TOXICITY PROFILE OF PARENTERAL ARTESUNATE FOLLOWING SUBACUTE TREATMENT IN RATS AND DOGS

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Subchronic data for repeated intravenous and intramuscular artesunate in animals are available in the literature for a maximum duration of 14-days. Published results of genotoxicity studies *in vitro* in bacteria or cells of animal or human origin and *in vivo* in animals are equivocal, with a couple of negative results following oral administration and positive results in human cells.

With the studies performed in rats and dogs for a treatment period of 28-days including measures of safety pharmacology, toxicokinetic evaluation and *in vivo* genotoxicity data, a comparison of toxicity and exposure of intravenous versus intramuscular artesunate for a prolonged treatment period is possible, delivering important information, when a decision between both routes has to be made for use in patients. Results of the peripheral blood micronucleus assay in rats were positive, suggesting genotoxic potential for the intravenous route of administration of artesunate, what is, beside patients, likewise relevant for production and health care personnel.

ABSTRACT

OBJECTIVES The objectives of these studies were to investigate the toxicity, safety and toxicokinetics of single and multiple doses of artesunate for injection in rats and dogs.

METHODS Sprague-Dawley rats and Beagle dogs were treated intravenously or intramuscularly for 28 consecutive days with doses of up to 30 mg/kg artesunate, evaluating toxicity, kinetics, genotoxicity, and cardiovascular and central nervous safety parameters after single and 4-week repeated administrations. Furthermore, respiratory parameters were evaluated after a single intravenous administration in rats.

RESULTS Artesunate was well tolerated with no mortality and only minor effects on clinical pathology parameters. Following repeated intramuscular administration, local reactions at the injection site became evident. Signs of regenerative anaemia were evident in both rats and dogs and are attributed to the pharmacological effect of artesunate (effective against blood stages of malaria parasites). No severe toxicity or any effects on safety measures were noticed.

CONCLUSIONS The results obtained in these studies support the safe use of intravenous and intramuscular artesunate for a period beyond the commonly used three (to maximum seven) days in humans. Cardiovascular, central nervous and respiratory safety measures indicate no risk at clinically used doses.

INTRODUCTION

Artesunate, an artemisinin-derivative, has been used for decades in humans for the treatment of severe *falciparum* malaria, which is a uniformly lethal disease if not treated promptly with potent antimalarial drugs. The WHO recommends the use of parenteral artesunate for the treatment of severe *falciparum* malaria since 2005.[1] Although the mechanism of action remains to be completely elucidated, various data prove the efficacy of artesunate *in vitro*, *in vivo* in rodent and monkey challenge models, and in clinical and field isolates, by the number of successfully cured patients. Adsorption, distribution and elimination data are known for several routes of administration in commonly used laboratory species, including pregnant animals. Metabolism data are reported *in vitro* for animal and human cells, for animals as well as drug-drug interaction data for humans.

For intravenously administered artesunate, tolerability and efficacy were investigated in rats for three consecutive days. These studies, performed by Xie and Li, evaluated known haematotoxic and potential nephrotoxic effects of high doses of artesunate in uninfected and *plasmodium berghei*-infected rats.[2,3] Using the intramuscular route of administration in rats, studies of a duration of up to 7 days are available in the literature for artesunate as well as for the active metabolite dihydroartemisinin (DHA).[4,5] For nonrodents, intravenous artesunate data for up to 14 days of treatment in dogs and in monkeys are reported.[6,7]

Extensive work was done evaluating reproductive and developmental effects *in vitro*, using whole embryo cultures, in rats, rabbits, and monkeys using the oral, intravenous, intramuscular or intraperitoneal route of administration.[7-26] However, to date, no *in vivo* genotoxicity data for the intravenous route of administration are available in the literature.

During the last decade, nearly no additional work has been published to close the gap of nonclinical toxicity and safety pharmacology data for intravenous and intramuscular administered artesunate for a treatment duration beyond 14 days. In contrast, oral artesunate was investigated more recently for subchronic treatment (durations of one to three months) in mice, rats, rabbits and dogs.[27,28,29,30]

The aim of the studies presented in the following was to evaluate the toxicity of intravenous and intramuscular injected artesunate, in order to address potential risks by prolonged intravenous use, safety measures and for genotoxicity.

Materials and methods Two repeated dose toxicity studies and one respiratory safety study with administration of artesunate *via* the intravenous (IV) or intramuscular (IM) route were conducted in rats and dogs. Details of the study designs are shown in Table 1.

Table 1: Study Designs

Study	Duration	Routes	Endpoints
Repeated dose toxicity, rat	28-day treatment, 28-day recovery	IV and IM	General toxicity, neuro-behavioral changes (modified Irwin Test), body temperature, genotoxicity (micronucleus test)
Repeated dose toxicity, dog	28-day treatment, 28-day recovery	IV and IM	General toxicity, safety pharmacology (cardiovascular effects), body temperature
WBP, rat	Single dose	IV	Respiratory system

Study	Duration	Routes	Endpoints

Abbreviations: IM = intramuscularly; IV = intravenously; WBP = whole-body plethysmography.

Test article Artesunate (artesunic acid, ZA1200207) was obtained from Guilin Pharmaceutical Co., Ltd. Purity of the batch used was ≥ 99%. Formulations of artesunate for intravenous or intramuscular administration were prepared using sodium bicarbonate as solvent (60 mg/mL) for the stock solution, and dilutions were made with sodium chloride to the final required concentrations for injections. Concentration and stability of the test item in the dosing formulations were analysed in representative samples from the animal studies by using an HPLC method, validated compliant with international guidelines and methods.

Animal studies Dose levels were selected based on a preliminary 5-day repeated dosing study in rats. For the repeated dose toxicity studies in rats and dogs, intravenous doses of 3, 10, 30 mg/kg/d and an intramuscular dose of 30 mg/kg/d were selected. Control animals received the vehicle (mixture of sodium bicarbonate and sodium chloride) by IV injection. The application volume was 5 mL/kg in rats and 2 mL/kg in dogs for intravenous and 0.5 mL/kg (stock solution 60 mg artesunate /L).

Purpose-bred conventional naïve Beagle dogs, 6-10 months of age (5-10 kg bw), and specific pathogen-free Sprague-Dawley rats, 6-9 weeks of age (males: 180-300 g bw; females: 160-200 g bw) were included in the studies. The animals were received from licensed providers, quarantined and acclimatised for at least 5 (rats) or 14 days (dogs) before inclusion into the studies. 21 dogs and 92 rats of either sex were randomly assigned (based on body weights) to the five groups (n=3 dogs/sex; n=10 rats/sex) by using a Provantis module for grouping. Additional animals (see Table 2) were kept for a recovery period of 4 weeks or used for TK blood samplings in rats. Animals were treated for 28 consecutive days intravenously into the tail vein (rats) or into peripheral veins of the limbs (dogs) or intramuscularly into the quadriceps femoris.

Table 2: Repeated Dose Toxicity Groups and Numbers of Animals

		Rat (N per sex)			Dog (N per sex)	
Dose	**Route**	**Main**	**Recovery**	**TK**	**Main**	**Recovery**
Control	IV	10	5	3	3	2
3 mg/kg/d	IV	10	-	6	3	-
10 mg/kg/d	IV	10	-	6	3	-
30 mg/kg/d	IV	10	5	6	3	2
30 mg/kg/d	IM	10	5	6	3	2

Abbreviations: IM = intramuscular; IV = intravenous; N = number; TK = toxicokinetic.

The animals were group housed by sex in plastic solid-bottom cages (≤ 5 rats/cage) or in stainless steel cages (dogs), with single housing for collection of feces and urine samples, and for dogs additionally during designated procedures. Room temperature was kept between 18-26°C (dogs) or 20-26°C (rats) with a relative room humidity of between 40 and 70%, air changes > 8/h (dogs) or 15/h (rats), and a 12-hour

light/dark cycle. Dogs were fed with approx. 250 g Dog Maintain Feed per day, and rats received SPF Rodent Maintenance Feed *ad libitum*. Animals were provided with purified water prepared in-house in the facility *ad libitum*. A pre-test health screening by a veterinarian was conducted for all animals used in the studies.

Animals (rats and dogs) were observed once daily for mortality and twice (during treatment) or once (during recovery) daily for clinical findings (study procedures are listed chronically in supplementary Table 3). Body weights were recorded once before dosing and once weekly thereafter, and body weight gains calculated. Food consumption was checked once daily in dogs and at least once weekly in rats, and average food consumption (g/animal/day) calculated for rats. All animals (rats and dogs) were subjected to ophthalmologic examinations prior to first dose and at the end of the treatment and recovery periods.

Blood for clinical pathology examinations was collected twice before the first dose in dogs, and on D28 or D29 and D56 (recovery period) in rats and dogs. For clinical chemistry, blood was collected into tubes with separation gel and coagulant, centrifuged to obtain serum and analyzed with a Hitachi-7180 Automatic Clinical Analyzer. Samples for haematology or coagulation analysis were collected into tubes containing EDTA-K2 or citrate sodium as anticoagulant and analyzed by the Sysmex XN-1000V or Sysmex CS-5100 Automated Analyzers. Urine and faeces (dogs only) were collected before dosing (dogs only) and once at the end of the dosing and recovery periods. Urine samples were analysed by the URIT-500B Urine Analyzer and dog faeces were analysed for occult blood in addition. Parameters determined are listed in supplementary Table 5.

Blood for determination of artesunate or its main metabolite dihydroartemisinin (DHA) was collected prior to dosing and 5, 30 min, 1, 3, 6 and 24 hours after dosing on D1. On D27 (dogs) or D28 (rats), samples were collected from all animals prior to dose and at 15, 30, 45 min, 1, 3, and 6 hours post dose. Blood was collected into pre-cooled heparinized tubes and stabilized by adding 40 mg/mL sodium fluoride (NaF) in water. Sample analysis was done using LC-MS/MS, validated compliant with international guidelines (ICH) and methods. Primary TK parameters, maximum plasma concentration (C_{max}), time point of C_{max} (T_{max}), and area under the concentration-time curve ($AUC_{(0-t)}$), were calculated.

All rats and dogs scheduled for necropsy at the end of the treatment and recovery periods were anaesthetized (approx. 30-45 mg/kg pentobarbital sodium or ketamine in combination with xylazine [mixture of 20 mg/kg / 10 mg/kg for dogs; 90 mg/kg / 6 mg/kg for rats]), exsanguinated and subjected to macroscopic pathological examinations (see supplement Table 4), and organ weights analysis. Organs and tissues (see supplement Table 4) were taken for histopathological investigations (reported elsewhere). In addition, bone marrow samples were collected from the femurs of rats and from the ribs of dogs and smears were prepared for analysis.

Safety pharmacology (cardio-vascular, neuro-behavioral and respiratory) ECG parameters (see supplement Table 4) and body temperature were determined on D1 (1.5 h post dose), D2, D27 (prior to dose and 1.5 h post dose) and D28 as well as D56 (recovery period) by non-invasive telemetry in all dogs.

Rats were investigated using a modified Irwin Test (see supplement Table 6) to check for neuro-behavioural effects and measurements of body temperature 5, 30,

60, 240 minutes and 24 hours after the first and second to last dose.

A separate study to evaluate effects on respiratory parameters (see supplement Table 4) was conducted in Sprague-Dawley rats. Based on the 5-day preliminary toxicity study, dose levels of 10, 30 and 60 mg artesunate/kg bw were selected for single intravenous administration. Five animals per sex and group received a dose volume of 5 mL/kg into the tail vein. Control animals received the vehicle. Body weights were recorded on the day of dosing and respiratory function measurements (see supplementary Table 4) were evaluated by whole-body plethysmography with data acquired immediately for baseline (90 minutes before dosing). Post-dosing data were generated at 10 min, 0.5, 1, 4 and 24 hours after dosing. Animals were stratified to five evaluation blocks, with at least one animal of each group represented in each block of measurement.

Genotoxicity At the end of the dosing period (D29), the first five rats/sex/group were selected from scheduled necropsy animals for micronucleus analysis using the MicroFlow® PLUS Rat Blood Micronucleus Analysis Kit (Litron Laboratories, USA). In peripheral blood, approximately 20,000 CD71 high-positive reticulocytes (RET) were counted for each animal to determine the number of micronucleated reticulocytes (MN-RET) and frequencies (%MN-RET). As a measurement of cytotoxicity, the percent of reticulocytes (%RET) were calculated from total erythrocytes. Negative and positive controls were included and mean and standard deviation of %RET and %MN-RET calculated.

Data and statistical analysis Statistical comparison was performed between treatment groups and control groups. Group means and standard deviations were calculated, separately for each sex using the statistical methods described in supplementary

Table 7. The data of mortality, clinical observations, food consumption, ophthalmology, urinalysis, occult blood in feces (dogs only) and bone marrow smears were not statistically analyzed.

Ethics and compliance statements All animal work in this study was conducted complying with the "Guide for Care and Use of Laboratory Animals" issued in 2011 by the National Research Council, USA and protocols, amendment(s) and procedures reviewed and approved by the Institutional Animal Care and Use Committee (IACUC). The studies were conducted according to OECD GLP regulations in a GLP certified laboratory and in consideration of internationally accepted testing guidelines (OECD, ICH) for preclinical safety studies.

RESULTS

Formulation analysis Results of the validation study demonstrated good specificity of the HPLC method, and DHA did not interfere with the detection of artesunate. The calibration curves of artesunate showed good linearity in the range of 10~80 μg/mL, acceptance criteria for sensitivity, carryover, intra-accuracy, precision and homogeneity were met. The dose formulations of artesunate and post-processed samples were stable during the period and conditions used.

Representative samples for formulation analysis were taken in all three studies on the first, second and last day of formulation. The results of system suitability tests, stock solution comparisons, performance checks and standard curves etc. met the requirement of the analytical method. No artesunate was

detected in the control dose formulation of all three studies. Measured concentrations of the dose formulation samples of the first preparation in the rat study were out of specification (88.5, 88.4, 87.4 or 82.1%, respectively of the nominal concentrations) in the low, mid and high dose IV and the undiluted stock for IM preparations, including backup samples used for re-analysis. The mean measured concentrations of artesunate dose formulations from the second preparation, as well as all other samples determined for the rat and dog studies were within 90.0%-110.0% of the nominal concentrations, with a relative deviation of no more than 2.0%, and thus within the acceptance criteria.

***In vivo* examinations** None of the animals died before scheduled termination. Clinical signs indicative of general toxicity were not observed in rats and dogs. In rats, swelling at the injection sites was observed (first time observed on D10) in all male and female animals of the group receiving intramuscular injections (30 mg/kg/d). Swelling of the hind limb (left and/or right) was seen in 8 of 15 male and 10 of 15 female rats (accompanied by limping in 4 females) and in all dogs during the second half of the treatment period. All swellings recovered during the treatment-free period. One male dog showed additionally decreased activity (D12-28), which was associated with an abscess and injury on the right hind limb and limping.

In rats, mean body weights and body weight gains were not affected in male and female animals receiving intravenous doses of 3, 10 and 30 mg/kg/d in comparison to control animals, while intramuscular treated animals gained less body weight (males statistically significant D7 to D35, females below 10%), caused by a significantly reduced food consumption (males whole treatment period, females D17-21). The food consumption and body weight (partly in males) completely recovered.

In dogs, body weights were slightly (below 5% difference) lower in female animals of the high dose IV and IM treatment groups (30 mg/kg/d) compared to the other treatment groups and the control group. In the female IM group, occasionally food was left over. Body weights recovered in females of the IM group, but remained slightly lower in females of the high dose IV group.

No ophthalmological effects were observed in rats and dogs at any time point. Following single and repeated dosing, body temperature was not affected in both species.

Clinical pathology In rats, haematological changes affected the animals of the 10 and 30 mg/kg/d IV group and of the 30 mg/kg/d IM group. Red blood cell parameters indicative of anaemia (red blood cell count RBC, hemoglobin HGB and hematocrit HCT) were about 50% decreased with a concomitant 2-4-fold increase of the reticulocytes (RET). The interrelated parameters (MCV, MCH, MCHC and RDW) were also affected. In addition, the number of platelets (PLT) and the platelet Crit (PCT) were up to 50% increased in these groups. The number of WBCs was increased reaching statistically significance (10 mg/kg/d only males), by an increase of the absolute and relative neutrophiles (NEUT, 2-4-fold), lymphocytes (LYMPH, up to 2-fold), monocytes (MONO) and basophiles (BASO, both up to 10-fold). The thrombin time (TT) and the activated partial thromboplastin time (APTT) but not the prothrombin time (PT) were statistically significantly shortened. Fibrinogen was statistically significantly increased in the IM (male and female) treatment group. The absolute and relative numbers of nucleated red blood cells were up to 200-fold increased in a dose-related manner in all artesunate groups. Apart, in the low dose

group treated with 3 mg/kg/d IV, only a few of the interrelated parameters were affected (see supplementary Table 8). Except the RDW in males and the MCV and MCH in females of the high dose IV and IM groups, all changes recovered.

Clinical chemistry examination revealed a statistically significant decrease of cholesterol (CHOL) levels in male rats in the mid and high dose IV groups (10 and 30 mg/kg/d) and the IM group (30 mg/kg/d), while triglyceride (TG, excluding females of the 10 mg/kg/d group), total bilirubin (T-BIL) and direct bilirubin (D-BIL, bilirubin including females of the 3 mg/kg/d group) values were increased (statistically significant in the IM group). Total bile acids (TBA) and phosphate (P) were increased in male and female animals of the mid (only P) and high dose IV and IM groups, reaching statistical significance only in males. The level of albumin (ALB) was decreased in animals (statistically significantly in females) of the IM group with statistically significant elevated globulin (GLO) values and a decreased ratio of A/G in male and female rats. Additional statistically significant findings in the treatment groups compared to controls appear to be incidental and not related to treatment with artesunate. For details please see supplementary Table 10. Except P and TBA (still increased in males of the IM group), all parameters completely recovered.

As observed in rats, haematological parameters indicative of anaemia were affected in dogs. The values of RBC, HGB, HCT were ~30-40% decreased in animals of the IV (10, 30 mg/kg/d) and IM (30 mg/kg/d) treatment groups. MCH, MCHC, PLT, and PDW were affected only slightly and not in all groups. The effect on RET was not as clear cut as in rats, with 2-3-fold increased values in the low and mid dose groups and ~50-70% decreased levels in the high dose IV and IM treatment groups. However, MPV was increased in the mid and high dose IV and the IM groups. For details please see supplementary Table 9. The effects on white blood cell parameters did not indicate a clear effect, also pre-treatment WBCs showed quite some variability. The NEUTs were minimally increased, the absolute and relative MONOs about 3-fold increased, and EOSs minimally decreased. As observed in rats, the absolute and relative numbers of nucleated red blood cells (NRBCs) were up to 300-fold increased in all artesunate groups. In contrast to rats, coagulation parameters (PT, APTT and TT) were not affected by the treatment in dogs. The only finding observed was an increase (partially statistically significant) of the fibrinogen values in the high dose IV and IM groups. Except fibrinogen levels (lower in males, but higher in females of the 30 mg/kg/d IV and IM groups) all hematological parameters recovered back to control or baseline values.

Aspartate aminotransferase (AST), alkaline phosphatase (ALP except females of the IM group), LDH (only females) and CK (excluding males of the high dose IM group) were increased in the 30 mg/kg/d IV and IM groups. T-BIL values were statistically significantly increased and above the range of pre-dose level in the 30 mg/kg/d IV group. For details please see supplementary Table 11. Further affected parameters were creatinine and potassium levels (statistically significantly decreased only in male dogs of the IM group), and decreased calcium levels in females in the 10 or 30 mg/kg/d IV group and the IM group. ALB was decreased, and globulin increased with affected A/G ratios in males of the IM treatment group (the same as observed in the rats), while this effect was seen in the high dose IV males to a lower degree. A trend towards lower ALB and higher GLO as well as lower A/G ratios was seen in females of these dose groups (30 mg/kg/d IV or IM). Females of the IM

treatment group showed also a statistically significant increase (above control and pre-dose ranges) of amylase. A complete recovery was observed for all the affected parameters, except ALP and CK (still higher in males of the IM group), and CREA (higher in males of the 30 mg/kg/d IV and IM groups).

No treatment-related findings were observed in urinalysis in male and female rats and dogs. No occult blood was determined in the faeces of dogs.

Post-mortem evaluations Terminal body weights at necropsy were lower in the IM treatment group (30 mg/kg/d). Liver weights were increased in the 10 and 30 mg/kg/d IV groups and the IM group (absolute or relative, statistically significant in the 30 mg/kg/d groups), and marginally in both sexes of the low dose group (3 mg/kg/d). Absolute and relative spleen weights were statistically significantly increased in the mid and high dose IV and the IM groups. Heart weights (absolute and relative) were increased in the high dose IV group. In addition, a few statistically significant differences were observed: decreased absolute and relative thymus weights and increased relative testes weights in males of the IM group. For details see supplementary Table 12. Increased spleen weights (both sexes), in females of the IM group increased liver weights and in males of the IM group decreased kidney weights showed no or incomplete recovery.

At the end of the treatment period, no macroscopic observations were noted in male and female rats of the control and low dose IV groups (3 mg/kg/d). Macroscopic findings of enlarged spleen were observed in in 3/10 males in the 10 mg/kg/d IV group, 7/10 males and 5/10 females in the 30 mg/kg/d IV group and 7/10 males and 6/10 females in the 30 mg/kg/d IM dose group. In addition, enlarged administration sites (5/10 males) and crusts (1/10 males) as well as red discoloration at the administration site (1/10 females) were observed in rats of the IM dose group. At the end of the recovery period, no macroscopic findings were observed in any of the animals.

In dogs, terminal body weights at necropsy were lower without dose-relation in females of all dose groups. Absolute and relative spleen and liver (males only) weights were dose-dependently increased in the 10 and 30 mg/kg/d IV groups and the IM group. Absolute heart weights were not affected in male animals, while they were dose-dependently lower in female animals of all dose groups (including relative weights). For details see supplementary

Table 13. No or incomplete recovery was observed for liver and spleen weights (still higher in male animals of the high dose IV and IM groups). In female animals, heart and liver weights were higher in the high dose IV group and spleen weights were still higher in the IM group.

Macroscopic examination of dogs at the end of the treatment period did not reveal any findings in animals of the IV treatment groups (3, 10, 30 mg/kg/d). All three male and female animals of the IM group (30 mg/kg/d) showed enlargements at the administration sites, and one male animal showed injury at the administration site in addition. No further macroscopic findings were observed. At the end of the recovery period, no macroscopic findings were observed in any of the animals.

Bone marrow analysis revealed no treatment-related findings in rats and dogs of the control and low dose IV group (3 mg/kg/d). In all rats and dogs of the mid and high dose IV (10 or 30 mg/kg/d) and the IM group (30 mg/kg/d) groups, erythroid dysplasia was noted and the granulocyte to erythroid ratio was inverted (GE ratio <1: 1)

characterized by active proliferation of bone marrow, and/or increased segmentation cells (rats only) and erythron, and/or relatively decreased granulocytes and lymphocytes. In dogs, hemophagocytic cells were observed in most of the smears (16/18 cases). Furthermore, increased erythroblastic islets were noted in 4 of 20 rats of the IM dose group, but not in the IV dose group animals. In addition, for one dog of the IM group, the GE ratio was 12.14:1 with significantly decreased erythroid hyperplasia, increased granulocytes (mainly neutrophile granulocytes), high proportions of naked nuclei in megakaryocytes, and a trend of stagnated hematopoietic function. In another dog of this group, the quality of smear was poor and the smear was thick; the proportion of naïve granulocytes in granulocytes was increased. In two dogs of the high dose IV group (30 mg/kg/d), and single rats of the large 10 mg/kg/d IV group and the IM group, smudge cells were observed.

Toxicokinetics All samples for the control group in rats and dogs on the first day of dosing (D1) and at the end of the treatment period (D28 rat, D27 dog) were below the quantitative lower limit of detection (10.00 ng/mL). The TK results for rats and dogs are presented in detail in supplement Table 14 and Table 15.

As expected after intravenous injection of artesunate, C_{max} was obtained at the first sampling time point 5 minutes after dosing in rats and dogs. Artesunate was rapidly metabolised to DHA within minutes after intravenous injection and C_{max} was also observed at the first sampling time point of 5 minutes in all IV dose groups (3, 10, 30 mg/kg/d), except in the high dose IV group (30 mg/kg/d) at the end of the treatment period, where C_{max} was determined 15 minutes after dosing. After IM injection of artesunate (30 mg/kg/d), C_{max} levels were reached within 5 (rats) and up to 15 (dogs) minutes after dosing, but metabolism to DHA was somewhat slower with T_{max} levels achieved within the first 30 minutes after dosing.

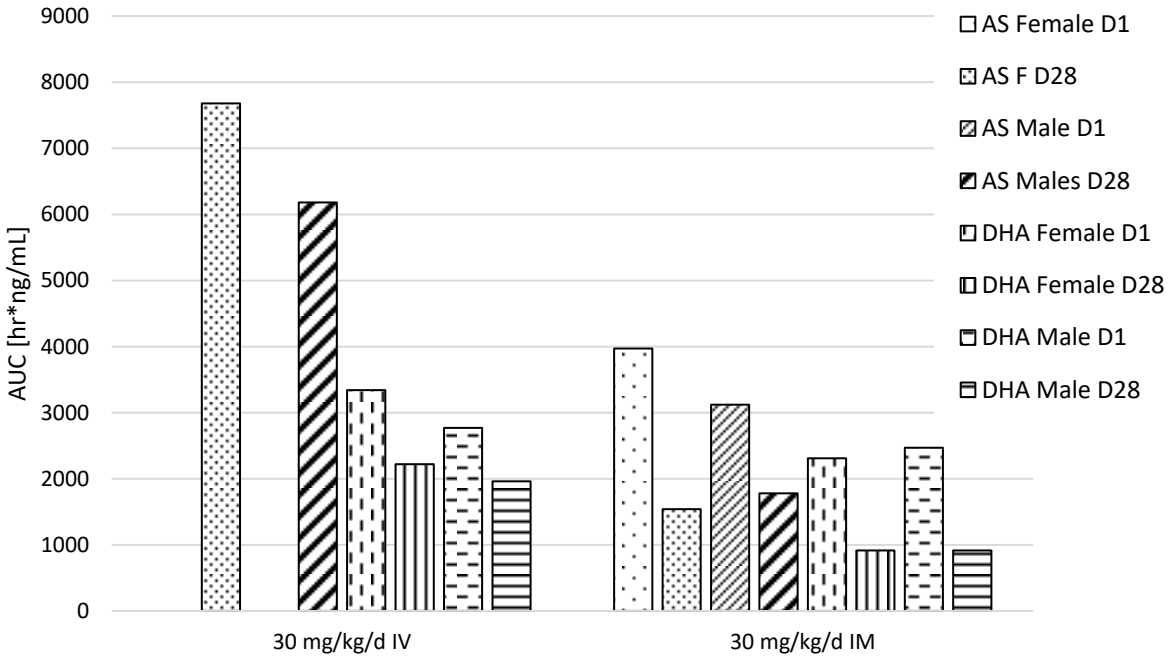
In rats, the increase of artesunate C_{max} was almost dose-proportional between the 3 and 10 mg/kg/d groups (range 2.9-4.9), and higher than dose proportional between the 10 and 30 mg/kg/d IV groups (range 5.9-14). With respect to DHA, the increase of the metabolite levels in the IV dose groups reflect the increase of doses in an almost proportional way. Animals of the IM dose group treated with 30 mg/kg/d showed lower C_{max} levels than in the high dose IV group (30 mg/kg/d). With the exception of D1 values in females (15%), plasma levels of males and females were about 62-69% lower in the IM group. This is also reflected in the levels of DHA which were about 55-78% lower.

Although the reanalysis of artesunate dosing solutions on the first day of dosing (D1) in rats was only about 82-88% of nominal, this was not reflected in the C_{max} levels of female animals of all dose groups and routes and in the male 30 mg/kg/d IV and IM groups. All C_{max} levels were lower after 28 days of treatment with artesunate, except in low and mid dose IV male rats (3 or 10 mg/kg/d) which were higher than the D1 C_{max} values.

For artesunate on D1 in rats, AUCs could only be calculated for the IM dose group (30 mg/kg/d) because most of the data at the various time points were below the limit of detection for the IV dose groups due to the fast metabolism of artesunate to DHA. In general, AUCs for artesunate and DHA were lower than D1 levels after 28 days of treatment with 30 mg/kg/d IM (see Figure 1). In the IV dose groups, AUCs increased with dose, and lower values were seen in female animals in the low and mid dose IV groups (3, 10 mg/kg/d) compared to male animals, while there was no difference

between male and female animals of the high dose IV and IM dose groups (30 mg/kg/d). In animals of all treatment groups, except males of the low dose group (3 mg/kg/d), the AUCs for DHA were somewhat lower on D28 than at the start of dosing on D1.

Figure 1: Comparison of plasma exposure in rats



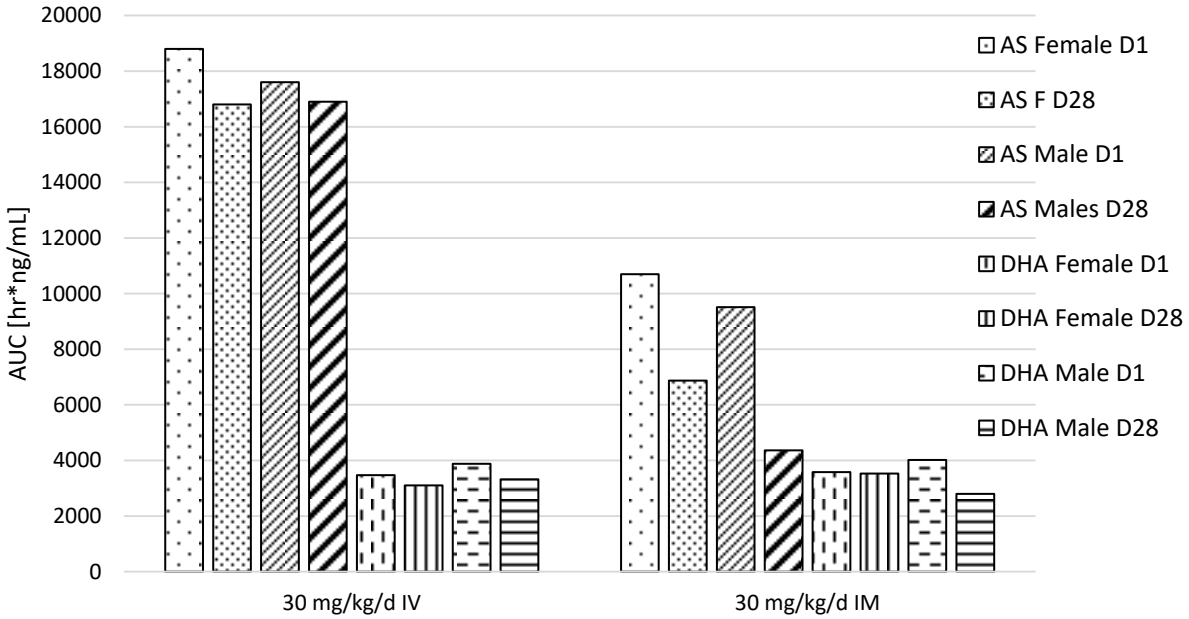
Exposure in terms of AUC is displayed for female and male rats after a single (D1) and repeated (D28) intravenous (IV) or intramuscular (IM) treatment with 30 mg artesunate/kg bw/day for artesunate (AS, left four bars per dose group) and its main metabolite dihydroartemisinin (DHA, right four bars per dose group). No AUC was calculated for males and females on the first day of treatment (D1) for artesunate due to fast metabolism to DHA. For the last sampling, additional early sampling time points were included, to enable calculating AUC values.

In dogs, plasma levels of artesunate (C_{max} and AUCs) were higher than in rats. There was no clear difference between D1 and D27 for the IV groups (3, 10, 30 mg/kg/d), but the D27 plasma levels were lower in the IM dose groups (30 mg/kg/d) as compared to D1. C_{max} and AUC increased slightly higher than dose proportional (4.1-9.2) with a slightly lower increase between the low and mid dose IV females on D27 (C_{max}: 2.4, AUC 2.1). As observed in rats, plasma levels in dogs (C_{max} and AUC) were lower in the IM group treated with 30 mg/kg/d than in the corresponding high dose IV group. C_{max} values were in the range of 64 to 92% and AUCs in the range of 43 to 74%.

The C_{max} levels of the metabolite DHA in dogs were almost in the same range as in rats with the exception of the 30 mg/kg/d IV group, for which the levels were lower in dogs than in rats. The increase in C_{max} and AUC was either dose proportional in females on D27 or less than dose proportional (range 2.1-2.7, 2.5-3.8, respectively) in most cases. The IM C_{max} level of DHA in animals treated with 30 mg/kg/d in comparison to the corresponding high dose IV group were about 29-68% lower. However, AUCs were

higher in the IM dose group compared to IV in females on D1 and D27 and males on D1 (range 3-12%) and lower in males on D27 (16%, see Figure 2).

Figure 2: Comparison of plasma exposure in dogs



Exposure in terms of AUC is displayed for female and male dogs after a single (D1) and repeated (D27) intravenous (IV) or intramuscular (IM) treatment with 30 mg artesunate/kg bw/day for artesunate (AS, left four bars per dose group) and its main metabolite dihydroartemisinin (DHA, right four bars per dose group).

Results did not indicate an obvious gender differences between artesunate and DHA levels in male and female rats and male and female dogs.

Safety pharmacology Cardio-vascular safety was investigated in male and female dogs on D1 (1.5 h post dose) and D2 and on D27 (prior to dose and 1 h post dose) and D28 and D56 (recovery period). No treatment or test item-related effect were observed in animals in the IV or IM treatment groups in comparison to the control group. PR and RR intervals, wave duration, QRS duration, and QT interval, heart rate and QTcv were within normal limits for all groups and no pathologic arrythmias were noted at any of the time points. One individual statistically significant increase of the corrected QT interval (9.4%) observed in female animals of the high dose IM group (30 mg/kg/d) on D28 of the study period can be regraded as incidental because this value was in the range of the values observed on D27 pre-dose and 1 hour post-dose.

Neuro-behavioural assessments were conducted in male and female rats pre-dose and at several time points (5, 30, 60 and 240 minutes and 24 hours) after the first (D1/2) and second to last dose (D27/28) by applying a modified Irwin screen. No treatment or test item-related findings were observed in animals of the treatment groups in comparison to control animals.

In a separate study, respiratory safety was investigated by whole-body plethysmography in male and female rats treated with a single intravenous (10, 30 or 60 mg/kg) artesunate injection prior to

dosing and at several intervals post-dose (10 min, 0.5, 1, 4 and 24 hours). The results did not indicate a test item or treatment-related effect on the tidal volume, minute volume and respiratory rates. 10 minutes following administration, respiratory parameters were increased in all animals independent of the treatment, some becoming statistically significant compared to their respective pre-dose values. All values returned back to baseline 0.5 to 1 hour post-dose and were therefore assumed to be related to the intravenous dosing procedure rather than to treatment with the test item.

Genotoxicity The genotoxic potential of the test item was investigated in male and female rats. Blood samples for a micronucleus assay in peripheral erythrocytes were collected at the end of the treatment period on the day of necropsy (D29). The mean percentage of reticulocytes (%RET) was increased in the mid and high dose IV and the IM treated animals (13.6%, 7.1%, 5.8% for males, 6.4%, 9.9%, 9.2% for females, respectively) compared to 0.7% and 1.6% in males and 1.3% and 1.9% in females of the control and low dose IV group. The percentage of micronucleated reticulocytes (%MN-RET) was 0.54%, 3.34%, 2.99% in males and 0.64%, 0.66%, 0.56% in females of the mid and high dose IV and the IM treated animals ($p<0.01$ for the IV groups, $p<0.05$ for the male IM group), respectively compared to 0.16%, 0.18% (males) and 0.11%, 0.13% (females) %MN-RET in the control and low dose IV group, respectively (being in the range of historical and assay negative control data). As the average values for micronucleated reticulocytes exceeded the upper of the historical ranges of negative/solvent controls and reached values of the assay internal positive control, artesunate was classified positive for the *in vivo* peripheral blood micronucleus assay.

DISCUSSION

Artesunate has been shown to exhibit relatively minor effects at high doses on the central nervous, cardiovascular and respiratory systems. The dose levels of artesunate used in the presented studies (up to 30 mg/kg/d intravenously or intramuscularly for 4 weeks or a single intravenous dose of 10, 30 or 60 mg/kg) were below doses known to cause central nervous or breathing effects. Following intravenous administration of artesunate, doses of 250 mg/kg and above caused decreased activity, analgesia, muscle relaxation, and hypnosis in mice.[31] Intramuscular administration of 150 mg/kg resulted in neurologic effects and mortality.[32] In rats, rabbits, guinea pigs, dogs and monkeys, intravenous doses reported lowering body temperature, causing EEG abnormalities, convulsions and breathing arrest between 160 to 640 mg/kg.[31] A no effect level (NOEL) was determined for 80 mg/kg intravenously in monkeys and dogs. In our studies, no effects on behaviour or cardiovascular effects were noted for 4-week repeated intravenous or intramuscular injections. As well, no breathing abnormalities following a single intravenous dose of up to 60 mg/kg artesunate were determined.

After repeated oral doses of artesunate, the levels of exposure decreased in rats and rabbits, this was especially evident in the high doses.[22,33] A decline in exposure in terms of C_{max} and AUC was determined as well in our studies following intravenous (only in rats) and intramuscular administration of 30 mg/kg/d for artesunate and for its main metabolite DHA. Reduced plasma protein binding (normally between 73-82% in rats) was demonstrated at higher doses (66% at DHA concentrations > 125

ng/mL).[34] Furthermore, the binding capacity of artesunate was significantly different between male and female rats in the study by Li et al., which could explain the differences observed between the sexes especially in the 3 mg/kg/d group and the supra-proportional increases in exposure in the 30 mg/kg/d IV group in rats.

A bioavailability of intramuscular administered artesunate is reported with 85-105% for artesunate and DHA.[35] In our studies, bioavailability of artesunate based on AUC ranged between 26 to 57% in rats and between 20 and 29% in dogs, although AUC values are of limited value, as they were based on single data due to the fast metabolism of artesunate to DHA. Bioavailability of DHA was 69% and 89% in females and males after the first treatment and only 41% and 47% at the end of the 4-week treatment period. In dogs, bioavailability of DHA following intramuscular administration ranged between 84-114% after a single and repeated injections, thus being in the range determined by Li et al.

Comparing human and animal exposure and the resulting effects following intravenous artesunate treatment, plasma concentration is not considered to be a good indicator of tissue exposure (measured by biologic effects, e.g. reticulocyte reductions) for artesunate across species.[26] In our studies, reticulocyte levels were reduced for absolute counts in dogs only at the end of the 4-week treatment period, but increased in absolute and relative counts in rats. Due to the mechanism of action, (affecting the blood stages of *plasmodium*, i.e. red blood cells), thus leading to anaemia (as demonstrated by our results in both rats and dogs), the increased reticulocyte levels determined in rats are indicative of regenerative anaemia by increased reticulocyte maturation. The inversed granulocyte to erythroid ratio (GE ratio <1:1) might be the result of an increased release of granulocytes, as indicated by increased plasma leukocyte counts. Increased spleen and liver weights, especially in rats but also noted in dogs, are indicative of extramedullary haematopoiesis to compensate anaemia, complying with results of other groups, e.g. as seen in a subchronic oral toxicity study for artesunate in dogs and a short-term intramuscular study in mice.[30,36]

Effects on clinical chemical parameters in rats and dogs were mainly characterized by reduced albumin levels, and increased globulin and bilirubin levels. The bilirubin is associated with an increased breakdown of red blood cells. In addition, an increase in liver parameters (AST, ALT) was seen in dogs, which was partly still evident at the end of the recovery period.

The effects described in our studies above were all of a minor toxic nature and most of them demonstrating a nearly to complete recovery, demonstrating a good tolerability and safety profile for artesunate. Comparing the intravenous to intramuscular route of administration, despite differing systemic exposure, the effects on target organs were nearly comparable to even more exaggerated in the intramuscular groups, demonstrating a good efficacy of this route, despite the local effects, but only arising after one week of repeated daily treatment. A good tolerability is known for intravenous or intramuscular use of artesunate in humans as well. In open-label randomised controlled trials in patients admitted to hospital with severe *falciparum* malaria in Bangladesh, India, Indonesia, and Myanmar (performed by the South East Asia Quinine Artesunate Malaria Trial, SEAQUAMAT group) and in paediatric patients admitted to hospital in the sub-Saharan Africa (AQUAMAT trial), superior efficacy, and a significantly reduced mortality were shown over quinine.[37,38] A better tolerability compared to intramuscular artemether has been demonstrated in several

studies. Following administration of 2.4 mg/kg artesunate at 0, 12 and 24 hours intravenously continued once daily until oral tolerance in adults (maximum 7 days) demonstrated a good tolerability. Beside a rare type-1 hypersensitivity reaction in about 1 in 3,000 treated patients, no serious adverse effects were noted. Our present studies demonstrated that artesunate, applied over a period of four weeks, was safe and produced only minor and to a great extent reversible effects in rats and dogs. Despite varying plasma levels comparing the intravenous to the intramuscular route of administration, effects on target organs were to the same extent, suggesting a comparable exposure, and potential effectivity, of artesunate for both routes, and despite some local effects during extended (beyond one week) duration of treatment for the intramuscular route, a comparable safety profile.

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Declaration of interests Y Xiong and Q Huang are employed at Guilin Pharmaceutical Co, Ltd. All other authors declare that they have no conflicts of interest.

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S - Supporting information

Table 3: Study Procedures

Study	Study Day	Parameter
Repeated dose toxicity rat	-2	Ophthalmology
	1 - 28	Daily intravenous and intramuscular treatment
	1	Modified IRWIN test incl. body temperature, TK sampling
	2	Modified IRWIN test incl. body temperature, TK sampling
	27	Modified IRWIN test incl. body temperature, ophthalmology
	28	Modified IRWIN test incl. body temperature, TK sampling, urine
	29	Clinical pathology, micronucleus assay, organ weights, macroscopic evaluation
	56	Urine
	57	Clinical pathology, organ weights, macroscopic evaluation

Study	Study Day	Parameter
Repeated dose toxicity dog	-8	Ophthalmology, clinical pathology, ECG incl. body temperature
	-6	Urine
	-3	Clinical pathology, body temperature, feces
	1 - 28	Daily intravenous and intramuscular treatment
	1	TK sampling, body temperature
	2	TK sampling, body temperature
	26	Urine
	27	TK sampling, ECG incl. body temperature
	28	Ophthalmology, clinical pathology, body temperature, feces
	29	Organ weights, macroscopic evaluation
	55	Urine
	56	Ophthalmology, clinical pathology, ECG incl. body temperature, feces
	57	Organ weights, macroscopic evaluation

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1226 **Table 4: Study Parameters**

Species	Endpoint	Parameter	Unit
Dog	Electrocardiogram (ECG)	PR interval	ms
		RR intervals	ms
		QRS waves	ms
		QT interval	ms
		Corrected QT interval (QTcv)	ms
		HR	bpm
Dog/rat	Ophthalmology	Eyelid, eyeball, conjunctiva, sclera, cornea, anterior chamber, iris, pupil, crystal, vitreous body and fundus	
	Organ weights	Adrenal, brain, epididymidis, heart, kidneys, liver, ovaries, oviduct, uterus, spleen, testes, thymus, thyroid and parathyroid	
	Necropsy	Body and musculoskeletal system, body surface and natural orifices; cranial cavity and the outer surface of the brain, thoracic cavity, abdominal cavity, pelvic cavity and visceral organ, all gross lesions (location, color, shape, or size)	
	Histopathology (collected organs)	Administration site, adrenals, aorta, bone (femur), bone marrow (sternum), brain (cerebrum), brain (cerebellum), brain (medulla), cecum, colon, duodenum, epididymides, esophagus, eyes, gall bladder (dogs), heart, ileum, jejunum, kidneys[a], liver, lungs (with main-stem bronchi), lymph node (mesenteric), lymph node (submandibular), mammary gland (dog female only, rat male and female), nerve (optic), nerve (sciatic, dog only), uterus, ovaries, oviduct, pancreas, parathyroid, Peyer's patch, pituitary, prostate, rectum, salivary glands (submandibular), skeletal muscle (biceps femoris), skin (abdominal), spinal cord (cervical), spinal cord (mid-thoracic), spinal cord (lumbar), spleen, stomach, testes, thymus, thyroid, tissues with any gross lesions, trachea, urinary bladder, vagina	
Rat	Whole-body plethysmography	TV (tidal volume)	mL
		MV (minute volume)	mL
		RR (respiratory rate)	bpm

Abbreviations: ms = milliseconds; bpm = beats per minute

[a.] All dog renal tissue slice used for immunohistochemical detection.

1227

1228 **Table 5: Study Parameters (Clinical Pathology)**

Endpoint	Parameter	Unit	Parameter	Unit
Haematology and Coagulation	Red Blood Cell Count (RBC)	10^{12}/L	Platelet Distribution Width (PDW)	fL
	Hemoglobin (HGB)	g/dL	Platelets Crit (PCT)	%
	Hematocrit (HCT)	%	White Blood Cell Count (WBC)	10^9/L
	Mean Corpuscular Volume (MCV)	fL	Neutrophils (Absolute) (NEUT)	10^3/µL
	Mean Corpuscular Hemoglobin (MCH)	pg	Lymphocytes (Absolute) (LWMPH)	10^3/µL
	Mean Corpuscular Hemoglobin Concentration (MCHC)	g/dL	Monocytes (Absolute) (MONO)	10^3/µL
	Reticulocyte Absolute (#RET)	10^9/L	Eosinophils (Absolute) (EOS)	10^3/µL
	Reticulocyte Percent (RET%)	%	Basophils (Absolute) (BASO)	10^3/µL
	Red cell Distribution Width (RDW-CV)	%	Neutrophils Percent (NEUT%)	%

Endpoint	Parameter	Unit	Parameter	Unit
	Platelet Count (PLT&O)	10^9/L	Lymphocytes Percent (LYMPH%)	%
	Mean Platelet Volume (MPV)	fL	Monocytes Percent (MONO%)	%
	Nucleated Red Blood Cells (Relative) (NRBC%)	%	Basophils Percent (BASO%)	%
	Nucleated Red Blood Cells (Absolute) (#NRBC)	10/µL	Eosinophils Percent (EOS%)	%
	Prothrombin Time (PT)	sec	Thrombin Time (TT)	sec
	Activated Partial Thromboplastin Time (APTT)	sec	Fibrinogen (Fbgc)	g/L
Clinical chemistry	Alanine Aminotransferase (ALT)	U/L	Total Protein (TP)	g/L
	Aspartate Aminotransferase (AST)	U/L	Albumin (ALB)	g/L
	Alkaline Phosphatase (ALP)	U/L	Globulin (GLO)	g/L
	Creatine Kinase (CK)	U/L	Albumin/Globulin Ratio (A/G)	N/A
	Gamma glutamyl transpeptidase (GGT) (dog only)	U/L	Amylase (AMY)	U/L
	Total cholesterol (CHOL)	mmol/L	Lipase (LIP)	U/L
	Triglyceride (TG)	mmol/L	Total Bile Acids (TBA)	µmol/L
	Total Bilirubin (T-BIL)	µmol/L	Lactic Dehydrogenase (LDH)	U/L
	Bilirubin, Direct (D-BIL)	µmol/L	Potassium (K)	mmol/L
	Glucose (GLU)	mmol/L	Chloride (Cl)	mmol/L
	Blood urea Nitrogen (BUN)	mmol/L	Calcium (Ca)	mmol/L
	Creatinine (CREA)	µmol/L	Inorganic Phosphate (P)	mmol/L
	Sodium (Na)	mmol/L		
Urinalysis	Specific gravity	Glucose	Urobilinogen	Color (visual)
	Protein	Bilirubin	White blood cells	Urine pH
	Ketones	Nitrite	Vitamin C	Urine sediment (microscopic)
	Occult blood	Clarity (visual)	Urine volume (approx. 16 hours)	mL

Abbreviations: sec = seconds; ms = milliseconds; g = gram; L = liter; dL = deciliter; µL = microliter; fL = femtoliter; pg = picogram; % = percent; U = unit; N/A = not applicable, mmol = millimole; µmol = micromole.

1229

1230 **Table 6: Study Parameters (Irwin Screen) in Rats**

Parameter	Score	Description
A. Observations in Removal from Cage and Handling		
Ease of removal	1-3	1 = normal, 2 = easier, 3 = difficult
Handling reactivity	1-3	1 = normal (with squeaking sound or moderate resistance), 2 = less (staying quietly in the hands or slightly resistance), 3 = greater (struggling intensely or twisting in the hands)
Body tone	1-3	1 = normal, 2 = decreased, 3 = increased
Palpebral closure		1 = normal opening of eyelids, 2 = partially closed eyes, 3 = eyelids shut
Eye observations	1-6	1 = normal, 2 = chromodacryorrhea or red tears, 3 = exophthalmos or protrusion of eyeball, 4 = nystagmus or unusual, repetitive eye movement, 5 = opacity, 6 = lacrimation

Parameter	Score	Description
Pupil size	<0.5~ 3.5	
Pupil response	1-3	1 = normal, 2 = reduced, 3 = no response
Palpebral reflex	1-4	1 = normal, 2 = reduced, 3 = no response, 4 = exaggerated
Salivation	1-3	1 = none, 2 =s light, 3 = severe
Piloerection	1-2	1 = not present 2 = present (coat does not lie down after stroking)
B. Observations in open field		
Involuntary motor movements	1-2	1 = no present 2 = present (a = fasciculation, b = tremors, c = clonic (test discontinued), d = tonic (test discontinued), e = clonic/tonic (test discontinued)
Arousal (the level of alertness)	1-3	1 = normal head or body movement, 2 = decreased head or body movements, 3 = increased head or body movement
Ambulation activity	1-4	1 = normal, 2 = slight impairment, 3 = severe impairment
Gait	1-2	1 = normal, 2 = abnormal (a = ataxia, b = forelimbs drag, are extended or unable to support weight, c = hindlimbs drag, are extended or unable to support weight, d = walks on tiptoes, e = hunched or crouched body position, f = rat is ambulatory, body is flat, and legs are unable to support its weight, g = rat is ambulatory, body is flat to the ground, and legs are unable to support its weight, h = recumbency (test discontinued), i = unable to asses for no moving
Excessive behavior	1-2	1 = not present, 2 = present (a = licking/biting, b = grooming, c = scratching, d = chewing, e = head-bobbing, f = circling, g = sniffing, h = other)
Bizarre behavior (such as straub tail, retropulsion, and writhing)	1-2	1 = not present, 2 = present
Respiration	1-2	1= normal 2 = abnormal (a = slowed, b = rapid, c = dyspnea, d = noisy)
C. Stimulus Reactivity		
Startle response	1-4	1 = normal reactivity (short jerk), 2 = hypo-reactivity (weak reaction), 3 = absent (no reaction), 4 = hyperreactivity (jumping with all paws off the ground)
Extensor thrust	1-4	1 = normal response, 2 = reduced response, 3 = no response, 4 = exaggerated response
D. Manipulations Test		
Air righting reflex	1-4	1 = normal, lands on all four feet, 2 = lands on all four feet, slightly uncoordinated, 3 = lands on side, 4 = lands on back
E. Physiological parameters		
Loose/watery feces	1-2	1 = not present, 2 = present

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1232 **Table 7: Statistical Methods**

Species	Endpoint	Statistical methods
Dog/rat	body weights, body weight gains, food consumption (during dosing period), body temperature, ECG parameters, haematology, coagulation, clinical chemistry, organ weights, ratios of organ-to-body weight and organ-to-brain weight	Data within groups were evaluated for homogeneity of variance by Levene's test. For data whose variances are homogeneous ($p>0.05$), a one-way analysis of variance (ANOVA) was performed on the data; for non-homogeneous data ($p\leq0.05$), a logarithmic transformation was automatically applied to obtain log data, and a Levene's test was applied to the log data again. For log data whose variances were homogeneous ($p>0.05$), a one-way analysis of variance (ANOVA) was performed on the log data; for non-homogeneous log data ($p\leq0.05$), a rank transformation was applied on the log data to obtain rank data before Kruskal-Wallis test being performed. Differences between groups were further tested by Dunnett t test for pairwise comparisons (at the 0.05 and 0.01 levels) only if the ANOVA was significant ($p\leq0.05$); otherwise no further analyses was performed. If significant results were obtained in the Kruskal-Wallis test ($p\leq0.05$), Dunnett t test on rank data were used for pairwise comparisons between groups (at the 0.05 and 0.01 levels); If no significant results were obtained in the Kruskal-Wallis test ($p>0.05$), no further analysis was performed.
Dog/rat	Binary data like lacrimation, paralysis, convulsions, splayed hindlegs, urination, defecation, death	SPSS statistics 21. Pearson's Chi-square was used for analysis of binary data; ranked data were analyzed by Kruskal-Wallis test. When there was a statistical significance ($p\leq0.05$), Dunnett't test was conducted (at the level of 0.05 and 0.01) after rank transformation of the data. When there was no statistical significance ($p>0.05$), no additional statistical analysis was made.
Rat (respiratory study)	Respiratory rate RR, tidal volume TV, and minute volume MV	SPSS statistics 21, groups 2-4 were compared with group 1, or the data at each time point after dosing with the baseline value: 1) Data within groups were evaluated for homogeneity of variance by Levene's test. When Levene's test indicated homogeneous variances ($p>0.05$), comparison between test article treated and control groups were made using a one-way analysis of variance (ANOVA); when variances are significant ($p\leq0.05$), Dunnett's T3 test were applied (at the 0.05 and 0.01 levels). 2) When analysis of variance was significant ($p\leq0.05$), a comparison of treated groups to control group by Dunnett's test for multiple comparisons was performed (at the 0.05 and 0.01 levels); when analysis of variance was not significant ($p>0.05$), statistical analysis was completed.

Abbreviations: ms = milliseconds; bpm = beats per minute.

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1236 **Table 8: Haematology and Coagulation Data Rats (D29), Means ± SD**

		Control		3 mg/kg/d IV		10 mg/kg/d IM		30 mg/kg/d IV		30 mg/kg/d IM	
Parameter	**Sex**	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
WBC	M	6.57	1.09	6.44	1.40	10.05	1.70**	14.46	3.12**	14.67	3.60**
(10^9/L)	F	4.53	1.83	4.42	1.60	6.68	2.38	9.28	2.45**	11.79	3.71**
NEUT	M	0.79	0.16	0.78	0.27	1.76	0.51**	3.34	0.52**	4.06	1.73**
(10^9/L)	F	0.71	0.61	0.50	0.23	1.49	0.89**	2.31	0.65**	2.91	1.21**
LYMPH	M	5.39	0.90	5.32	1.20	7.32	1.41*	8.45	2.42**	7.44	2.34**
(10^9/L)	F	3.57	1.36	3.66	1.33	4.65	1.84	4.91	1.23	6.90	2.55**
MONO	M	0.36	0.12	0.31	0.07	0.92	0.30**	2.49	0.88**	2.98	0.94**
(10^9/L)	F	0.22	0.10	0.23	0.10	0.50	0.25*	1.94	0.87**	1.85	0.80**
BASO	M	0.01	0.01	0.01	0.00	0.03	0.01**	0.12	0.09**	0.16	0.07**
(10^9/L)	F	0.01	0.01	0.01	0.01	0.01	0.01	0.08	0.06**	0.09	0.08**
RBC	M	8.00	0.26	7.94	0.56	5.88	0.78**	4.15	0.43**	3.87	0.50**
(10^{12}/L)	F	7.01	0.32	7.28	0.33	5.55	0.70**	3.64	0.53**	3.75	0.86**
HGB	M	14.95	0.78	14.07	0.92	10.31	0.90**	7.69	0.64**	7.37	0.95**
(g/dL)	F	13.74	0.60	13.19	0.51	9.77	1.00**	6.74	0.98**	6.91	1.71**
HCT	M	43.42	2.01	41.45	2.72	33.04	2.65**	26.86	1.97**	24.45	2.67**
(%)	F	40.38	2.47	39.82	1.38	31.14	2.84**	22.71	2.95**	22.71	5.42**
MCV	M	54.27	1.86	52.20	1.05*	56.62	3.89	65.05	3.38**	63.36	2.43**
(fL)	F	57.57	2.00	54.74	2.06	56.46	3.30	62.62	3.09**	60.68	2.82
MCH	M	18.70	0.78	17.73	0.40**	17.65	1.00**	18.61	0.99	19.06	0.51
(pg)	F	19.60	0.42	18.13	0.39**	17.67	0.78**	18.54	0.29**	18.35	0.55**
MCHC	M	34.42	0.39	33.96	0.26	31.20	0.84**	28.64	1.52**	30.11	1.01**
(g/dL)	F	34.09	1.15	33.12	0.80	31.35	0.97**	29.65	1.36**	30.30	1.60**
PLT&O	M	1112.9	108.2	1136.4	124.8	1246.3	81.4	1465.4	258.5**	1637.7	260.1**
(10^9/L)	F	1081.2	118.3	1145.4	130.0	1315.9	80.8*	1546.0	168.1**	1590.9	328.4**
PCT	M	0.80	0.08	0.85	0.08	0.95	0.05**	1.06	0.22**	1.16	0.20**
(%)	F	0.75	0.12	0.82	0.10	0.95	0.08*	1.07	0.13**	0.99	0.22**
RET%	M	3.22	0.40	3.71	0.62	15.71	3.11**	15.44	6.66**	10.67	6.69**
(%)	F	3.87	0.80	4.77	0.95	11.88	2.06**	10.77	2.87**	9.47	6.74**
#RET	M	257.77	30.73	293.51	47.89	860.30	126.6**	589.87	220.6**	424.77	327.8*
(10^9/L)	F	270.33	51.70	345.56	61.93	642.89	111.9**	390.62	115.29	350.94	297.4
RDW-CV	M	12.24	0.42	17.49	1.45**	25.67	2.49**	25.40	2.93**	23.79	2.33**
(%)	F	10.84	0.53	13.58	0.99*	19.05	1.10**	19.01	2.93**	20.12	3.85**
NRBC%	M	0.19	0.11	2.04	1.06**	81.39	34.75**	142.58	59.01**	136.13	45.39**
(/100 WBC)	F	0.13	0.17	1.91	1.87**	78.66	25.59**	197.72	38.09**	127.73	63.30**
#NRBC	M	0.01	0.01	0.14	0.08**	8.15	3.89**	20.05	7.87**	19.53	5.88**
(10^9/L)	F	0.01	0.01	0.09	0.10**	4.98	2.24**	18.37	5.58**	15.28	9.78**
APTT	M	17.15	1.68	17.62	1.13	16.70	0.71	16.42	0.69	14.69	1.06**
(Sec)	F	15.86	1.47	16.11	1.74	13.86	1.75*	13.91	0.99*	13.96	0.92*
Fbgc	M	2.46	0.23	2.49	0.15	2.40	0.17	2.70	0.25	3.63	0.24**
(g/L)	F	1.95	0.21	1.85	0.17	1.98	0.26	2.08	0.21	3.07	0.56**
TT	M	43.67	1.95	42.90	2.13	41.38	1.51*	37.59	1.91**	34.33	0.94**
(Sec)	F	39.46	2.07	39.46	2.25	36.47	2.80**	34.68	1.24**	32.58	0.73**

Abbreviations: SD = standard deviation; M = male; F = female; g = gram; kg = kilogram; bw = body weight; d = day; L = liter; dL = deciliter; fL = femtoliter; pg = picogram; % = percent; Sec = seconds; WBC = white blood cells; RBC = red blood cells; HGB = hemoglobin; HCT = hematocrit; MCV = mean corpuscular volume; MCH; mean corpuscular hemoglobin; MCHC; mean corpuscular hemoglobin concentration; PLT&O = platelet count; RDW-CV = red cell distribution width; PCT = platelet crit; NEUT = neutrophiles; LYMPH = lymphocytes; MONO = monocytes; BASO = basophiles; RET% = reticulocytes percent; #RET = reticulocytes absolute; NRBC% = nucleated red blood cells relative; #NRBC = nucleated red blood cells absolute; APTT = activated partial thromboplastin time; Fbgc = fibrinogen; TT = thrombin time; IV = intravenously; IM = intramuscular.
*p<0.05; **p<0.01

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1239 **Table 9: Haematology and Coagulation Data Dogs (D28), Means ± SD**

		Control		3 mg/kg/d IV		10 mg/kg/d IM		30 mg/kg/d IV		30 mg/kg/d IM	
Parameter	**Sex**	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
WBC	M	10.18	2.14	13.28	1.25	13.39	2.78	12.82	2.55	16.90	5.32
(10^9/L)	F	11.74	2.07	13.17	2.47	14.91	3.48	15.76	2.75	19.27	8.17
RBC	M	6.45	0.56	6.19	0.30	5.35	0.31*	4.37	0.57**	4.13	0.26**
(10^{12}/L)	F	6.53	0.53	6.80	0.90	5.41	0.23	4.55	0.45**	4.56	0.90**
HGB	M	14.84	1.24	14.03	0.31	11.53	0.46**	9.48	1.23**	9.00	0.72**
(g/dL)	F	15.26	1.26	14.83	1.60	11.87	0.42*	10.04	0.98**	9.76	1.84**
HCT	M	44.82	3.63	42.60	0.85	36.07	1.10**	28.96	3.64**	27.74	2.30**
(%)	F	46.00	3.70	45.50	5.01	36.73	1.07*	31.18	2.65**	30.06	5.30**
MCH	M	23.00	0.75	22.70	0.62	21.60	0.61*	21.70	0.52*	21.78	0.78*
(pg)	F	23.40	1.39	21.87	0.95	21.97	0.51	22.08	0.65	21.44	0.72*
MPV	M	10.16	1.21	10.67	0.76	12.27	0.38	12.84	0.54**	12.32	1.27*
(fL)	F	10.36	0.42	10.87	0.86	11.97	0.38*	13.30	1.11**	11.92	0.84**
NEUT	M	4.97	0.84	7.21	1.18	7.54	1.48	6.67	1.73	7.46	3.71
(10^9/L)	F	6.46	1.43	6.59	1.42	8.70	4.16	7.93	3.58	9.06	6.50
MONO	M	1.14	0.33	1.45	0.44	2.38	0.22*	2.74	0.98**	3.07	1.21**
(10^9/L)	F	0.95	0.18	1.24	0.40	1.20	0.96	2.66	1.12*	2.62	1.21*
EOS	M	0.36	0.24	0.54	0.08	0.50	0.33	0.38	0.20	0.25	0.10
(10^9/L)	F	0.64	0.12	0.82	0.52	0.46	0.25	0.38	0.31	0.26	0.15
RET%	M	0.81	0.59	1.22	0.52	1.73	0.17*	0.45	0.34	0.37	0.36
(%)	F	0.63	0.29	1.22	0.99	2.87	1.51**	0.69	0.34	0.42	0.20
#RET	M	53.42	40.48	76.30	34.65	93.03	14.60	20.02	14.83	16.00	16.90**
(10^9/L)	F	41.28	19.53	87.90	77.58	156.87	88.13*	30.76	13.06	18.68	9.67
NRBC%	M	0.04	0.05	1.10	0.92*	220.90	108.93**	82.76	63.74**	26.10	42.90**
(/100 WBC)	F	0.10	0.10	0.43	0.21	208.27	79.25**	126.12	60.16**	47.08	46.93**
#NRBC	M	0.004	0.005	0.16	0.14	27.56	10.14**	11.51	11.38	5.30	8.79
(10^9/L)	F	0.01	0.01	0.06	0.03	30.00	11.29**	20.93	12.21**	7.61	7.99**
Fbgc	M	2.08	0.38	2.01	0.35	2.48	0.44	2.87	0.32*	3.24	1.01*
(g/L)	F	1.73	0.45	1.59	0.29	1.99	0.10	2.89	0.82*	2.85	0.92

Abbreviations: SD = standard deviation; M = male; F = female; g = gram; kg = kilogram; bw = body weight; d = day; L = liter; dL = deciliter; fL = femtoliter; % = percent; WBC = white blood cells; RBC = red blood cells; HGB = hemoglobin; HCT = hematocrit; MPV = mean platelet volume; NEUT = neutrophiles; MONO = monocytes; RET% = reticulocytes percent; #RET = reticulocytes absolute; NRBC% = nucleated red blood cells relative; #NRBC = nucleated red blood cells absolute; Fbgc = fibrinogen; IV = intravenously; IM = intramuscular.
*p<0.05; **p<0.01

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1241 **Table 10: Clinical Chemistry Data Rats (D29), Means ± SD**

		Control		3 mg/kg/d IV		10 mg/kg/d IM		30 mg/kg/d IV		30 mg/kg/d IM	
Parameter	**Sex**	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
AST	M	121.10	28.40	116.60	26.18	124.40	29.80	161.40	51.66	151.40	36.35
(U/L)	F	105.67	22.55	113.56	37.82	106.30	24.56	103.10	11.74	143.10	45.40
ALP	M	146.60	21.04	141.30	28.99	143.10	23.00	123.90	25.27	104.90	17.51**
(U/L)	F	74.22	18.01	74.44	16.26	70.70	11.93	55.60	8.63*	53.70	7.04**
CHOL	M	1.75	0.32	1.59	0.29	1.38	0.30*	1.42	0.23*	1.35	0.26**
(mmol/L)	F	1.80	0.50	1.92	0.45	1.84	0.43	1.83	0.32	1.72	0.29**
TG	M	0.10	0.03	0.10	0.02	0.14	0.07	0.14	0.03	0.20	0.07**
(mmol/L)	F	0.11	0.03	0.13	0.04	0.11	0.03	0.16	0.14	0.17	0.07
T-BIL	M	0.61[n]	0.15	0.94[n]	0.51	1.69[n]	0.53	2.91[n]	0.83	2.15[n]	0.51
(µmol/L)	F	1.05	0.30	1.73	0.61*	2.45	0.45**	3.03	0.66**	2.63	1.37**
D-BIL	M	0.46[n]	-	0.39 [n]	0.04	0.77 [n]	0.30	1.22[n]	0.48	0.99[n]	0.34
(µmol/L)	F	0.43	0.10	0.59	0.17	0.82	0.14*	1.01	0.36**	0.93	0.56*
BUN	M	4.64	0.52	4.60	0.55	4.71	0.65	5.22	0.41	5.46	1.46
(mmol/L)	F	5.07	0.41	5.05	0.54	5.25	0.60	6.17	0.98*	6.76	1.50**
P	M	2.66	0.21	2.59	0.19	2.91	0.29	2.97	0.24*	3.01	0.27**
(mmol/L)	F	2.68	0.32	2.66	0.24	2.83	0.28	2.86	0.40	2.70	0.25
TP	M	57.80	2.49	59.10	1.52	58.30	2.87	59.80	3.01	58.20	3.08
(g/L)	F	64.11	4.26	68.00	2.96*	65.50	4.06	67.00	2.75	61.80	1.62
ALB	M	37.80	1.87	38.70	1.77	38.90	2.13	39.50	1.84	36.30	2.11
(g/L)	F	47.33	3.81	50.22	3.23	48.80	3.29	49.80	2.74	41.90	2.47**
GLO	M	20.00	1.56	20.40	1.35	19.40	1.58	20.30	1.64	21.90	1.45*
(g/L)	F	16.78	1.39	17.78	0.97	16.70	1.25	17.20	1.23	19.90	1.52**
A/G	M	1.90	0.19	1.91	0.19	2.02	0.20	1.95	0.13	1.66	0.10**
	F	2.84	0.30	2.84	0.29	2.93	0.22	2.91	0.29	2.12	0.27**
TBA	M	9.39	4.64	9.03	3.54	12.14	4.50	16.15	9.93	25.36	20.85*
(µmol/L)	F	10.90	6.06	12.76	10.55	11.08	7.05	22.24	17.92	22.11	17.36

Abbreviations: SD = standard deviation; M = male; F = female; g = gram; kg = kilogram; bw = body weight; d = day; L = liter; U = units; mmol = millimole; µmol = micromole; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CHOL = total cholesterol; TG = triglycerides; T-BIL = total bilirubin; D-BIL = direct bilirubin; BUN = blood urea nitrogen; K = kalium; P = phosphate; TP = total protein; ALB = albumin; GLO = globulin; A/G = albumin/globulin ratio; TBA= total bile acids; IV = intravenously; IM = intramuscular.
[n] = inappropriate for statistics; *p<0.05; **p<0.01

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1243 **Table 11: Clinical Chemistry Data Dogs (D28), Means ± SD**

		Control		3 mg/kg/d IV		10 mg/kg/d IM		30 mg/kg/d IV		30 mg/kg/d IM	
Parameter	**Sex**	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
ALT	M	29.80	9.36	36.67	9.45	20.67	3.79	23.20	2.39	36.00	8.86
(U/L)	F	29.80	3.90	27.67	4.16	29.00	6.00	19.80	6.94	41.60	11.19
AST	M	29.60	3.13	29.00	3.00	27.67	2.31	32.00	5.24	61.40	31.28*
(U/L)	F	25.60	5.03	27.33	3.79	26.00	2.65	37.00	7.68*	65.80	33.52**
ALP	M	89.00	18.72	98.67	33.08	80.67	44.38	84.80	7.05	119.40	45.11
(U/L)	F	79.00	15.86	50.00	17.35	75.67	6.11	107.60	27.68	161.60	100.2*
CK	M	171.20	34.30	185.33	68.06	182.00	61.83	154.00	40.24	655.00	631.89
(U/L)	F	136.20	43.80	152.00	19.16	168.00	38.31	287.80	50.33**	680.80	540.5**
T-BIL	M	0.83	0.34	0.67	0.12	1.06	0.25	1.92	0.42**	1.31	0.39
(µmol/L)	F	0.72	0.20	0.97	0.51	1.10	0.11	2.15	0.57**	1.31	0.60
D-BIL	M	N/A	N/A	0.40	N/A	N/A	N/A	0.51	0.11	0.54	0.17
(µmol/L)	F	N/A	N/A	N/A	N/A	N/A	N/A	0.70	0.38	0.46	0.08
CREA	M	56.60	4.93	53.67	7.37	50.67	2.31	50.40	4.04	39.80	7.43**
(µmol/L)	F	55.40	2.88	52.33	17.04	47.67	5.03	45.60	3.51	41.00	7.38
K	M	4.90	0.14	4.96	0.26	4.68	0.12	4.58	0.33	4.46	0.26*
(mmol/L)	F	4.95	0.14	4.85	0.13	4.74	0.13	4.87	0.35	4.54	0.27
Ca	M	2.49	0.10	2.53	0.05	2.44	0.05	2.43	0.04	2.38	0.09
(mmol/L)	F	2.58	0.04	2.55	0.08	2.42	0.09*	2.44	0.10**	2.39	0.06**
ALB	M	31.80	3.27	30.67	1.53	30.33	1.53	29.00	1.87	25.80	1.92**
(g/L)	F	32.80	1.64	31.67	3.51	29.67	0.58	29.80	1.10	26.60	3.91**
GLO	M	31.00	1.41	30.33	2.52	34.00	3.46	35.80	3.35	41.00	3.08**
(g/L)	F	29.80	0.84	32.33	3.51	31.33	3.79	35.80	2.49	35.60	7.09
A/G	M	1.03	0.14	1.02	0.12	0.90	0.14	0.82	0.10*	0.63	0.07**
	F	1.10	0.06	0.99	0.19	0.96	0.14	0.84	0.08	0.79	0.25
AMY	M	738.22	170.13	707.90	24.26	831.53	123.65	857.62	144.29	908.80	104.74
(U/L)	F	625.80	50.75	677.57	81.52	791.23	153.65	709.38	117.39	903.90	114.52**
LDH	M	80.20	19.74	109.67	41.31	149.00	67.29	115.00	51.87	151.20	55.20
(U/L)	F	81.40	32.00	79.67	26.10	20.67	3.79	329.60	111.2**	246.80	86.14**

Abbreviations: SD = standard deviation; M = male; F = female; N/A = not available; g = gram; kg = kilogram; bw = body weight; d = day; L = liter; U = units; mmol = millimole; µmol = micromole; ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALP = alkaline phosphatase; CK = creatin kinase; T-BIL = total bilirubin; D-BIL = direct bilirubin; CREA = creatinine; K = kalium; Ca = calcium; ALB = albumin; GLO = globulin; A/G = albumin/globulin ratio; AMY = amylase; LDH = lactate dehydrogenase; IV = intravenously; IM = intramuscular.
*p<0.05; **p<0.01

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1245 **Table 12: Organ Weights Rats (D29), Means ± SD (n=10/sex/group)**

		Control		3 mg/kg/d IV		10 mg/kg/d IV		30 mg/kg/d IV		30 mg/kg/d IM	
Parameter	**Sex**	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Terminal bw (kg)	M	338.7	10.30	338.8	15.86	341.3	13.94	326.6	21.55	293.5	28.92**
	F	236.5	16.95	240.0	20.68	241.2	16.90	231.4	18.64	219.6	17.73
Heart abs (g)	M	1.30	0.12	1.30	0.10	1.46	0.18	1.52	0.21**	1.24	0.13
	F	0.93	0.06	0.96	0.09	0.98	0.07	1.03	0.11	0.94	0.11
Heart to bw (%)	M	0.38	0.03	0.38	0.03	0.43	0.05	0.47	0.07**	0.42	0.04
	F	0.39	0.02	0.40	0.03	0.41	0.02	0.44	0.04	0.43	0.04
Heart to brain (%)	M	65.11	6.13	64.34	5.05	70.72	7.96	74.71	10.48*	62.0	7.12
	F	48.02	4.17	48.96	4.39	50.58	4.25	53.53	6.22	50.03	4.74
Liver abs (g)	M	8.76	0.42	9.01	1.04	9.21	0.77	9.60	0.65	9.12	1.09
	F	6.80	0.72	7.43	1.26	7.19	0.68	7.85	1.10	7.40	0.60
Liver to bw (%)	M	2.59	0.08	2.65	0.21	2.70	0.18	2.94	0.12**	3.10	0.14**
	F	2.89	0.41	3.09	0.43	2.98	0.12	3.38	0.27	3.37	0.14
Liver to brain (%)	M	437.96	23.62	445.68	55.25	445.44	28.24	471.55	37.60	457.79	59.68
	F	349.90	34.26	379.41	60.49	371.10	38.11	409.89	63.62	394.28	24.46
Spleen abs (g)	M	0.69	0.05	0.70	0.12	1.59	0.31**	3.33	0.87**	2.72	0.83**
	F	0.56	0.10	0.57	0.10	1.04	0.19**	2.09	0.63**	1.85	0.40**
Spleen to bw (%)	M	0.20	0.02	0.21	0.03	0.47	0.09**	1.01	0.21**	0.91	0.23**
	F	0.24	0.05	0.24	0.03	0.43	0.08**	0.90	0.24**	0.86	0.22**
Spleen to brain (%)	M	34.47	3.01	34.41	6.31	76.94	13.20**	163.42	40.06**	136.69	42.66**
	F	28.93	5.63	28.91	5.06	53.67	9.38**	109.27	33.69**	99.02	21.48**

Abbreviations: SD = standard deviation; M = male; F = female; abs = absolute; bw = body weight; d = day; g = gram; mg =milligram; kg = kilogram; IV = intravenously; IM = intramuscular; % = percentage.
*p<0.05; **p<0.01

1246

1247 **Table 13: Organ Weights Dogs (D28), Means ± SD (n=3/sex/group)**

		Control		3 mg/kg/d IV		10 mg/kg/d IM		30 mg/kg/d IV		30 mg/kg/d IM	
Parameter	**Sex**	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Terminal bw (kg)	M	9.93	0.67	9.60	0.26	9.97	0.42	9.50	0.26	10.00	0.72
	F	9.73	0.40	9.43	0.23	9.50	0.62	9.37	0.32	9.03	0.55
Heart abs (g)	M	83.60	3.70	90.07	3.57	86.20	4.16	82.30	10.98	80.57	11.50
	F	88.00	11.63	69.27	4.71	68.77	8.49	67.70	7.33	67.53	12.49
Heart to bw (%)	M	0.85	0.08	0.94	0.05	0.87	0.08	0.87	0.11	0.80	0.06
	F	0.90	0.08	0.74	0.06	0.72	0.05	0.73	0.10	0.75	0.13
Heart to brain (%)	M	106.77	4.70	116.89	9.05	108.97	10.81	106.85	12.27	100.68	14.37
	F	124.27	5.86	100.12	14.24*	98.43	7.45*	87.73	9.79**	95.49	8.18*
Liver abs (g)	M	237.00	9.40	254.53	26.82	292.93	14.25	299.37	12.03	316.70	16.99
	F	306.13	20.33	277.97	29.73	274.90	15.59	298.17	28.98	291.50	40.56
Liver to bw (%)	M	2.39	0.10	2.65	0.23	2.95	0.25	3.15	0.05	3.17	0.10
	F	3.15	0.27	2.95	0.29	2.90	0.19	3.18	0.21	3.22	0.26
Liver to brain (%)	M	302.70	12.52	331.30	51.55	370.40	38.11	389.02	5.26	395.80	23.11
	F	436.10	54.95	398.62	23.56	395.36	33.24	386.51	41.07	416.86	68.56
Spleen abs (g)	M	26.57	4.01	27.53	4.90	52.33	7.33	55.83	16.21	61.07	11.24
	F	23.80	4.78	23.87	3.13	61.83	33.58	97.87	23.80	46.60	4.59
Spleen to bw (%)	M	0.27	0.02	0.29	0.05	0.52	0.05	0.59	0.16	0.61	0.07
	F	0.24	0.05	0.25	0.04	0.64	0.32	1.05	0.29	0.52	0.08
Spleen to brain (%)	M	33.94	5.26	35.86	7.59	65.85	7.83	72.35	19.34	76.31	14.06
	F	33.78	7.19	34.27	3.66	88.67	47.50	126.48	29.11	66.59	8.52
	F	1.01	0.43	1.31	0.24	1.34	0.18	1.28	0.26	1.53	0.31

Abbreviations: SD = standard deviation; M = male; F = female; abs = absolute; bw = body weight; d = day; g = gram; mg = milligram; kg = kilogram; IV = intravenously; IM = intramuscular; % = percentage.
*p<0.05; **p<0.01

1248

1249 **Table 14: Toxicokinetic Parameters in Rats (D1 and D28)**

			3 mg/kg/d IV		10 mg/kg/d IM		30 mg/kg/d IV		30 mg/kg/d IM	
Parameter	**Sex**	**Day**	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Artesunate										
C_{max} (ng/mL)	F	1	467[a]	174	2296[a]	693	14413[a]	4151	12300	3620
		28	287	197	999	326	13400	4360	5030	2140
	M	1	341[a]	72	1395[a]	516	19777[a]	6941	6140	1460
		28	770	583	2230	856	13200	3850	4920	1480
T_{max} (hr)	F	1	0.0830[a]	N/A	0.0830[a]	N/A	0.0830[a]	N/A	0.0830	N/A
		28	0.0830	N/A	0.0830	N/A	0.0830	N/A	0.0830	N/A
	M	1	0.0830[a]	N/A	0.0830[a]	N/A	0.0830[a]	N/A	0.0830	N/A
		28	0.0830	N/A	0.0830	N/A	0.0830	N/A	0.0830	N/A
$AUC_{(0-t)}$ (hr*ng/mL)	F	1	NC	NC	NC	NC	NC	NC	3970	912
		28	98.4	72.7	313	100	7680	2940	1540	555
	M	1	NC	NC	NC	NC	NC	NC	3120	470
		28	405	347	1070	448	6180	2700	1780	80.8
Dihydroartemisinin										
C_{max} (ng/mL)	F	1	569	90.2	2190	124	7680	944	3240	567
		28	408	113	1610	238	7260	1440	1660	483
	M	1	546	73.5	1680	613	5650	641	2020	276
		28	575	126	1960	408	5460	1290	1180	351
T_{max} (hr)	F	1	0.0830	N/A	0.0830	N/A	0.0830	N/A	0.0830	N/A
		28	0.0830	N/A	0.0830	N/A	0.0830	N/A	0.0830	N/A
	M	1	0.0830	N/A	0.0830	N/A	0.0830	N/A	0.500	N/A
		28	0.0830	N/A	0.0830	N/A	0.0830	N/A	0.167	N/A

Parameter	Sex		3 mg/kg/d IV		10 mg/kg/d IM		30 mg/kg/d IV		30 mg/kg/d IM	
$AUC_{(0-t)}$	F	1	227	41.6	911	40.8	3340	416	2310	574
(hr*ng/mL)		28	153	26.0	526	95.7	2220	479	914	332
	M	1	198	19.1	737	214	2770	597	2470	400
		28	224	31.7	651	167	1960	703	915	69.7

Abbreviations: $AUC_{(0-t)}$ = area under the concentration time curve to the last time point; C_{max} = maximum plasma concentration; t_{max} = time point of C_{max}; SD = standard deviation; M = male; F = female; N/A = not available; NC = not calculated; ng = nanogram; mL = milliliter; hr = hours.

[a.] observed at T_{max} (calculation not possible, other time points below quantitative lower limit of 10.00 ng/mL).

Table 15: Toxicokinetic Parameters in Dogs (D1 and D27)

			3 mg/kg/d IV		10 mg/kg/d IM		30 mg/kg/d IV		30 mg/kg/d IM	
Parameter	**Sex**	**Day**	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Artesunate										
C_{max}	F	1	2170	445	10200	1150	53600	7540	19300	5250
(ng/mL)		27	2890	1180	6940	1270	63900	6000	8070	4060
	M	1	1740	461	12100	350	50200	5580	14400	5840
		27	2210	339	10100	2290	61700	9620	4890	2920
T_{max}	F	1	0.0830	N/A	0.0830	N/A	0.0830	N/A	0.0830	N/A
(hr)		27	0.0830	N/A	0.0830	N/A	0.0830	N/A	0.250	N/A
	M	1	0.0830	N/A	0.0830	N/A	0.0830	N/A	0.0830	N/A
		27	0.0830	N/A	0.0830	N/A	0.0830	N/A	0.0830	N/A
$AUC_{(0-t)}$	F	1	797	182	3740	363	18800	2670	10700	1500
(hr*ng/mL)		27	878	400	1880	398	16800	1600	6870	626
	M	1	621	165	4310	198	17600	1830	9510	241
		27	632	115	2740	632	16900	2540	4360	1440
Dihydroartemisinin										
C_{max}	F	1	685	73.0	1520	132	3800	420	2680	881
(ng/mL)		27	590	99.5	1770	199	5380	513	1890	567
	M	1	829	157	2220	336	4740	902	2740	791
		27	928	224	2020	161	4780	1030	1510	666
T_{max}	F	1	0.0830	N/A	0.0830	N/A	0.0830	N/A	0.500	N/A
(hr)		27	0.0830	N/A	0.0830	N/A	0.250	N/A	0.500	N/A
	M	1	0.0830	N/A	0.0830	N/A	0.0830	N/A	0.500	N/A
		27	0.0830	N/A	0.0830	N/A	0.250	N/A	0.500	N/A
$AUC_{(0-t)}$	F	1	298	50.1	921	228	3470	401	3580	784
(hr*ng/mL)		27	272	78.4	921	151	3100	392	3530	822
	M	1	368	102	1420	70.1	3880	630	4020	827
		27	384	68.5	1310	91.4	3320	387	2800	685

Abbreviations: $AUC_{(0-t)}$ = area under the concentration time curve to the last time point; C_{max} = maximum plasma concentration; t_{max} = time point of C_{max}; SD = standard deviation; M = male; F = female; N/A = not available; ng = nanogram; mL = milliliter; hr = hours.