters are addressed.

Dose Dependence:

On dosing Day 28, the exposure to BAY 123 increased with increasing dose. The increase in terms of both AUC(0-24) and C_{max} was slightly more than dose-proportionate from low to medium dose group. From low to high dose group, the increase was markedly more than dose-proportionate in terms of AUC(0-24) and slightly more than dose-proportionate in terms of C_{max}, respectively.

Time Course within the Dosing Interval:

On dosing Day 28, rather flat plasma concentration-time profiles were observed around t_{max}. Individual t_{max} were found to be between 1 and 7 h for all dose groups. Trough concentrations at the end of the observation period were ranging from 6.8 to 27.7% of the respective C_{max} with a tendency to higher values for the higher doses.

Influence of Repeated Dosing:

On dosing Day 28, no relevant change of exposure compared to dosing Day 1 was observed for low and medium dose groups. For high dose group, the exposure in terms of both AUC(0-24) and C_{max} was moderately higher on Day 28 when compared to Day 1.

Further details of the toxicokinetic investigation are given in Appendix - Toxicokinetics.

6. Discussion

In the present study, doses of 0, 2, 6 and 20 mg/kg BAY 123 were administered to male and female Beagle dogs once daily by oral (gavage) administration over a period of 4 weeks followed by a treatment-free observation period of 4 weeks.

The investigation revealed primary pharmacodynamic effects on hemostasis indicated by prolongation of activated partial thromboplastin time starting at the mid dose of 6 mg/kg. In addition, slight prolongation of thrombin time and transient decrease in thrombocytes (both at 20 mg/kg) were seen.

In clinical examinations, test item-related findings (salivation, vomiting, diarrhea) combined with reduced food intake in individual animals as well as body weight loss were noted at the high dose of 20 mg/kg.

The changes in biochemical parameters, such as the decrease in albumin in combination with the increases in alpha-, beta- and gamma-globulins as well as the increases in fibrinogen and monocytes noted at 20 mg/kg are indicative for an inflammatory response. The increases in total cholesterol and alkaline phosphatase mainly at 20 mg/kg are considered to be related to metabolic activation in the liver.

Postmortem examinations revealed different subsets of findings. The main complex is the cellular infiltration of the granulocytic cell lineage in liver and choroid plexus together with the increased granulopoiesis in the bone marrow and the extramedullary erythro- and

megakaryocytopoiesis in the spleen. The white blood cell lineage is predominant in the bone marrow and erythropoiesis seems to be decreased, correlating to decreases in erythrocytes, hemoglobin and hematocrit observed at the high dose of 20 mg/kg. The extramedullary erythro- und megakaryocytopoiesis in the spleen might be the attempt to compensate the lack of erythrocytes and megakaryocytes, however, significant effects on reticulocyte count were not observed. A granulocytosis in the blood could not be seen, neither could cellular infiltration of the granulocytic cell lineage be observed in other organs. The mechanism for the infiltration in just liver and choroid plexus and the reason for the shift to granulopoiesis in the bone marrow remain unclear.

In the spinal cord, the acute subarachnoidal hemorrhages might be correlated to the occurrence of the vascular infiltrates in the brain of these animals. They may cause a higher vulnerability of the affected blood vessels.

The tubular degeneration in the testes at the high dose is clearly more pronounced than background lesions in adolescent dogs at comparable age. The extent of testicular changes in the mid and low dose group however, were in the range of background findings of dogs of that age. Of note, the dogs used in this study were at the brink of adulthood, but not yet completely mature.

At the end of the four week post-treatment observation period, all test item-related effects showed a tendency towards reversibility or were no longer apparent.

Overall, a no-observed-adverse-effect-level (NOAEL) could not be established.