AN ABSTRACT OF THE DISSERTATION OF

Roopesh Kumar Neelkant for the degree of Doctor of Philosophy in Pharmacy presented on November 17, 2003.

Title: Pharmacokinetics of Ibuprofen and Phenylbutazone in Elephants following Oral Administration of Single and Multiple Doses.

Abstract approved:

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The pharmacokinetics and dose proportionality of ibuprofen were examined in African and Asian elephants after single dose administration of 4, 5 and 6 mg/kg and on multiple dose administration. Each elephant received all the three doses and plasma ibuprofen concentrations were measured by HPLC. Approximate linearity of ibuprofen pharmacokinetics in elephants was found in the dose ranges of 4-6 mg/kg. On dosing the African elephants at 7 mg/kg and Asian elephants at 6 mg/kg every 12 hours to steady state, the ibuprofen concentrations produced were comparable to therapeutic levels achieved in humans. On comparing the AUC and clearance/F obtained in the single dose study with AUC and clearance determined with multiple dosing to steady state, no significant differences were observed showing ibuprofen followed linear kinetics.

The pharmacokinetics and dose proportionality of phenylbutazone were examined in African and Asian elephants after single dose administration of 2, 3 and 4 mg/kg and on multiple dose administration. Each elephant received all the three doses and plasma phenylbutazone concentrations were measured by HPLC. Approximate linearity of phenylbutazone pharmacokinetics in elephants was found in the dose ranges of 2-4 mg/kg. The clearance/F of Asian elephants was significantly lower than African elephants, so proper care should be taken with the dose and dosing frequency in multiple dosing of phenylbutazone for Asian and African elephants. An oral dose of 2 mg/kg every 24 hours was administered to the African elephants and an oral dose of 3 mg/kg every 48 hours was administered to the Asian elephants and the pharmacokinetic parameters were determined at steady state.

Pharmacokinetics of Ibuprofen and Phenylbutazone in Elephants following Oral Administration of Single and Multiple Doses.

by

Roopesh Kumar Neelkant

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CONTRIBUTION OF AUTHORS

Dr. Ursula Bechert provided the plasma samples of elephants for ibuprofen and phenylbutazone study.

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DEDICATION

To my parents, brothers and sister for their love and support

Pharmacokinetics of Ibuprofen and Phenylbutazone in Elephants following Oral Administration of Single and Multiple Doses

Chapter 1 Introduction

Musculoskeletal disorders (eg. trauma, arthritis) occur commonly in captive elephants. To treat these and other conditions, non steroidal anti-inflammatory agents like ibuprofen and phenylbutazone are used strictly on an empirical basis in elephants. Clinical application of drug use in elephants for safe, reliable and effective results necessitates the establishment of a treatment response curve or blood concentration profiles for each individual drug in both species (African vs. Asian).

In chapter 2, the pharmacokinetics of ibuprofen, a widely used non steroidal anti-inflammatory in veterinary medicine, was studied in twenty healthy elephants after oral administration of single doses of 4, 5 and 6 mg/kg and on repeated oral administration of 6 mg/kg in Asian and 7 mg/kg in African elephants every 12 hours. Both compartmental and noncompartmental analysis of the data was performed after the three single dose treatments and the pharmacokinetic parameters averages with their standard deviations for each treatment are presented. Also the pharmacokinetic parameters averages with their standard deviations at steady state after multiple dosing are presented. Comparisons of pharmacokinetic parameters between Asian and African elephants and between male and female elephants were performed.

In chapter 3, the pharmacokinetics of another widely used non steroidal anti-inflammatory phenylbutazone was studied in 15 healthy elephants after oral administration of single doses of 2, 3 and 4 mg/kg. Noncompartmental analysis of the data was performed after the three single dose treatments of phenylbutazone and the pharmacokinetic parameter averages with their standard deviations are presented. Multiple dose study was performed on seven African elephants on repeated administration of 2 mg/kg every 24 h and in three Asian elephants on repeated administration of 3 mg/kg every 48 h. Pharmacokinetic parameter averages and their standard deviations at steady state are presented. Comparisons of pharmacokinetic parameters between Asian and African elephants and between male and female elephants were performed.

Chapter 2

Pharmacokinetics of Ibuprofen orally given at single doses 4 mg/kg, 5 mg/kg and 6 mg/kg and on multiple dosing in Healthy Asian and African Elephants

Roopesh K. Neelkant, Ursula Bechert and J. Mark Christensen

ABSTRACT

The aim of this study was to determine pharmacokinetics and dose proportionality of ibuprofen in the Asian and African elephants following single dose oral administration of 4, 5 and 6 mg/kg and on multiple dose administration. In this study, twenty healthy elephants, each received all three doses with a washout period of three weeks between each dose. Blood samples were taken over a 48 hour period and analyzed for measurement of plasma ibuprofen concentrations using an HPLC assay. Statistical comparisons were made using analysis of variance mixed effects model following necessary transformation. The means and standard deviations of peak plasma concentrations (C_{max}), the time to C_{max} (T_{max}), the area under the plasma concentration versus time curve (AUC), the apparent half life ($T_{1/2}$), the total body clearance (CL/F) after oral ibuprofen administration, and the terminal volume of distribution (Vd/F) were calculated using noncompartmental methods following the three doses of 4, 5 and 6 mg/kg ibuprofen yielding values of 16.47 ± 6.87, 18.28 ± 8.62, and 22.38 ± 11.96 mcg/ml, 4.14 ± 2.63, 4.08 ± 1.60 and 5.56 ± 3.65 hours, 142.40 ± 65.38, 177.09 ± 72.14 and 227.37 ± 117.30 mcg.h/ml, 4.73 ± 2.81, 5.11 ± 1.93, and 5.21 ± 2.53 hours, 35.94 ± 19.87, 33.08 ± 13.68, and 32.84 ± 15.03 ml/h/kg and 208.82 ± 87.53, 228.96 ± 100.36, and 228.96 ± 142.94 ml/kg respectively for the pharmacokinetic parameters. Approximate linearity of ibuprofen pharmacokinetics in elephants was found in the dose ranges of 4-6 mg/kg. The mean values of AUC were 142.40, 177.09, and 227.37 mcg.h/ml

following 4, 5, and 6 mg/kg dose, respectively. The dose normalized AUC's were lower in African elephants than in the Asian elephants, but the difference was not significant. Similarly the CL/F in Asian elephants was lower than in African elephants but not significant. Ibuprofen plasma concentrations were highly variable following oral administration in the three doses given. Since the study evaluated ibuprofen pharmacokinetics only in a limited number of elephants, firm conclusions on differences in ibuprofen pharmacokinetics between Asian and African elephants, found after oral administration of the three doses of ibuprofen, cannot be made. There was no gender difference in pharmacokinetic parameters in African and Asian elephants. Although clearance/F of African females was found to be less than the African male elephants the difference was not statistically significant. On dosing the African elephants at 7 mg/kg and Asian elephants at 6 mg/kg every 12 hours to steady state, the ibuprofen concentrations produced were comparable to therapeutic levels achieved in humans and were maintained. On comparing the AUC and clearance/F obtained in the single dose study with AUC and clearance determined with multiple dosing to steady state, no significant differences were observed showing ibuprofen followed linear kinetics.

INTRODUCTION

The Asian elephant is a critically endangered species, and populations declined from an estimated 100,000 in 1900 to approximately 35,000 today (1). Contributing to the poor rate of reproductive success among captive populations of elephants is a lack of knowledge regarding basic husbandry requirements. Maintaining elephants in safe and healthy captive environments will contribute to the success of conservation program goals for this species. The endangered and threatened states respectively of Asian and African elephants dictates that each individual elephant remaining on this earth be given the best veterinary care as possible.

Musculoskeletal disorders (eg. trauma, arthritis) occur commonly in captive elephants, affecting 73% of the animals studied in 69 zoos in North America (2). To treat these and other conditions, non steroidal anti-inflammatory agents like ibuprofen are used strictly on an empirical basis in elephants. Choosing an appropriate drug and dosage is difficult for veterinarians due to lack of research in elephant physiology and how drugs interact in their system. Clinical application of drug use in elephants for safe, reliable and effective results necessitates the establishment of a treatment response curve or blood concentration profiles for each individual drug in both species (African versus Asian). There is some evidence of possible species (African versus Asian) difference, but it is poorly documented (3). Evidence suggests that there are species differences in drug

metabolism between African and Asian elephants for the drugs ketamine hydrochloride and sulfamethoxazole and trimethoprim (4, 5). Ketamine hydrochloride is an anesthetic used in elephants and African elephants required three times the dose of the Asian elephant to immobilize the animal (4). African and Asian elephants differ in a number of ways morphologically including: height, weight, ear size, distal trunk processes, and number of digits per foot, ribs and caudal vertebrae and also the food intake (6) table 2.1.

The following clinical signs may suggest rheumatoid arthritis in elephants: 1) lameness fluctuating – good and bad days – often more noticeable after resting and in cold weather with seasonal patterns; 2) swollen and warm joints where swelling and lameness may migrate, particularly at onset; 3) generalized weakness, lethargy, depression with behavioral changes being the presenting symptoms; 4) failure to gain or hold normal weight with or without a good appetite; 5) abnormal immunoglobulin levels with a rise in rheumatoid factor activity and/or mycoplasma antibody; 6) mycoplasma antibody titer rise especially following a flare reaction; 7) temporary worsening with onset of antimycoplasma therapy; 8) arthritis brought under control through long term intermittent antimycoplasma therapy supplemented with anti-inflammatory therapy (2).

Physiologic diversity and large body size compared to other species, makes dosages based on metabolic scaling calculations unreliable. The enormous body size and dissimilar physiological metabolism of elephants compared to other

Table 2. 1 Differences between African and Asian elephants (6).

	African elephant	Asian elephant
Weight	8000 - 15000 lbs	6000 - 11000 lbs
Skin	Wrinkly	Smoother
Height at shoulder	10-13 ft	6-10 ft
No. of ribs	Up to 21 pairs	Up to 20 pairs
Highest point	On shoulder	On head
Line of back	Concave	Convex or straight
Line of belly	Slopes down toward hind legs	Either almost straight or sagging in middle
Shape of head	Not foreshortened from front to back, no bumps, no hollow	foreshortened from front to back, with bumps on top of head, hollow forehead
Size of ears	Larger extend beyond neck	Smaller do not extend beyond neck
Top edge of ear muscle in adults	Folded in middle	Folded outward
Teeth	Diamond-shaped lamella section of molars	Very foreshortened lamella section
Tusks	Present in both sexes, larger in the male	Mostly in males, rudimentary or absent in females
Trunk	More rings, less rigid	Little ringing, more rigid
Tip of trunk	Two 'fingers'	Only one 'finger'
No. of nail-like structures	4 or 5 on front foot 3, 4, or 5 on hind foot	5 on front foot 4 or 5 on hind foot
Food	Mainly leaves	Mainly grass

species (eg horses and cows) complicate estimation of dosing requirements based on metabolic scaling calculations. The elimination rate and half life of a drug may be profoundly affected by such variations in metabolic rate between species. Doses of Amikacin and the combination of sulfamethoxazole and trimethoprim when extrapolated from the reported horse dose, these doses were normalized to be similar to human doses on a mg/kg basis, did not achieve the recommended serum levels in elephants (5, 7, 8).

Dosages for anti-inflammatories used empirically in elephants were close to reported equine levels. Although ibuprofen is used with elephants, there is not an established dosage for equines the normal model species used in metabolic scaling of doses to elephants (3). There are no reported pharmacokinetic studies with anti-inflammatories in elephants, and is an area in need of research.

Ibuprofen is one of the most commonly used drugs for the treatment of inflammatory conditions in captive elephants. Empirical dosages of ibuprofen administered to captive elephants today range between 0.5-4.0 mg/kg, and the median dosing frequency used by zoo veterinarians for ibuprofen is 24 hours (3). No pharmacokinetic trials have been conducted for anti-inflammatory agents in elephants; although several studies have been published for various antibiotics (5, 7, 9, 10, 11). Results from the antibiotic studies suggest that metabolic scaling calculations for elephants are unreliable, mostly under estimating the dosage and dosing interval requirements (5, 7).

The objective of this study was to determine the pharmacokinetics of ibuprofen in elephants after giving single doses of 4, 5, and 6 mg/kg orally and after repeated oral administration of 6 mg/kg every 12 hours in Asian elephants and 7 mg/kg every 12 hours in African elephants. From the pharmacokinetic data obtained appropriate dosing regimens can be determined for ibuprofen given orally in elephants.

Ibuprofen is a propionic acid derivative non steroidal anti-inflammatory drug (NSAID) that has a number of beneficial actions in addition to its analgesic and antipyretic effects. Ibuprofen, (±) –(R,S)-2-(4-isobutylphenyl)-propionic acid, is a chiral 2-aryl propionic (2-APA) derivative nonsteriodal anti-inflammatory drug figure 2.1. The pharmacodynamic properties of propionic acid derivatives do not differ significantly. All are effective cyclooxygenase inhibitors, although there is considerable variation in their potency. Ibuprofen is a potent inhibitor of prostaglandin synthesis with S (+) enantiomer possessing the majority of pharmacological activity. Their total mode of action is not known. Ibuprofen possesses an asymmetric carbon atom and can therefore occur either as the (+)-isomer or the (-)-isomer, there is an almost complete inversion of poorly active (-)-isomer form to the much more active (+)-isomer in the body (12).

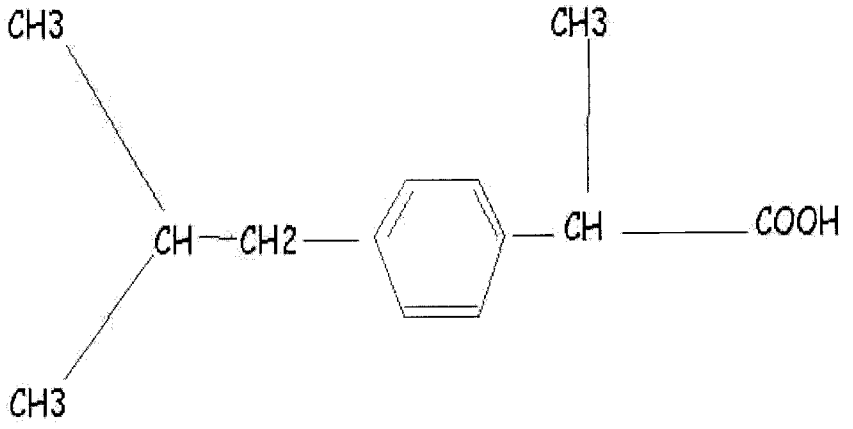

Figure 2. 1 (±) –(R,S)-2-(4-isobutylphenyl)-propionic acid.

Ibuprofen in humans is used in the management of mild to moderate pain and inflammatory in conditions such as dymenorrhoea, head ache including migraine, postoperative pain, dental pain, musculoskeletal and joint disorders such as ankylosing spondylitis, osteoarthritis and rheumatoid arthritis including juvenile chronic arthritis, peri-articular disorders such as bursitis and tenosynovitis, and soft tissue disorder treatments of patent ductus arteriosus.

The absorption of ibuprofen is rapid and peak concentrations in plasma are observed after 1 to 2 hours when given orally. The half-life in plasma for humans is

about 2 hours (13,14). Ibuprofen is 90 to 99% bound to plasma proteins (15). About 60 % of an administered dose of R(-) ibuprofen was stereospecifically inverted to the S(+) enantiomer. There was no measurable inversion of the S(+) to R(-) ibuprofen (16). Substantial concentrations are attained in synovial fluid, which is a proposed site of action for NSAID drugs (17). Non-linear pharmacokinetics has been reported for ibuprofen, and have been attributed to saturation of plasma protein binding (18). Ibuprofen is eliminated following biotransformation to glucuride conjugate metabolites that are excreted in urine, with little of the drug being eliminated unchanged. Ibuprofen is extensively metabolized via formation of the major metabolites 2-hydroxy ibuprofen and carboxy ibuprofen figure 2.2 (14). Oxidative metabolism by CYP 450 isoenzyme CYP2C9 appears to be the major fate of ibuprofen, followed by acyl – glucuronidation of the oxidized metabolite (14). Hepatic disease and cystic fibrosis can alter disposition kinetics of ibuprofen.

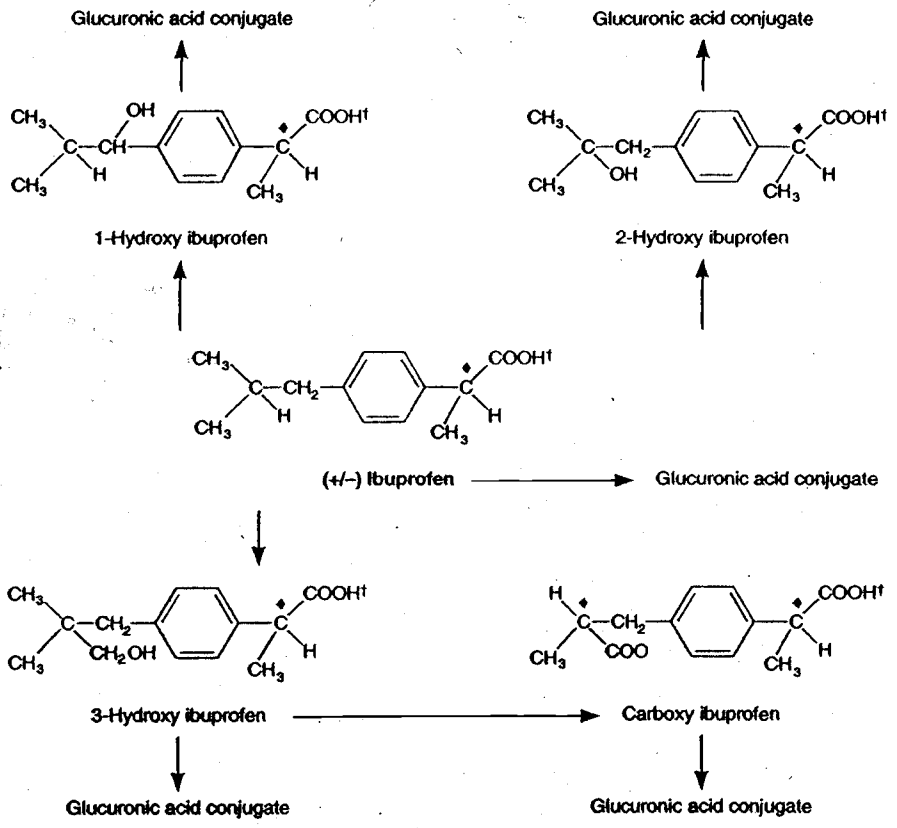

Figure 2. 2 Biotransformation of ibuprofen (14).
Symbols: ♦ = chiral center(s); † = sites of glucurondation.

MATERIALS AND METHOD

Materials

Ibuprofen was obtained from Pfizer (Kalamazoo, MI). Glacial acetic acid, acetonitrile and cyclohexane of HPLC grade were obtained from Fischer Scientific (Fair lawn, NJ). Gemfibrozil was obtained from Sigma Chemical Co. (St. Louis, MO).

Study Protocol

Twenty healthy elephants housed in zoos throughout North America were used in the study. Ages and weights were obtained prior to the initiation of the study and ranged from 2400 to 7400 kg. In the study African male and female elephants had an average weight of 4400 and 3400 kg, whereas the Asian male and female elephants weighed on average around 4900 and 3600 kg respectively. The elephants were of standard adult age and ranged from 14 to 44 years. Ibuprofen was given orally with food treats (e.g., apples, bread and peanut butter). Blood samples were taken from the superficial ear veins using 20 gauge butterfly catheters, which allowed for some animal movement during venipuncture. Catheter placement was generally not successful. None of the elephants received any medications for at least 4 weeks prior to the trials.

Single dose study: Pilot pharmacokinetic studies were conducted with one or two elephants using empirically derived dosing regimens and preceded both the single dose and multiple dose trials to ensure that proper ranges for dosage and dosing frequency would be utilized. Based on the pilot study results, the best therapeutic dose for ibuprofen for the single dose study was determined to be dosing at 4, 5 and 6 mg/kg to each animal. Washout periods between doses were 3 weeks in duration, thereby allowing complete elimination of residual drug metabolites. Blood samples were collected at –5, 15, 30, 45, 60 minutes, 1.5, 2, 4, 10, 12, 24 and 48 hours post administration from all the elephants. Blood samples were placed into red top tubes and centrifuged for 10 minutes at 13000 g. Serum was decanted into plastic screw-cap vials and kept frozen until time of analysis.

Multiple dose study: The optimal dosing frequency was determined by examining two different dosing intervals (12 and 24 h) utilizing the doses given in the single dose study. A simulation was performed to extrapolate ibuprofen plasma concentrations to steady state levels using the single dose ibuprofen plasma levels by superposition method. Based on the simulation study, a 12 h dosing interval was selected. Blood samples were collected hourly for four hours after each administration, then every six hours plus one hour prior to the next administration. A three week wash out was included between single dose and multiple dose studies.

Determination of plasma ibuprofen concentrations:

The HPLC system used to assay ibuprofen consisted of an HPLC pump (Model M-600 A; Water Associates, Inc. Milford, MA) with a WISP auto sampler (Model 712, Water Associates, Inc.) and a variable wavelength detector set at 229 nm and an automated data integrator (Hewlett Packard Model 3390A, Wilmington, DE).

To 0.5 ml of elephant serum, 100 μl of internal standard solution (25 mcg/ml of gemfibrozil) and three drops of glacial acetic acid were added and the mixture was vortexed for 30 seconds. To this solution, 0.5 ml of acetonitrile was added followed by vortexing for 30 seconds. Ibuprofen was double extracted using 2ml cyclohexane with each extraction and vortexing for 30 seconds followed by centrifugation for 10 minutes at 4500 rpm. The supernatant was separated, and the two cyclohexane extractions were pooled vacuum evaporated and dried. The dried samples were then reconstituted with 0.5ml of mobile phase (40% acetonitrile and 60% 0.1M acetic acid and the pH was adjusted to 3.5) and vortexed for 1 minute followed by centrifugation for 5 minutes at 4500 rpm. Of this solution, 100µl was injected onto an HPLC C-18 column having a flow rate of 1.5 ml per minute for the mobile phase. Calibration curves were obtained by plotting the peak areas against the corresponding concentrations of prepared ibuprofen standard solutions. The slope, intercept, and correlation coefficient of each calibration curve were determined by linear regression analysis. The concentration of ibuprofen in

experimental samples was calculated from the peak area of each experimental sample and the slope and intercept of the appropriate calibration curve. Correlation coefficients of calibration curves were greater than 0.998. Table 2.2 shows the calibration curve with the means, standard deviations and percent coefficient of variation.

Table 2. 2 Calibration curve values showing mean, SD and %CV.

Concentration (mcg/ml)	Area Ratio	Average	SD	%CV
0.50	0.10	0.10	0.02	20.09
	0.09			
	0.09			
	0.14			
	0.08			
1.00	0.16	0.17	0.01	5.90
	0.17			
	0.18			
	0.19			
	0.17			
5.00	0.83	0.80	0.02	2.64
	0.80			
	0.79			
	0.79			
	0.77			
	0.80			
10.00	1.55	1.59	0.07	4.56
	1.69			
	1.48			
	1.61			
	1.59			
25.00	3.44	3.75	0.19	5.06
	3.98			
	3.72			
	3.73			
	3.91			
50.00	7.03	7.46	0.38	5.07
	8.01			
	7.08			
	7.50			
	7.75			

SD: Standard deviation
% CV: Percent of coefficient variation

Pharmacokinetic analysis

Plasma ibuprofen concentration profiles were analyzed utilizing both compartmental and non-compartmental approaches, using WinNonlin pharmacokinetic-pharmacodynamic software (19). Plasma ibuprofen concentration profiles after oral doses were best described by a one compartment open model with first order input according to Akaike's information criterion (20). A weighting factor of (1/plasma concentration) was used. Elephant ibuprofen plasma concentration profiles gave comparatively good fits with a one compartment model.

The pharmacokinetic parameters were calculated from the final equations obtained. The areas under the plasma concentration time curves (AUC) were calculated from the coefficients and exponential terms of the equation best describing the data.

$$C_p = \sum_{i=1}^{n} C_i e^{(\lambda_i t)} \quad (1)$$

$$AUC = \sum_{i=1}^{n} C_i / \lambda_i \quad (2)$$

where n is the number of exponential terms in the equation. The total body clearance (Cl_T/F) was calculated according to

$$Cl_T / F = Dose/AUC \quad (3)$$

The MRT is the mean residence time and is equal to

$$MRT = AUMC/AUC \quad (4)$$

where AUMC is the area under the first momentum curve, and was calculated from

$$AUMC = \sum_{i=1}^{n} C \ / \lambda_i^2 \tag{5}$$

The apparent volume of distribution at steady (Vd_{ss}) was calculated according to

$$Vdss = {CL_T}/{MRT} \tag{6}$$

Half-life was calculated according to

$$\lambda_i t_{1/2} = 0.693 / \lambda_i \tag{7}$$

In noncompartmental analysis, AUCs and AUMCs were calculated using log linear trapezoidal rule method. T_{max} and C_{max} were determined from the fitted data. MRT and terminal half-lives were calculated as stated above from equations 4 and 7.

Following administration of ibuprofen given at 4, 5, and 6 mg/kg, dose proportionality of ibuprofen was determined using AUC. With respect to the lowest (4 mg/kg) dose AUC; AUC comparisons for the three doses was estimated by the following equation.

$$R = \frac{AUC_{4,5,or\,6\,mg/kg}}{AUC_{4\,mg/kg}} \tag{8}$$

R is dose proportionality ratio. If dose proportionality is linear, the ratios for the three doses should not be statistically different from 1.0 : 1.25 : 1.5. In addition, a power function relationship was used to describe the relationship between AUC and dose.

$$AUC = a \bullet (Dose)^b \quad (9)$$

In the above equation, a represents the coefficient, and b represents the exponent of the power function regression. If AUC exhibits linear dose proportionality, then b should be equal to unity. The parameters a and b were estimated by linear least square regression following a log-transformation as follows:

$$\ln (AUC) = \ln (a) + b \bullet \ln (DOSE) \quad (10)$$

A similar power function relationship was also established for C_{max}. Linearity was indicated if 95% confidence interval for the exponent included the value of 1 (21).

Multiple dose study: Pharmacokinetic parameters at steady state for multiple dose study were determined using Kinetica software (22). C_{max} and Cmin are the maximum and minimum steady state drug concentrations during a dosing interval at steady state. T_{max} is the maximum time corresponding to C_{max}. AUC_{ss} is the area under the curve during a dosing interval at steady state. AUC_{ss} was calculated using the trapezoidal rule. Cl_{ss} is the clearance at steady state. Cl_{ss} was calculated as follows.

$$Cl_{ss} = \frac{Dose}{AUC_{ss}} \quad (11)$$

$C_{average}$ is the mean or average steady state drug concentration expressed as:

$$C_{average} = \frac{AUC_{ss}}{Tau} \quad (12)$$

% AUCdf is the percentage-area-fluctuation expressed as:

$$\%AUCdf = 100\frac{AUC_{above} + AUC_{below}}{AUC_{ss}} = 100\frac{AUCbetweenC(t)andC_{average}}{AUC_{ss}} \quad (13)$$

Statistical analysis

Following oral administration of ibuprofen at three different doses, statistical comparisons of mean plasma concentrations at each sampling time and estimates of the pharmacokinetic parameters among the three doses were made using ANOVA. Statistical comparisons between Asian and African elephants were done using ANOVA mixed effects model:

$$\log(y_{ijkl}) = s_i + z_j + \theta_k + \gamma_l + \varepsilon_{ijkl} \quad (14)$$

If y_{ijkl} represents the measured pharmacokinetic parameter on the *k*th dose in the *j*th zoo for the *i*th elephant of *l*th species then the analysis of variance model would be as above. Where s_i is a random elephant effect, z_j is a fixed zoo effect, θ_k is the effect of the kth dose, γ_l is the effect of species and ϵ_{ijkl} is a normally distributed random error with mean value zero. The parameter for zoo was included in the model, as the elephants included in the study were from six different zoos. The number of elephants included in the study from each zoo was different. Also the number of Asian and African elephants differed between the zoos. Species was included in the model, as one of the objectives of the experiment was to see if differences exist between African and Asian elephants. Dose was included in the

model as the experiment was carried out at three different doses and the aim was to see if their was any difference in the parameters with different doses.

Statistical software SAS was used for analysis (23). A paired t test was used to compare the Asian and African elephant's AUC's and clearance/F of single dose studies for AUC and clearance at steady state for linear kinetic studies.

RESULTS AND DISCUSSION

Twenty-one elephants were used for the study with 20 elephants completing the four treatments. Elephant Smokey finished only one treatment (4 mg/kg). Demographic information of individual elephants is presented in table 2.3. Tables 2.4, 2.5, 2.6, 2.7, 2.8, and 2.9 show plasma concentrations of ibuprofen in each elephant after oral administration of 4, 5, and 6 mg/kg, along with the averages and standard deviations at each sampling time. Individual elephant plasma concentration profiles of ibuprofen after oral administration are shown in figures 2.3, 2.4, and 2.5, respectively, whereas figures 2.6, 2.7, and 2.8 show mean ibuprofen concentrations (with standard error) time curves.

Plasma concentration time profiles after oral administration were fitted to one and two compartment open models with first order input. None of these models gave a good fit to the concentration time profiles. Plasma concentration time profiles fitted with a one compartment open model with a lag time gave a comparatively better fit. Plasma concentrations of ibuprofen as a function of time after oral administration of the three doses were fitted to a bi-exponential equation (equation 1), using WinNonlin and weighting factor of (1/Cp) (figure 2.9, 2.10, and 2.11).

$$Cp = C_1 e^{-\lambda i \bullet t} \tag{14}$$

where Cp is ibuprofen plasma concentration. λ_i is the exponent, C_1 is the pre-exponential coefficient, and t is the time. Table 2.10 shows the mean and standard

deviation of the pharmacokinetic parameters after fitting the data of elephants to a one compartment model. The individual pharmacokinetic parameters at 4, 5, and 6 mg/kg of oral dose in each elephant after fitting to a one compartmental model are shown in table 2.11, 2.12 and 2.13.

Concentration-time profiles following the three doses were fitted to the non-compartmental model using WinNonlin. The obtained mean pharmacokinetic parameters following 4, 5, and 6 mg/kg are shown in table 2.14 and the pharmacokinetic parameters obtained from individual elephant after 4, 5, and 6 mg/kg oral dose are shown in table 2.15, 2.16 and 2.17. Because of the erratic results, pharmacokinetic parameters of elephants Hugo for all the doses, Smokey and Packy for 4 mg/kg and Toby for 6 mg/kg were excluded from the study. Data of these elephants is in appendix A and B. For further dose proportionality and for comparisons between African and Asian elephants, the pharmacokinetic parameters obtained with non-compartmental model were used.

The mean volume of distribution at steady state for lactating cows, lactating goats and in healthy foals was 0.14 L/kg, 0.16 L/kg and 0.14 L/kg when compared to of 0.30 L/kg in elephants (24, 25, 26). The mean total clearance/F for lactating cows and healthy foals was 86.2 ml/kg/h and 180 ml/kg/h (0.003 L/kg/min) when compared to of 33 ml/kg/h in elephants (24, 26). The mean elimination half-life in lactating diary cows, lactating goats and in healthy foals was 1.55 h, 1.08 h and 79 minutes when compared to 5.0 h in elephants (24, 25, 26). Pharmacokinetic parameters of ibuprofen in elephants were comparable to that of dogs. The mean

rate constant for elimination, elimination half life and total body clearance for dog was 0.16 h^{-1}, 4.6 h and 29.4 ml/kg/h when compared to 0.16 h^{-1}, 5.0 h and 33 ml/kg/h of elephants (27).

Table 2. 3 Demographic characteristics of elephants in study.

Elephant	Gender	Origin	Weight (lbs)	Age (years)	Zoo
Digger	Male	African	5100	18	Riddles
Solomon	Male	African	6800	19	Riddles
Toby	Male	African	6800	22	Riddles
Willie	Male	African	9000	23	Riddles
Angus	Male	African	13409	24	Bowmansville
Tattoo	Female	African	7400	*	Kansas City
Lea	Female	African	8000	*	Kansas City
Tasha	Female	African	8245	22	Pittsburgh
Sheba	Female	African	7856	17	Bowmansville
Felix	Female	African	6000	18	Riddles
Hugo	Male	Asian	10400	40	Oregon
Hank	Male	Asian	9000	14	Riddles
Packy	Male	Asian	13556	*	Oregon
Vance	Male	Asian	10184	27	Bowmansville
Tai	Female	Asian	8600	*	Have trunk
Dixie	Female	Asian	7600	*	Have trunk
Limba	Female	Asian	6976	39	Bowmansville
Peggy	Female	Asian	8000	44	Riddles
Shine	Female	Asian	8850	*	Oregon
Booper	Female	Asian	7700	30	Riddles

* No record

Table 2. 4 Ibuprofen plasma concentration profiles of African elephants at the oral dose of 4 mg/kg (n=10).

	Ibuprofen plasma concentration (mcg/ml)											
Time(h)	Digger	Solomon	Toby	Willie	Angus	Tattoo	Lea	Tasha	Sheba	Felix	Mean	SD
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.25	0.000	0.000	0.294	0.000	0.467	0.000	0.000	0.000	0.000	0.000	0.076	0.166
0.5	0.000	0.293	0.433	0.266	1.528	0.000	0.000	0.000	0.388	0.000	0.291	0.469
0.75	0.377	0.257	-	1.039	5.525	0.241	2.942	0.000	0.679	0.319	1.264	1.827
1	0.805	0.545	1.947	1.508	12.599	2.038	6.958	0.000	1.259	0.247	2.790	3.973
1.5	1.419	0.865	3.639	1.762	21.648	5.899	8.850	0.000	3.870	0.330	4.828	6.522
2	1.797	1.267	3.162	1.377	28.414	8.461	8.223	6.509	10.931	0.305	7.045	8.360
4	12.613	3.852	6.703	19.679	22.300	12.745	12.135	16.327	24.619	4.455	13.543	7.238
10	3.084	6.211	2.178	6.142	7.077	4.444	11.273	2.425	7.925	6.262	5.702	2.793
12	1.984	3.727	0.985	3.892	5.588	3.142	8.474	1.234	5.208	4.635	3.887	2.258
24	0.000	0.238	0.000	0.582	0.389	0.271	1.540	0.000	0.598	0.855	0.447	0.481
48	0.000	0.000	0.000	0.000	0.000	0.000	0.945	0.000	0.000	0.000	0.095	0.299

Table 2. 5 Ibuprofen plasma concentration profiles of Asian elephants at the oral dose of 4 mg/kg (n=8).

	Ibuprofen plasma concentration (mcg/ml)									
Time(h)	Vance	Hank	Tai	Dixie	Limba	Peggy	Booper	Shine	Mean	SD
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.25	0.000	0.796	0.392	0.299	0.000	0.000	3.644	0.325	0.682	1.227
0.5	2.302	1.179	0.265	2.412	0.945	2.205	10.199	0.926	2.554	3.185
0.75	9.974	3.264	0.459	8.198	1.351	1.826	22.675	2.477	6.278	7.442
1	15.244	3.418	0.760	13.025	2.130	3.626	23.415	3.959	8.197	8.092
1.5	19.187	13.501	4.753	13.582	4.583	16.777	19.612	6.643	12.330	6.240
2	23.015	11.733	4.868	14.887	5.759	15.010	17.392	7.558	12.528	6.270
4	21.259	11.625	18.270	16.675	10.718	25.669	19.500	19.564	17.910	4.922
10	11.652	3.518	9.263	7.392	7.104	8.963	9.215	8.650	8.220	2.349
12	7.117	2.109	7.767	5.999	4.195	6.907	5.739	6.439	5.784	1.831
24	2.133	0.000	1.224	0.790	0.429	1.513	2.011	1.434	1.192	0.745
48	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 2. 6 Ibuprofen plasma concentration profiles of African elephants at the oral dose of 5 mg/kg (n=10).

	Ibuprofen plasma concentration (mcg/ml)											
Time(h)	Digger	Solomon	Toby	Willie	Angus	Tattoo	Lea	Tasha	Sheba	Felix	Mean	SD
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.25	0.259	0.298	0.749	0.000	0.570	0.000	0.000	0.000	0.000	0.000	0.188	0.277
0.5	0.272	1.603	0.614	0.000	0.619	0.000	0.000	0.000	0.682	0.000	0.379	0.520
0.75	0.715	4.033	1.117	0.485	2.988	0.000	0.256	0.000	2.176	0.217	1.199	1.401
1	1.414	5.783	1.219	1.367	4.818	1.928	1.295	0.000	4.530	0.386	2.274	2.010
1.5	2.331	8.085	3.361	3.829	9.455	10.931	6.348	0.498	13.746	0.616	5.920	4.535
2	3.792	10.244	4.172	4.528	17.390	16.219	8.252	1.632	24.429	1.076	9.173	7.816
4	16.037	10.682	8.297	6.786	27.409	18.474	18.054	28.919	28.905	10.307	17.387	8.561
10	3.359	6.388	5.867	6.017	9.264	7.502	12.553	5.130	8.561	7.205	7.185	2.533
12	2.048	5.601	4.038	3.229	5.671	4.841	9.571	3.702	6.230	6.012	5.094	2.072
24	0.000	0.000	0.000	0.388	0.914	0.922	2.565	0.000	0.898	0.390	0.608	0.794
48	0.000	0.000	0.000	0.000	0.000	0.000	0.303	0.000	0.000	0.000	0.030	0.096

Table 2. 7 Ibuprofen plasma concentration profiles of Asian elephants at the oral dose of 5 mg/kg (n=9).

	Ibuprofen plasma concentration (mcg/ml)										
Time(h)	Packy	Vance	Hank	Tai	Dixie	Limba	Peggy	Booper	Shine	Mean	SD
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.25	0.000	0.000	0.281	0.399	0.687	0.665	0.272	0.000	0.000	0.256	0.282
0.5	0.000	3.423	0.689	1.316	1.218	2.838	0.924	1.818	0.628	1.428	1.099
0.75	0.000	10.035	0.980	1.532	1.422	4.436	1.516	3.969	0.234	2.680	3.145
1	0.452	21.545	1.619	1.525	1.376	5.681	2.739	3.653	0.758	4.372	6.642
1.5	3.111	30.385	4.165	6.474	1.207	8.759	4.944	10.373	1.073	7.832	9.024
2	5.133	30.049	4.800	8.577	2.589	11.783	7.647	9.080	1.550	9.023	8.530
4	36.986	29.707	12.678	21.542	18.891	-	12.198	11.066	6.664	18.716	10.319
10	12.737	13.410	5.870	13.015	15.640	6.522	7.921	8.667	17.917	11.300	4.224
12	8.724	11.868	3.807	8.560	10.879	3.798	6.716	6.324	14.094	8.308	3.537
24	1.803	3.267	0.000	1.651	2.937	0.818	0.812	0.833	2.762	1.654	1.135
48	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 2. 8 Ibuprofen plasma concentration profiles of African elephants at the oral dose of 6 mg/kg (n=9).

	Ibuprofen plasma concentration (mcg/ml)										
Time(h)	Digger	Solomon	Angus	Willie	Tattoo	Lea	Tasha	Sheba	Felix	Mean	SD
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.25	0.000	0.335	0.394	0.483	0.000	0.000	0.000	0.251	0.000	0.194	0.215
0.5	0.000	0.455	1.135	0.725	0.296	0.000	0.677	0.656	0.000	0.432	0.376
0.75	0.000	0.425	2.162	2.464	4.067	1.207	4.390	0.988	0.000	1.623	1.603
1	0.000	0.457	2.146	2.982	4.317	4.540	14.344	1.719	0.000	3.117	4.281
1.5	0.492	0.669	4.722	9.134	14.427	10.521	36.925	3.474	0.000	8.191	11.216
2	2.200	1.620	8.167	8.869	21.076	12.409	40.014	6.146	0.279	10.301	12.174
4	21.899	8.042	23.377	8.622	24.158	17.752	20.596	31.381	9.234	17.097	8.627
10	3.813	8.082	23.314	10.013	10.337	17.938	4.917	12.514	9.726	10.758	5.947
12	2.072	5.349	15.738	6.827	6.403	14.770	2.749	11.595	5.466	7.337	5.008
24	0.000	0.438	4.544	0.512	0.699	3.759	0.000	1.952	0.743	1.265	1.636
48	0.000	0.000	0.000	0.349	0.000	0.000	0.000	0.000	0.000	0.035	0.110

Table 2. 9 Ibuprofen plasma concentration profiles of Asian elephants at the oral dose of 6 mg/kg (n=9).

	Ibuprofen plasma concentration (mcg/ml)										
Time(h)	Vance	Hank	Packy	Tai	Dixie	Limba	Peggy	Booper	Shine	Mean	SD
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.25	9.958	0.000	0.434	0.000	0.000	4.570	0.000	0.449	0.000	1.712	3.429
0.5	14.811	0.246	0.335	0.695	1.848	11.435	1.348	0.536	1.012	3.585	5.496
0.75	36.458	0.263	0.362	1.562	5.354	15.848	4.517	0.932	2.261	7.506	11.890
1	50.867	0.571	0.902	2.337	8.391	17.359	5.743	1.441	6.254	10.430	16.051
1.5	47.397	2.371	0.989	2.631	13.702	15.918	7.949	4.325	24.718	13.334	14.975
2	48.762	4.435	1.680	4.096	18.353	14.968	10.130	11.594	40.978	17.222	16.677
4	31.634	12.643	2.205	19.689	21.732	15.729	8.046	13.358	27.710	16.972	9.297
10	13.407	9.222	24.547	15.650	16.729	6.052	12.094	9.615	8.842	12.907	5.550
12	10.332	6.945	27.603	9.681	11.166	3.461	6.744	8.207	6.685	10.092	6.966
24	2.130	0.704	13.586	1.472	1.686	0.306	1.223	2.234	1.207	2.728	4.119
48	0.385	0.000	1.898	0.000	0.000	0.000	0.000	0.000	0.000	0.254	0.630

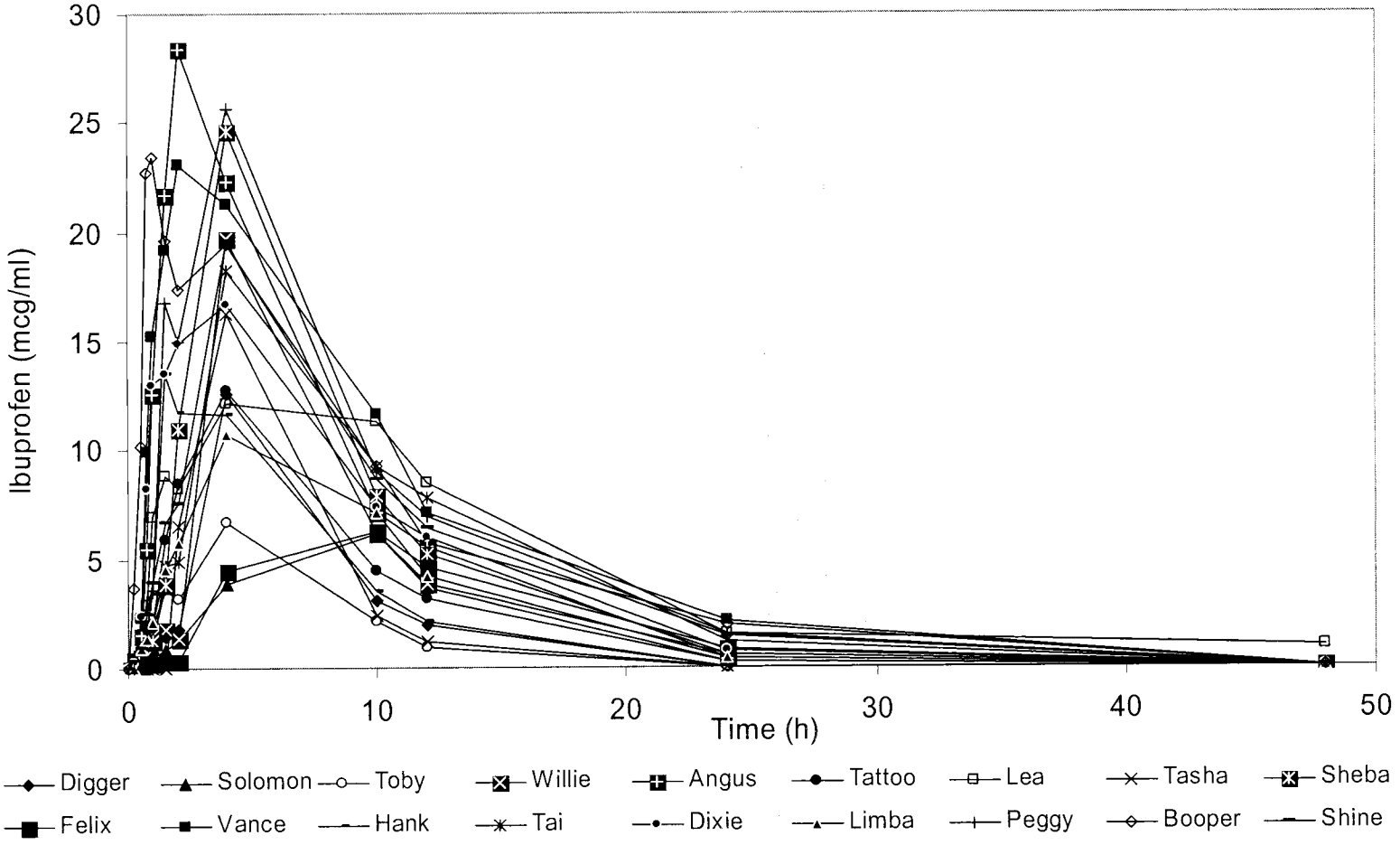

Figure 2. 3 Individual elephant plasma ibuprofen concentration (mcg/ml) versus time (h) curves after an oral dose of 4 mg/kg.

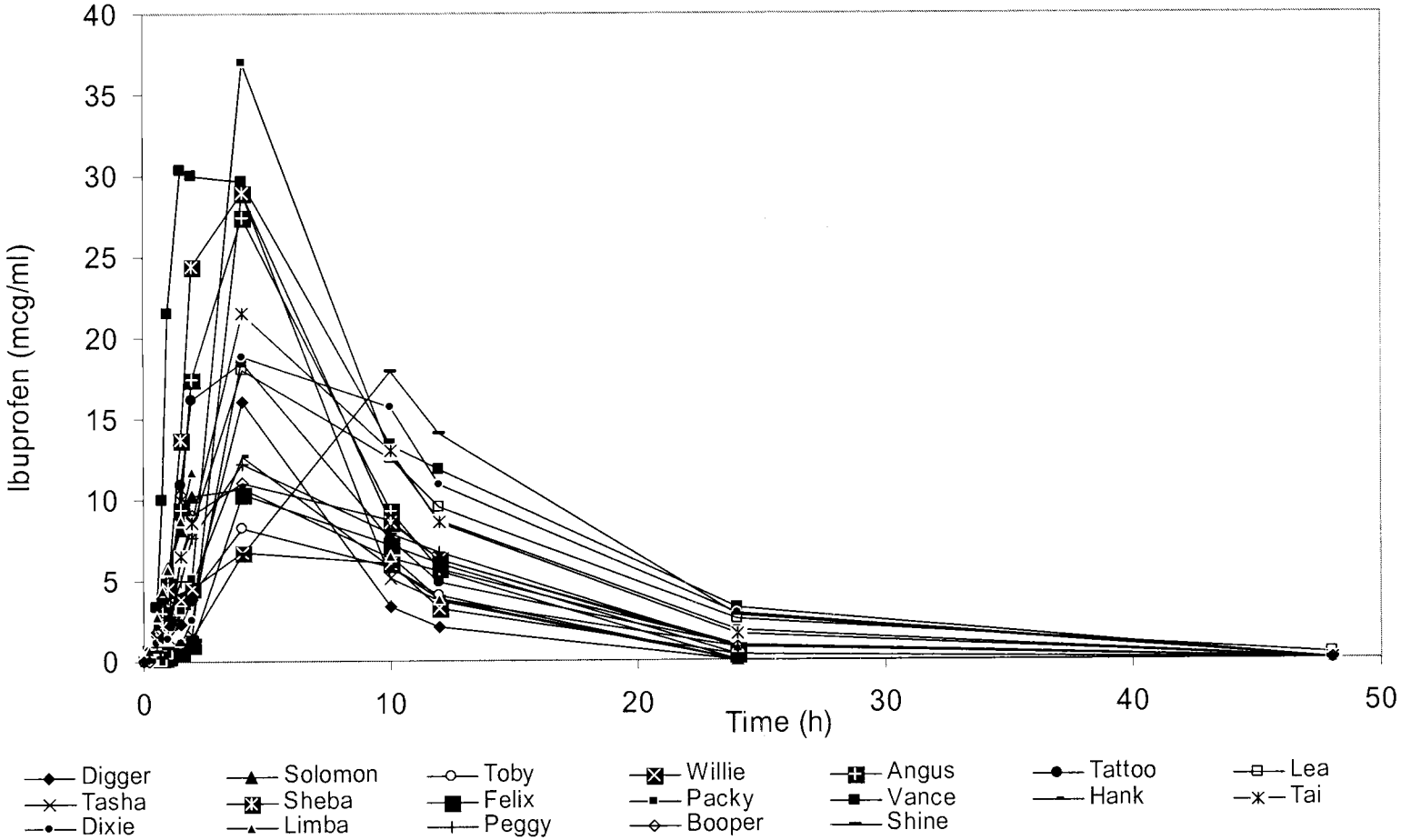

Figure 2. 4 Individual elephant plasma ibuprofen concentration (mcg/ml) versus time (h) curves after an oral dose of 5 mg/kg.

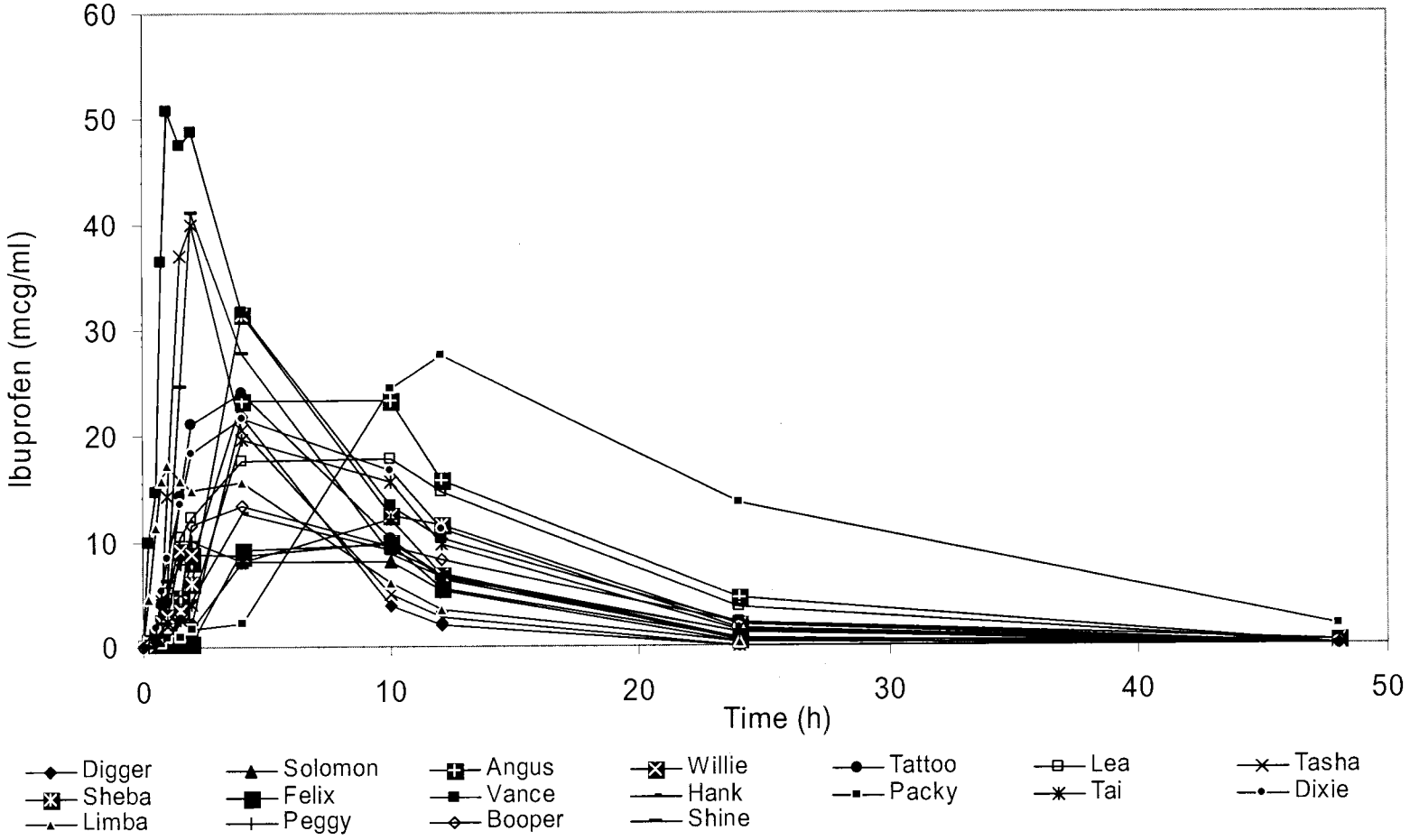

Figure 2. 5 Individual elephant plasma ibuprofen concentration (mcg/ml) versus time (h) curves after an oral dose of 6 mg/kg.

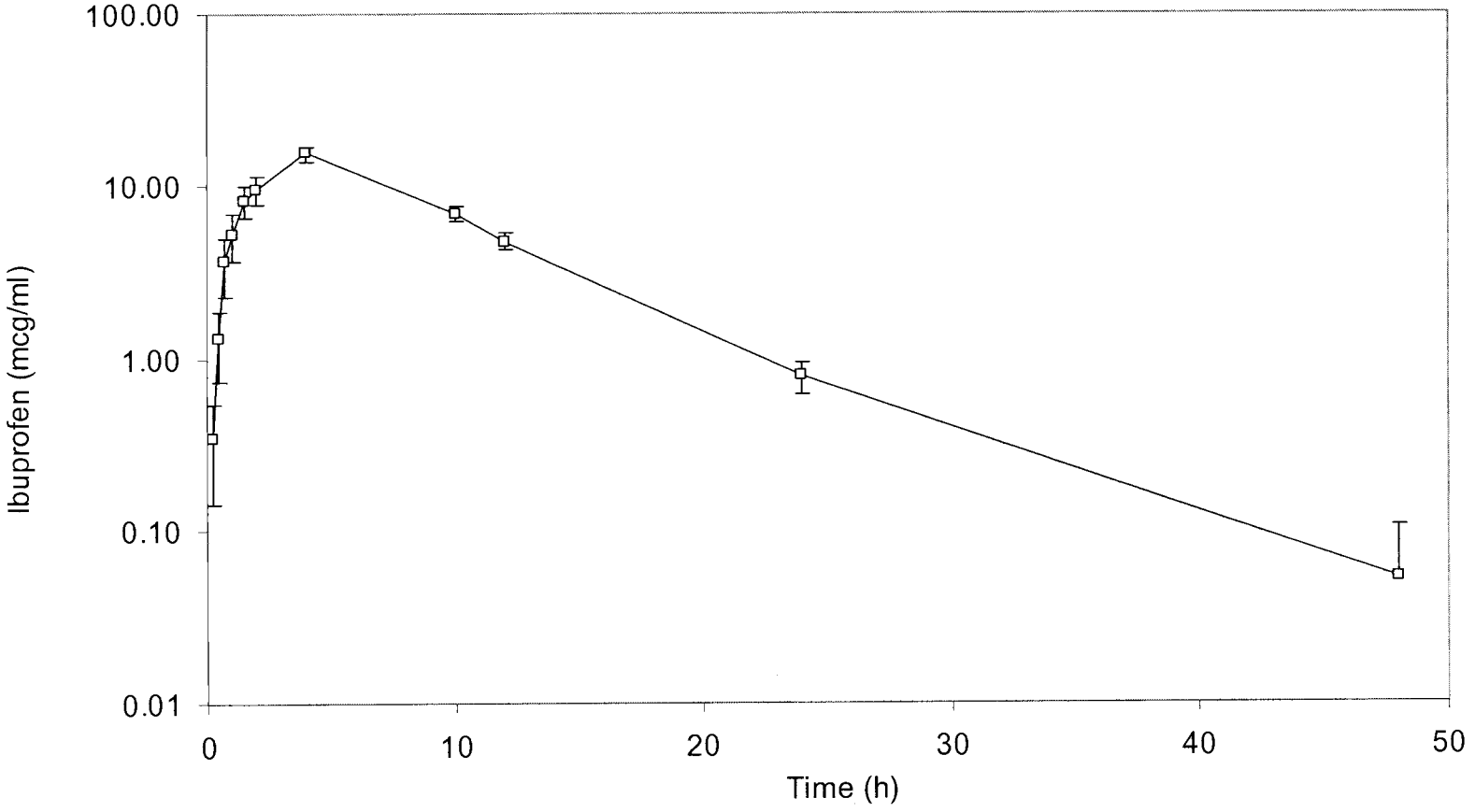

Figure 2. 6 Semilogarthmic plot of the mean ibuprofen concentration with standard error versus time after an oral dose of 4 mg/kg of ibuprofen was given orally to elephants (n = 18).

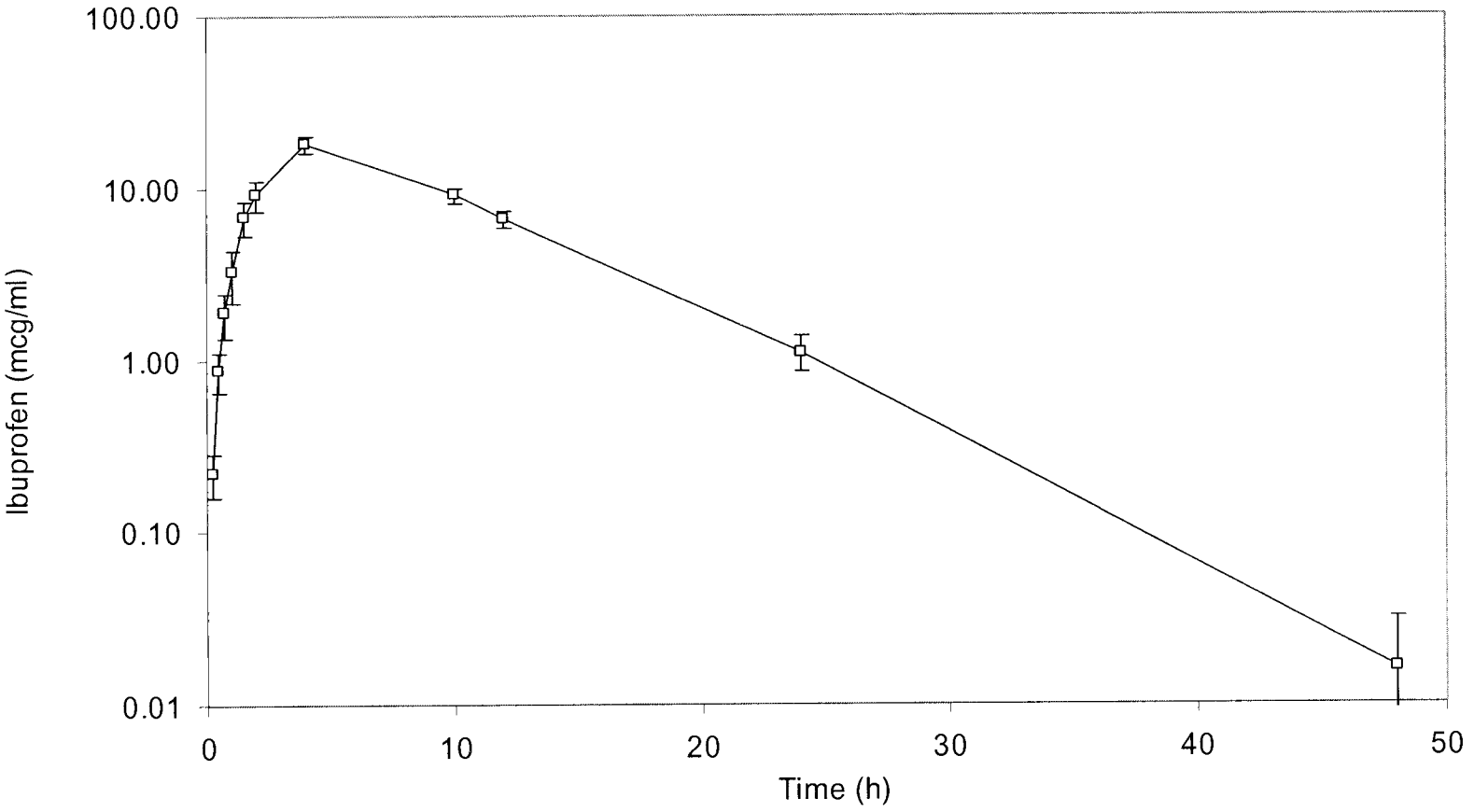

Figure 2. 7 Semilogarthmic plot of the mean ibuprofen concentration with standard error versus time after an oral dose of 5 mg/kg of ibuprofen was given orally to elephants (n = 19).

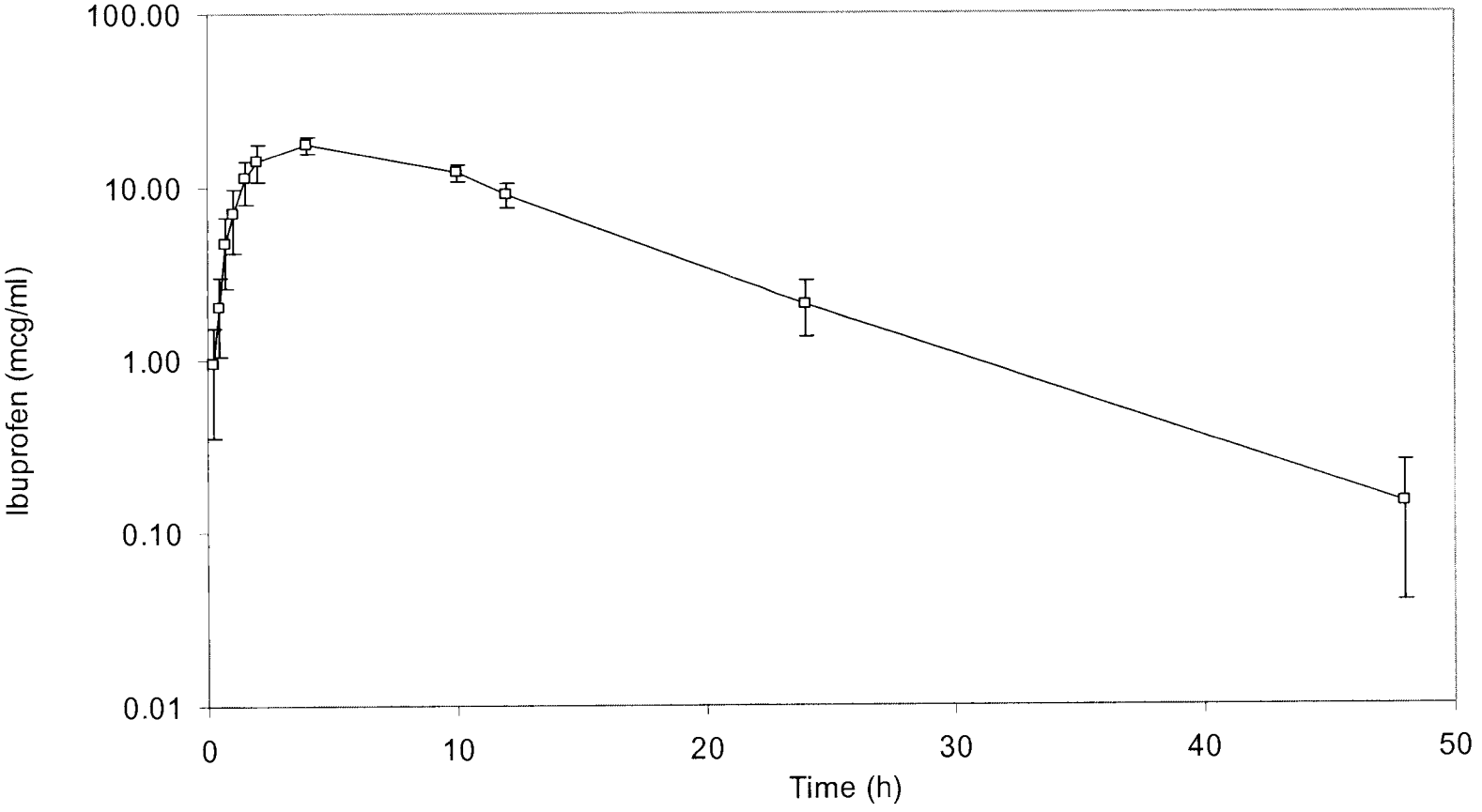

Figure 2. 8 Semilogarthmic plot of the mean ibuprofen concentration with standard error versus time after an oral dose of 6 mg/kg of ibuprofen was given orally to elephants (n = 18).

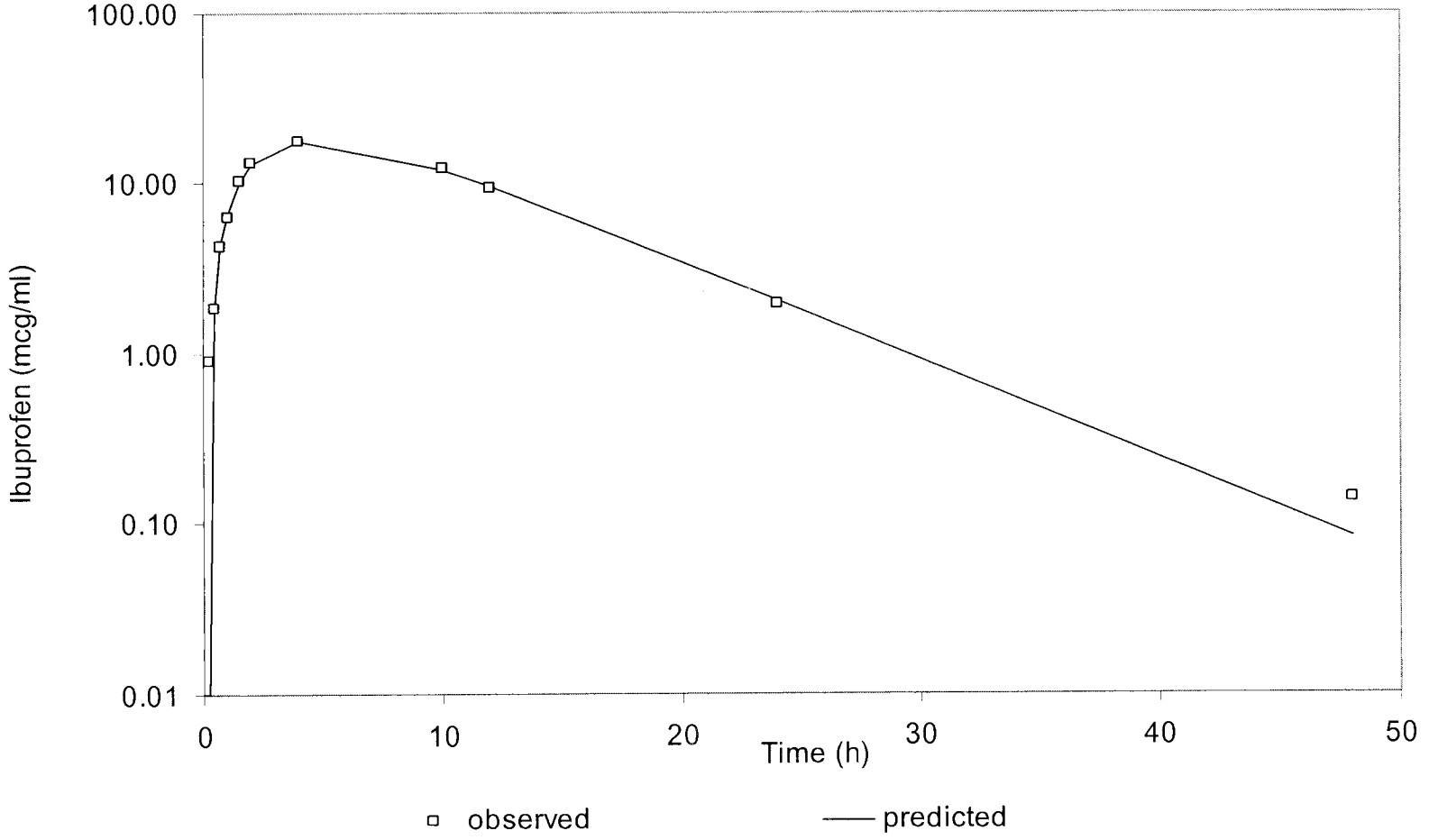

Figure 2. 9 Semilogarthmic plot of observed and predicted ibuprofen plasma concentrations versus time at 4 mg/kg oral dose.

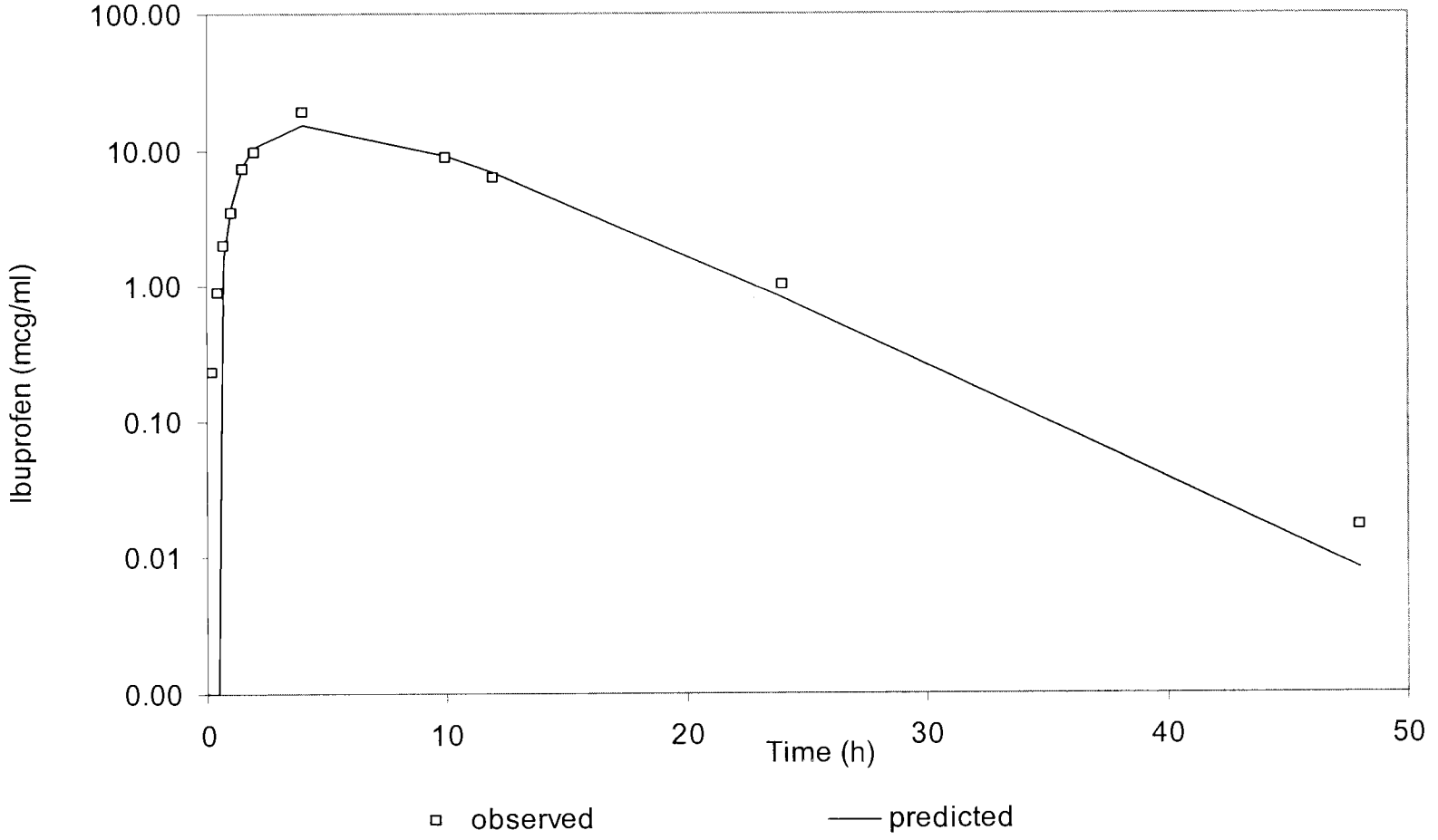

Figure 2. 10 Semilogarthmic plot of observed and predicted ibuprofen plasma concentrations versus time at 5 mg/kg oral dose.

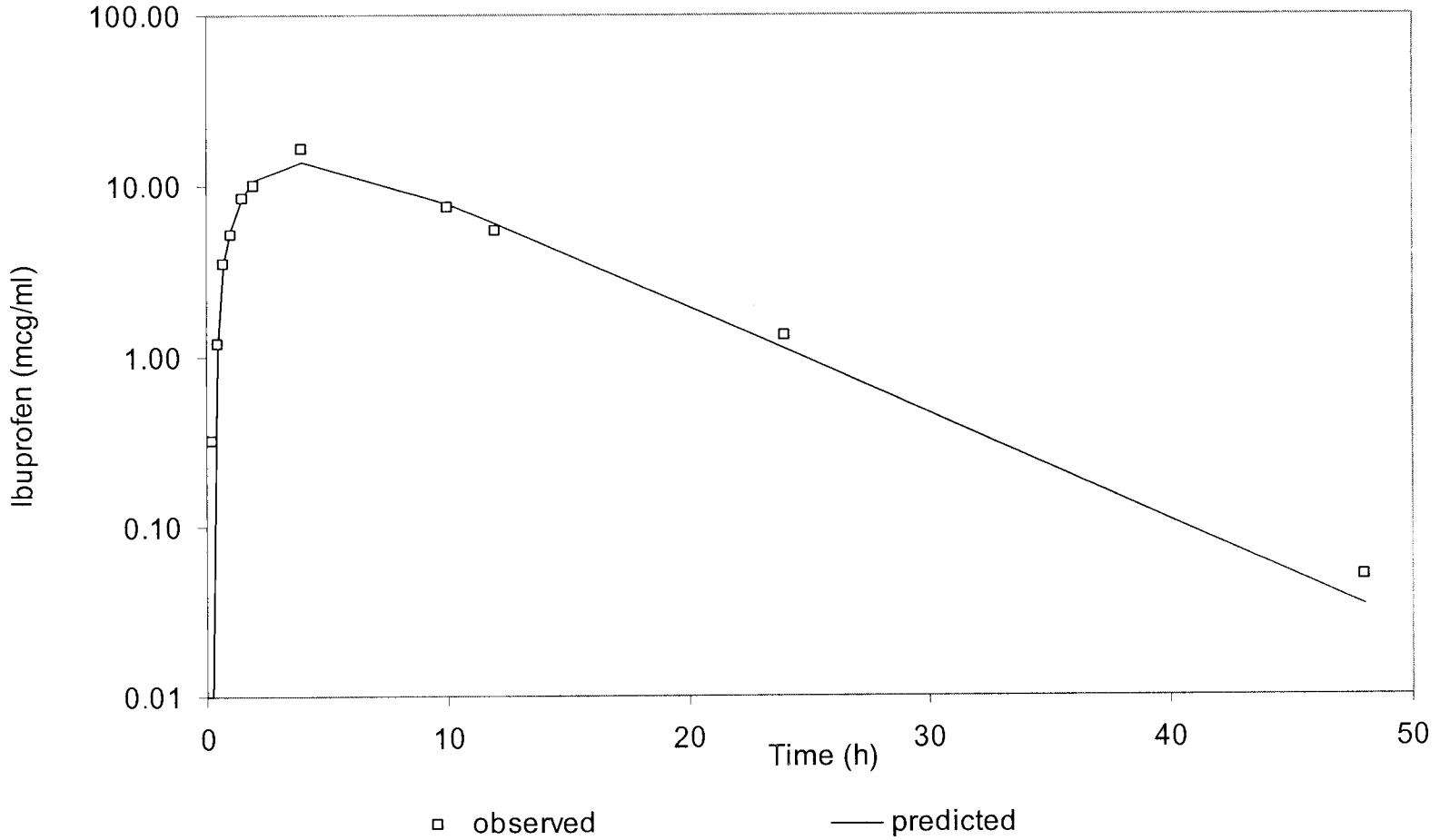

Figure 2. 11 Semilogarthmic plot of observed and predicted ibuprofen plasma concentrations versus time at 6 mg/kg oral dose.

Table 2. 10 Ibuprofen compartmental pharmacokinetic parameters after oral administration of 4, 5, and 6 mg/kg doses.

	4 mg/kg* (n = 18)	5 mg/kg[$] (n = 17)	6 mg/kg[%] (n = 18)
Parameter	Mean ± SD	Mean ± SD	Mean ± SD
Absorption rate constant (1/h)	1.90 ± 3.24	1.05 ± 1.64	0.97 ± 1.14
T_{lag} (h)	0.86 ± 0.76	1.05 ± 0.86	1.26 ± 1.62
C_{max} (mcg/ml)	15.14 ± 7.95	18.46 ± 9.60	21.04 ± 10.89
T_{max} (h)	3.79 ± 1.61	4.15 ± 0.99	4.87 ± 2.49
Terminal Rate Constant (1/h)	0.23 ± 0.16	0.19 ± 0.06	0.18 ± 0.06
Terminal Half-Life (h)	3.88 ± 1.60	4.03 ± 1.53	4.21 ± 1.55
Harmonic Mean Half-Life (h)	3.01	3.65	3.85
Vd/F (ml/kg)	173.42 ± 80.98	178.00 ± 66.58	189.85 ± 90.61
CL/F (ml/h/kg)	35.74 ± 21.52	33.37 ± 15.02	33.92 ± 16.37
AUC (mcg.h/ml)	145.04 ± 64.27	177.09 ± 72.05	220.65 ± 112.46
MRT (h)	4.74 ± 2.75	4.75 ± 2.38	4.81 ± 2.09

*Hugo, Tasha and Packy – Not included [%]Hugo – Not included [$]Hugo and Toby – Not included

Table 2. 11 Ibuprofen compartmental pharmacokinetic parameters after oral administration of 4 mg/kg dose in individual elephant.

Subject	K_a (1/h)	T_{lag} (h)	K_{el} (1/h)	AUC (mcg.h/ml)	MRT (h)	C_{max} (mcg/ml)	T_{max} (h)	t-half life (h)	Vd/F (ml/kg)	CL/F (ml/h/kg)
Digger	0.27	0.89	0.23	55.72	3.40	5.16	4.85	2.97	308.05	71.79
Solomon	0.19	0.71	0.19	56.42	4.65	3.86	6.09	3.72	380.55	70.89
Toby	0.30	0.57	0.36	43.72	2.20	5.31	3.59	1.92	253.37	91.49
Willie	0.18	0.44	0.17	84.71	5.61	5.40	6.20	4.19	285.36	47.22
Angus	1.15	0.62	0.20	197.28	4.47	26.93	2.47	3.53	103.15	20.28
Smokey	3.94	0.63	0.09	227.99	10.28	19.11	1.61	7.56	191.42	17.54
Tattoo	0.48	0.82	0.23	100.64	3.46	11.91	3.73	2.96	169.96	39.75
Lea	0.26	0.18	0.15	192.57	6.49	13.53	5.24	4.63	138.66	20.77
Sheba	7.54	1.95	0.19	186.13	3.31	32.17	2.45	3.65	113.06	21.49
Felix	0.14	3.49	0.84	92.68	-2.30	9.22	6.03	0.82	51.37	43.16
Vance	1.29	0.44	0.12	248.13	7.96	23.19	2.47	5.82	135.35	16.12
Hank	0.37	0.43	0.36	92.71	2.38	12.31	3.20	1.95	121.39	43.14
Tai	0.34	1.14	0.17	173.75	4.62	14.97	5.18	3.99	132.68	23.02
Dixie	1.10	0.39	0.14	169.83	6.94	17.24	2.55	5.08	172.69	23.55
Limba	0.24	0.58	0.24	110.86	3.67	9.79	4.74	2.94	153.18	36.08
Peggy	0.53	0.69	0.18	210.33	4.78	21.99	3.75	3.79	104.06	19.02
Booper	12.41	0.45	0.11	217.18	8.91	22.26	0.84	6.49	172.45	18.42
Shine	0.27	0.41	0.18	168.77	5.03	13.60	4.90	3.77	128.93	23.70
Mean	1.90	0.86	0.23	173.42	4.74	15.14	3.79	3.88	173.42	35.74
SD	3.24	0.76	0.16	80.98	2.75	7.95	1.61	1.60	80.98	21.52

Table 2. 12 Ibuprofen compartmental pharmacokinetic parameters after oral administration of 5 mg/kg dose in individual elephant.

Subject	K_a (1/h)	T_{lag} (h)	K_{el} (1/h)	AUC (mcg.h/ml)	MRT (h)	C_{max} (mcg/ml)	T_{max} (h)	t-half life (h)	Vd/F (ml/kg)	CL/F (ml/h/kg)
Digger	0.32	0.88	0.28	70.61	2.64	7.87	4.17	2.44	249.22	70.82
Solomon	0.86	0.36	0.09	154.52	10.45	10.91	3.27	7.50	349.92	32.36
Willie	0.23	0.65	0.24	85.12	3.51	7.42	4.87	2.88	244.46	58.74
Angus	0.31	0.63	0.29	196.76	2.79	21.88	3.94	2.38	87.07	25.41
Tattoo	0.97	0.93	0.17	165.96	5.13	19.07	3.12	4.20	182.57	30.13
Lea	0.42	0.89	0.12	238.08	7.73	16.87	5.14	5.97	180.92	21.00
Tasha	2.32	1.98	0.27	163.25	1.65	33.70	3.03	2.52	111.41	30.63
Sheba	0.68	0.86	0.22	221.52	3.70	28.30	3.33	3.16	102.89	22.57
Felix	0.29	1.87	0.26	115.84	1.98	11.65	5.53	2.67	166.19	43.16
Packy	6.93	3.02	0.17	247.82	2.96	37.78	3.57	4.15	120.72	20.18
Vance	2.03	0.59	0.11	348.86	8.61	32.11	2.12	6.38	131.98	14.33
Hank	0.27	0.64	0.22	102.84	3.89	9.26	4.71	3.14	220.21	48.62
Tai	0.31	0.88	0.18	221.03	4.67	18.68	5.08	3.85	125.67	22.62
Dixie	0.98	2.87	0.12	256.76	5.58	22.72	5.33	5.86	164.66	19.47
Limba	0.48	0.19	0.17	127.65	5.81	12.08	3.58	4.16	235.11	39.17
Peggy	0.23	0.42	0.20	142.54	4.63	11.07	5.14	3.50	177.12	35.08
Booper	0.27	0.25	0.19	151.27	5.07	12.45	4.64	3.69	175.82	33.05
Mean	1.05	1.05	0.19	177.08	4.75	18.46	4.15	4.03	178.00	33.37
SD	1.64	0.86	0.06	72.05	2.38	9.60	0.99	1.53	66.58	15.02

Table 2. 13 Ibuprofen compartmental pharmacokinetic parameters after oral administration of 6 mg/kg dose in individual elephant.

Subject	K_a (1/h)	T_{lag} (h)	K_{el} (1/h)	AUC (mcg.h/ml)	MRT (h)	C_{max} (mcg/ml)	T_{max} (h)	t-half (h)	Vd/F (ml/kg)	CL/F (ml/h/kg)
Digger	1.55	1.96	0.30	116.67	1.32	23.88	3.27	2.27	168.63	51.43
Solomon	0.25	1.75	0.25	110.22	2.28	10.04	5.79	2.79	219.43	54.44
Angus	0.17	0.67	0.15	363.94	6.05	21.07	7.01	4.65	110.71	16.49
Willie	0.23	0.39	0.22	141.72	4.11	11.67	4.86	3.12	190.71	42.34
Tattoo	0.40	0.63	0.24	213.84	3.56	23.86	3.81	2.90	117.55	28.06
Lea	0.33	0.63	0.11	328.29	8.13	21.30	5.58	6.08	160.20	18.28
Tasha	3.41	0.89	0.27	178.71	2.85	38.48	1.70	2.59	125.56	33.57
Sheba	0.23	0.92	0.18	239.90	4.62	18.04	5.77	3.84	138.66	25.01
Felix	2.12	3.80	0.19	140.47	1.44	21.14	5.05	3.63	223.57	42.71
Vance	3.72	0.42	0.15	374.67	6.26	49.03	1.32	4.63	106.94	16.01
Hank	0.24	1.24	0.24	147.28	2.92	12.90	5.44	2.89	169.57	40.74
Packy	0.37	6.98	0.08	523.77	5.33	27.68	12.27	8.53	141.04	11.46
Tai	0.17	0.66	0.18	203.24	4.88	13.06	6.39	3.84	163.69	29.52
Dixie	0.32	0.38	0.17	283.77	5.45	23.82	4.55	4.04	123.20	21.14
Limba	2.18	0.14	0.16	137.73	6.26	17.58	1.44	4.43	278.52	43.56
Peggy	0.34	0.22	0.13	163.36	7.37	11.79	4.78	5.26	278.74	36.73
Booper	0.53	0.88	0.11	198.17	8.55	14.05	4.68	6.54	285.50	30.28
Shine	1.69	0.92	0.19	250.47	4.41	35.74	2.38	3.69	127.61	23.96
Mean	0.97	1.26	0.18	220.65	4.81	21.04	4.87	4.21	189.85	33.92
SD	1.14	1.62	0.06	112.46	2.09	10.89	2.49	1.55	90.61	16.37

Table 2. 14 Ibuprofen noncompartmental pharmacokinetic parameters after oral administration of 4, 5, and 6 mg/kg doses.

	4 mg/kg* (n=18)	5 mg/kg[$] (n=19)	6 mg/kg[%] (n=18)
Parameter	Mean ± SD	Mean ± SD	Mean ± SD
C_{max} (mcg/ml)	16.47 ± 6.87	18.28 ± 8.62	22.38 ± 11.96
T_{max} (h)	4.14 ± 2.36	4.08 ± 1.60	5.56 ± 3.65
Terminal Rate Constant (1/h)	0.18 ± 0.06	0.15 ± 0.05	0.16 ± 0.06
Terminal Half-Life (h)	4.73 ± 2.81	5.11 ± 1.93	5.21 ± 2.53
Harmonic Mean Half-Life (h)	3.96	4.55	4.39
Vd_{ss} (ml/kg)	307.34 ± 159.14	323 ± 127.55	313.94 ± 141.53
CL/F (ml/h/kg)	35.94 ± 19.87	33.08 ± 13.68	32.84 ± 15.03
AUC (mcg.h/ml)	142.40 ± 65.38	177.09 ± 72.14	227.37 ± 117.30
MRT (h)	9.05 ± 2.87	10.11 ± 2.55	10.21 ± 3.64

- *Hugo, Smokey and Packy – Not included
- [$]Hugo – Not included
- [%]Hugo, Toby – Not included

Table 2. 15 Ibuprofen noncompartmental pharmacokinetic parameters after oral administration of 4mg/kg dose in individual elephant.

Subject	C_{max} (mcg/ml)	T_{max} (h)	AUC (mcg.h/ml)	K_{el} (1/h)	$T_{1/2}$ (h)	MRT (h)	CL/F (ml/h/kg)	Vd_{ss} (ml/kg)
Angus	28.41	2.00	192.46	0.21	3.26	6.52	20.78	135.55
Booper	23.41	1.00	227.35	0.10	6.64	9.73	17.59	171.11
Digger	12.61	4.00	70.01	0.23	2.99	7.22	57.13	412.46
Dixie	16.67	4.00	167.22	0.16	4.25	8.15	23.92	194.93
Felix	6.26	10.00	80.99	0.14	4.89	12.41	49.39	613.07
Hank	13.50	1.50	92.26	0.21	3.30	6.33	43.35	274.59
Lea	12.13	4.00	218.29	0.05	14.91	18.38	18.32	336.88
Limba	10.72	4.00	107.33	0.20	3.52	8.56	37.27	318.95
Peggy	25.67	4.00	220.79	0.13	5.46	9.20	18.12	166.60
Sheba	24.62	4.00	171.09	0.18	3.79	7.86	23.38	183.80
Shine	19.56	4.00	181.19	0.13	5.45	10.03	22.08	221.43
Solomon	6.21	10.00	62.36	0.23	2.99	10.00	64.14	641.46
Tai	18.27	4.00	174.70	0.15	4.69	10.04	22.90	229.81
Tasha	16.33	4.00	75.62	0.32	2.15	5.89	52.90	311.66
Tattoo	12.74	4.00	97.30	0.20	3.44	7.47	41.11	307.16
Toby	6.70	4.00	45.85	0.23	3.05	6.74	87.25	588.03
Vance	23.02	2.00	251.28	0.11	6.20	9.83	15.92	156.49
Willie	19.68	4.00	127.19	0.16	4.21	8.53	31.45	268.15
Mean	16.47	4.14	142.40	0.18	4.73	9.05	35.94	307.34
SD	6.87	2.36	65.38	0.06	2.81	2.87	19.87	159.14

Table 2. 16 Ibuprofen noncompartmental pharmacokinetic parameters after oral administration of 5mg/kg dose in individual elephant.

Subject	C_{max} (mcg/ml)	T_{max} (h)	AUC (mcg.h/ml)	K_{el} (1/h)	$T_{1/2}$ (h)	MRT (h)	CL/F (ml/h/kg)	Vd_{ss} (ml/kg)
Angus	27.41	4.00	208.63	0.16	4.31	7.91	23.97	189.58
Booper	11.07	4.00	141.68	0.17	4.13	9.30	35.29	328.17
Digger	16.04	4.00	84.61	0.26	2.69	6.65	59.10	393.15
Dixie	18.89	4.00	251.47	0.12	5.98	12.41	19.88	246.72
Felix	10.31	4.00	103.83	0.22	3.22	9.44	48.15	454.63
Hank	12.68	4.00	112.28	0.15	4.77	9.33	44.53	415.57
Lea	18.05	4.00	237.24	0.10	7.26	12.73	21.08	268.24
Limba	11.78	2.00	121.64	0.14	4.91	8.93	41.10	366.99
Packy	36.99	4.00	268.58	0.14	5.07	9.52	18.62	177.25
Peggy	12.20	4.00	138.42	0.17	4.14	9.55	36.12	344.83
Sheba	28.90	4.00	222.31	0.16	4.30	7.62	22.49	171.47
Shine	17.92	10.00	219.28	0.13	5.16	13.76	22.80	313.81
Solomon	10.68	4.00	177.62	0.07	10.34	15.86	28.15	446.44
Tai	21.54	4.00	221.42	0.14	4.82	9.98	22.58	225.41
Tasha	28.92	4.00	135.35	0.26	2.62	6.86	36.94	253.43
Tattoo	18.47	4.00	164.74	0.15	4.76	8.46	30.35	256.70
Toby	8.30	4.00	121.61	0.08	8.39	14.23	41.11	585.12
Vance	30.38	1.50	353.50	0.10	6.63	10.50	14.14	148.47
Willie	6.79	4.00	80.42	0.19	3.67	8.99	62.17	558.64
Mean	18.28	4.08	177.09	0.15	5.11	10.11	33.08	323.40
SD	8.62	1.60	72.14	0.05	1.93	2.55	13.68	127.55

Table 2. 17 Ibuprofen noncompartmental pharmacokinetic parameters after oral administration of 6mg/kg dose in individual elephant.

Subject	C_{max} (mcg/ml)	T_{max} (h)	AUC (mcg.h/ml)	K_{el} (1/h)	$T_{1/2}$ (h)	MRT (h)	CL/F (ml/h/kg)	Vd_{ss} (ml/kg)
Angus	23.38	4.00	364.41	0.11	6.18	12.77	16.47	210.19
Booper	13.36	4.00	193.42	0.11	6.56	12.31	31.02	381.73
Digger	21.90	4.00	99.80	0.29	2.36	6.49	60.12	390.46
Dixie	21.73	4.00	269.27	0.16	4.29	9.38	22.28	208.96
Felix	9.73	10.00	113.75	0.18	3.90	10.38	52.75	547.66
Hank	12.64	4.00	137.36	0.19	3.72	9.58	43.68	418.66
Lea	17.94	10.00	310.26	0.11	6.16	12.67	19.34	245.10
Limba	17.36	1.00	143.97	0.21	3.31	6.32	41.68	263.24
Packy	27.60	12.00	543.80	0.08	9.19	21.45	11.03	236.66
Peggy	12.09	10.00	153.52	0.16	4.44	10.38	39.08	405.85
Sheba	31.38	4.00	268.46	0.14	5.02	10.14	22.35	226.69
Shine	40.98	2.00	255.01	0.14	4.87	7.61	23.53	178.98
Solomon	8.08	10.00	98.15	0.21	3.33	9.70	61.13	592.80
Tai	19.69	4.00	219.10	0.16	4.21	10.06	27.39	275.49
Tasha	40.01	2.00	177.48	0.26	2.67	5.01	33.81	169.34
Tattoo	24.16	4.00	209.10	0.19	3.65	7.58	28.69	217.46
Vance	50.87	1.00	390.75	0.10	7.26	8.83	15.36	135.59
Willie	10.01	10.00	145.07	0.05	12.62	13.20	41.36	546.01
Mean	22.38	5.56	227.37	0.16	5.21	10.21	32.84	313.94
SD	11.96	3.65	117.30	0.06	2.53	3.64	15.03	141.53

Ibuprofen pharmacokinetics and dose proportionality following the oral single doses

Figure 2.12 and 2.13 compare the individual AUC and C_{max} following administration of 4, 5, and 6 mg/kg dose, respectively. The relationship between AUC versus dose and C_{max} versus dose, using the power model, are also presented in figure 2.12 and 2.13.

Approximate linear dose proportional increases were noted in mean AUCs which is supported by lack of statistically significant deviation from linearity in the dose normalized AUC (p-value: 0.97). Additionally with the power model the exponent of dose for AUC [$25.04 \bullet Dose^{1.16}$] was not significantly different from unity and 95% confidence interval of these exponents included 1 (0.4, 1.9). Whereas C_{max} does not appear to increase proportionally with increase in dose as was seen with the power model [$5.81 \bullet Dose^{0.69}$]. At higher doses the absorption appears to be slower as T_{max} tends to be longer as the dose increases. Likewise, there was no significant difference in dose independent pharmacokinetic parameters MRT and CL/F. (p-values of 0.25 and 0.99)

Statistical comparisons of dose-normalized plasma concentrations were also not significantly different following the three doses.

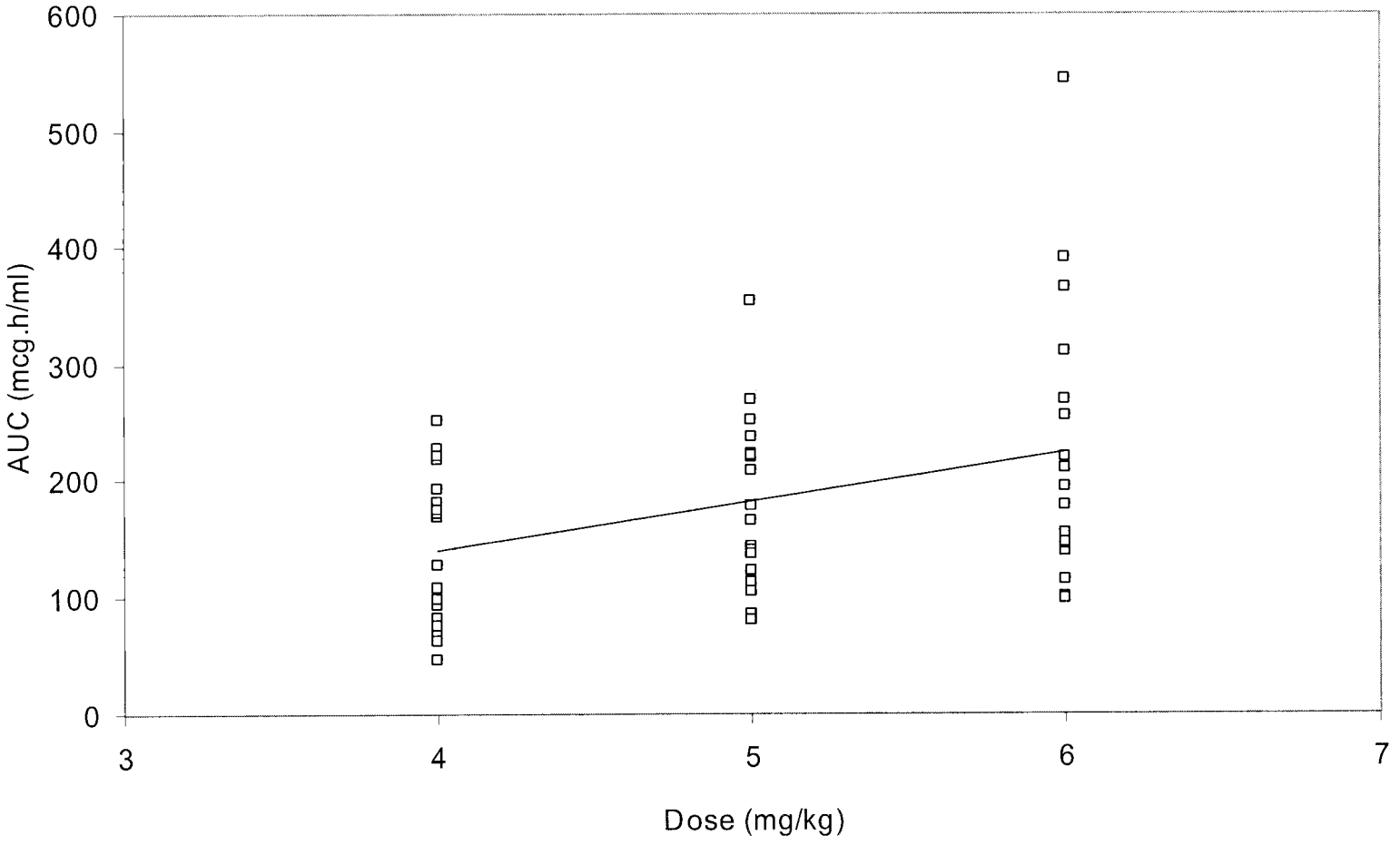

Figure 2. 12 Ibuprofen plasma AUC versus dose following single doses of 4, 5, and 6 mg/kg with fitted power function.

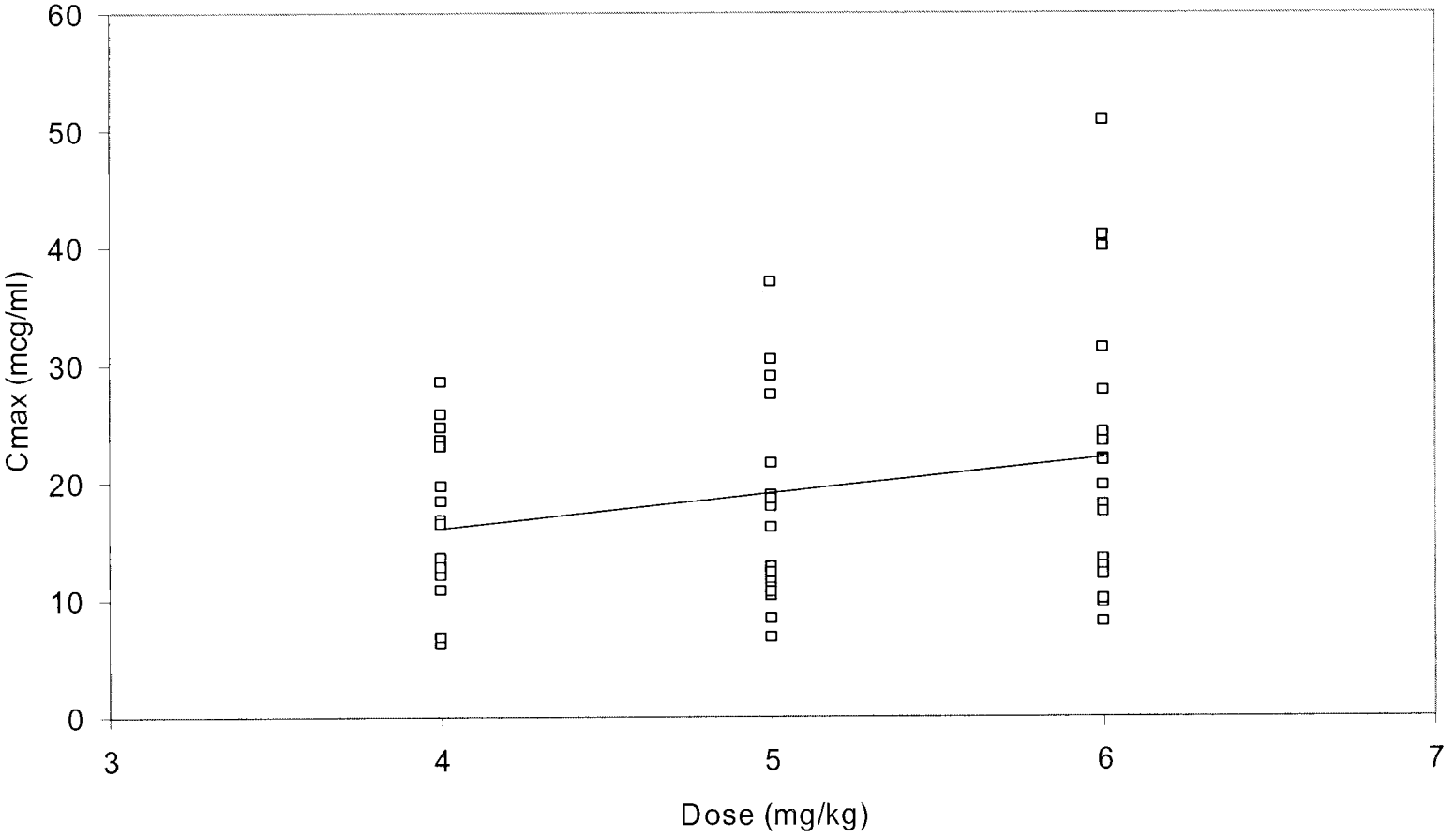

Figure 2. 13 Ibuprofen plasma C_{max} versus dose following single doses of 4, 5, and 6 mg/kg with fitted power function.

Comparison of ibuprofen pharmacokinetics between Asian and African elephants

Summary of the statistical comparison between doses and species effects on pharmacokinetic parameters following the three doses is shown in table 2.18. The plasma concentration time profiles for African and Asian elephants for the three doses are shown in figure 2.14, 2.15 and 2.16. Dose –dependent parameters such as AUC and C_{max} were dose normalized prior to comparison. Effects of dose, zoo and species groups were included in the ANOVA model. Although CL/F and AUC seem to be different between Asian and African elephants in table 2.18, they are not significantly different. Interestingly there appears to be differences between zoos and this might be due to various factors like how the dose is administered, or the food given or how the blood samples were withdrawn between the zoos etc. Although there is no significant difference between the African and Asian elephants in CL/F, the trend suggests that Asian elephants have lower CL/F than the African elephants. In humans ibuprofen is metabolized by CYP 450 enzyme CYP2C9. Genetic polymorphism is seen with CYP2C9 (28, 29). Ibuprofen is extensively metabolized by forming 2-hydroxy ibuprofen and carboxy ibuprofen. Ibuprofen is eliminated further following biotransformation to glucuronide conjugate metabolites that are excreted in urine, with little drug being eliminated unchanged. No firm conclusion can be made on differences in species due to large intra and intersubject variability and small sample size. The large intersubject variability and small sample size highly affect the power of hypothesis test. The

power of the test is the probability that the test does reject the null hypothesis when it is false. The result suggests the need of an additional study of larger sample size in each species group to confirm if there is a species difference. The plasma concentration time profiles for African male and female and Asian male and female elephants are shown in figures 2.17, 2.18, 2.19, 2.20, 2.21, and 2.22. The pharmacokinetic parameters between males and females in African and Asian elephants were not statistically significant. Although the CL/F of African females was less than the African males the difference was not statistically significant.

Table 2. 18 Pharmacokinetic parameters of ibuprofen given at 4, 5, and 6 mg/kg orally for African and Asian elephants.

	4 mg/kg		5 mg/kg		6 mg/kg		ANOVA
Parameter	African (n=10)	Asian (n=8)	African (n=10)	Asian (n=9)	African (n=9)	Asian (n=9)	P-value*
AUC (mcg.h/ml)	114.11 ± 60.13	177.76 ± 56.21	153.63 ± 57.15	203.14 ± 81.15	198.50 ± 97.03	256.24 ± 129.42	0.25
CL/F (ml/h/kg)	44.59 ± 21.97	25.14 ± 9.90	37.35 ± 14.98	28.34 ± 10.98	37.33 ± 17.36	28.34 ± 11.54	0.20
Terminal half-life(h)	3.54 ± 3.71	4.68 ±1.21	4.33 ± 2.62	4.95 ± 0.81	4.08 ± 3.14	4.95 ± 1.94	0.81
C_{max} (mcg/ml)	14.57 ± 7.72	18.85 ± 5.14	17.39 ± 8.56	19.27 ± 9.09	20.73 ± 10.66	24.04 ± 13.58	0.40
T_{max} (h)	5.00 ± 2.71	3.06 ± 1.32	4.00 ± 0.00	4.17 ± 2.40	6.44 ± 3.43	4.67 ± 3.84	0.45
Vd_{ss} (ml/kg)	379.81 ± 179.39	216.74 ± 56.93	357.74 ± 150.05	285.25 ± 90.35	349.52 ± 171.04	278.35 ± 102.27	0.10
MRT (h)	9.10 ± 3.78	8.98 ± 1.28	9.87 ± 3.24	10.36 ± 1.64	9.77 ± 2.91	10.66 ± 4.39	0.92

* P-value showing difference between African and Asian elephants

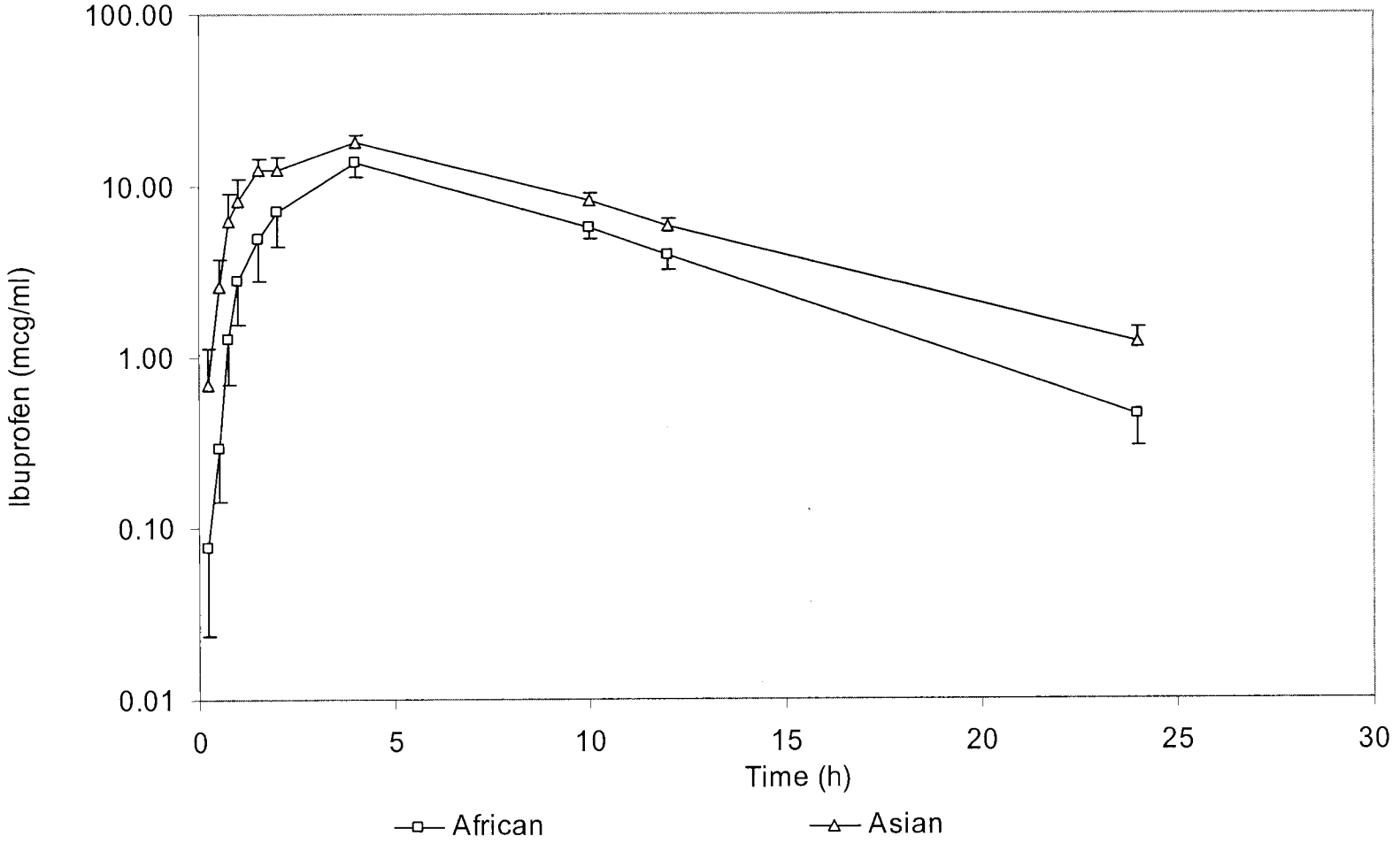

Figure 2. 14 Mean plasma concentration time profiles of ibuprofen with standard error in African (n=10) and Asian (n=8) elephants at 4 mg/kg oral dose.

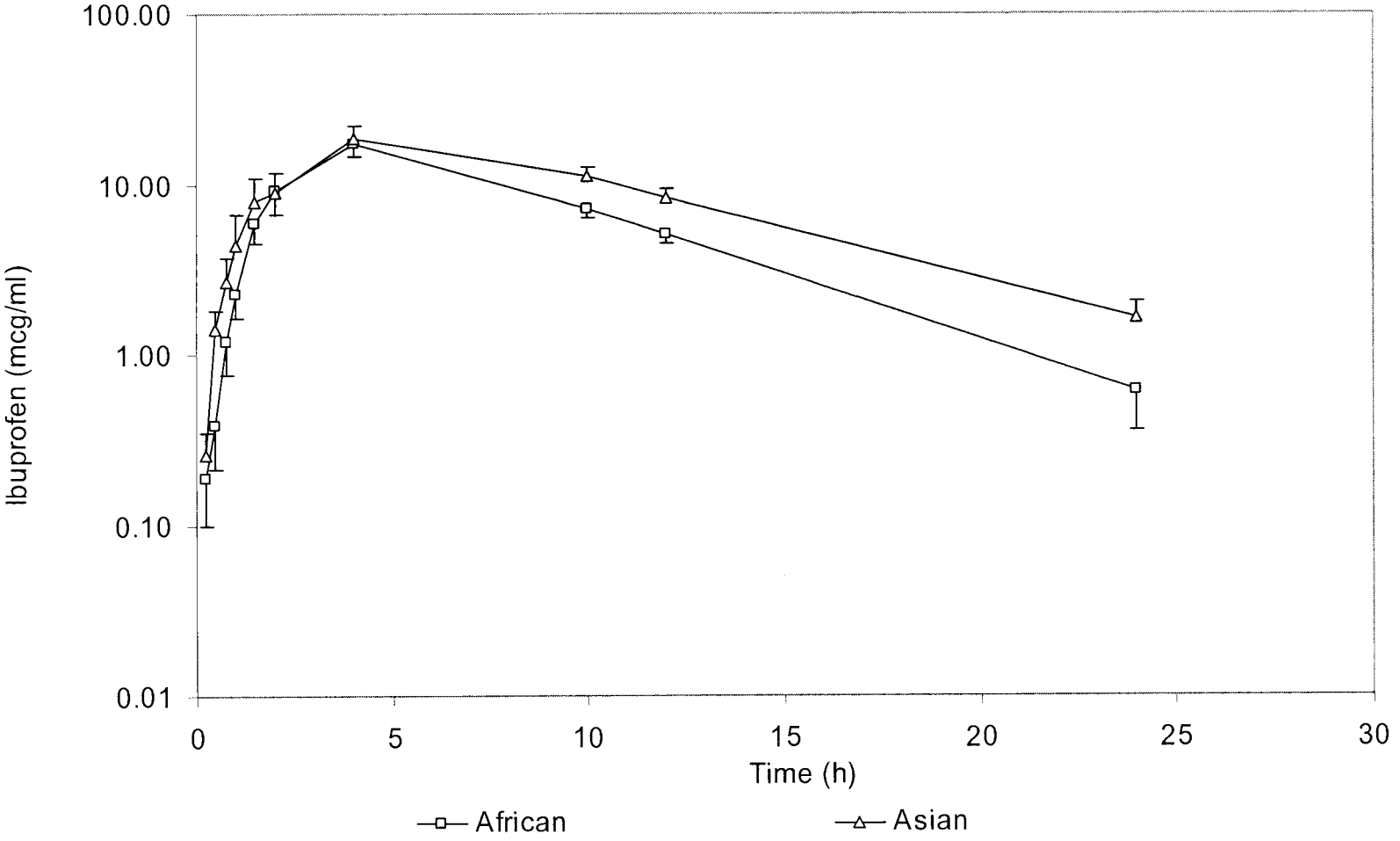

Figure 2. 15 Mean plasma concentration time profiles of ibuprofen with standard error in African (n=10) and Asian (n=8) elephants at 5 mg/kg oral dose.

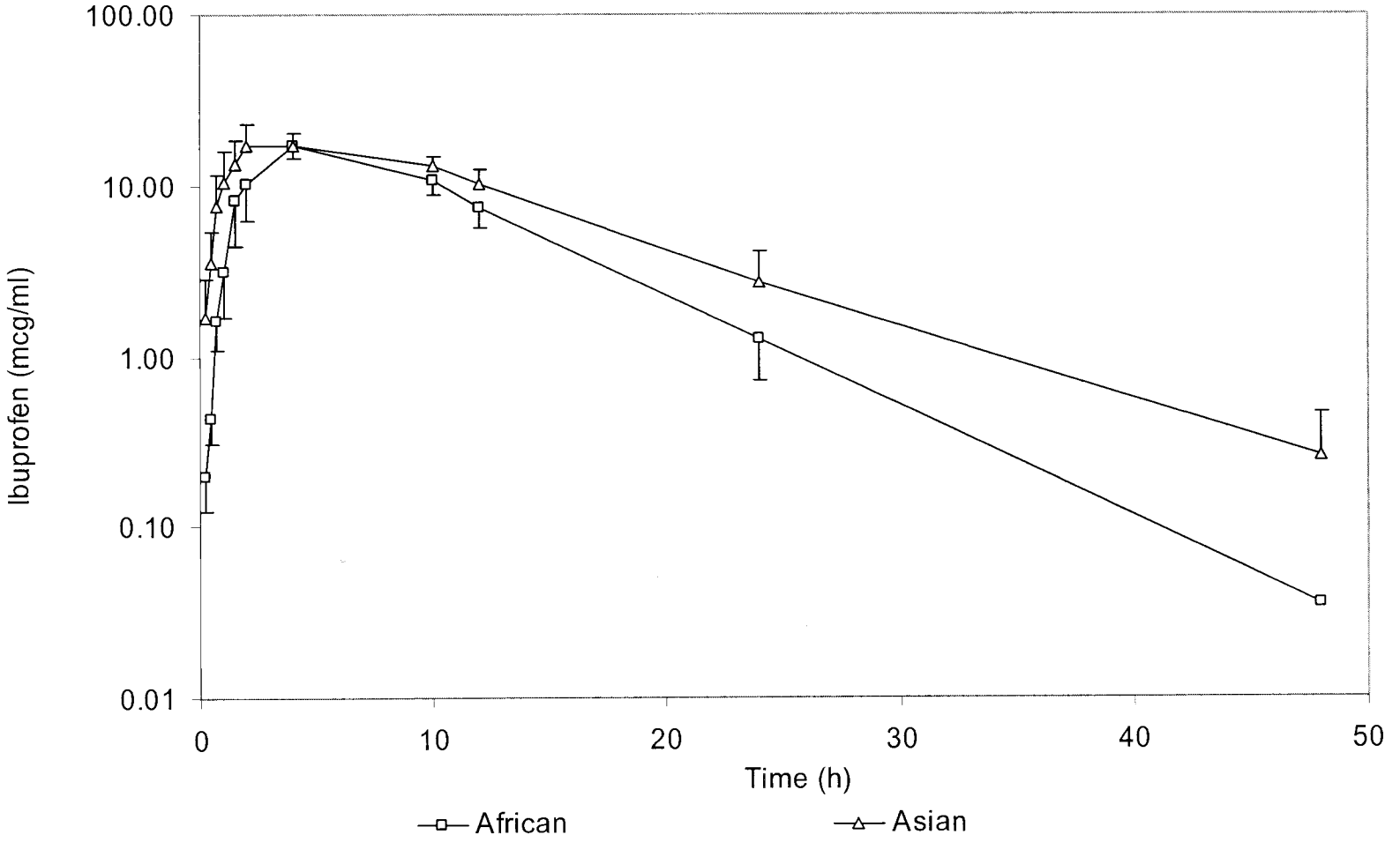

Figure 2. 16 Mean plasma concentration time profiles of ibuprofen with standard error in African (n=10) and Asian (n=8) elephants at 6 mg/kg oral dose.

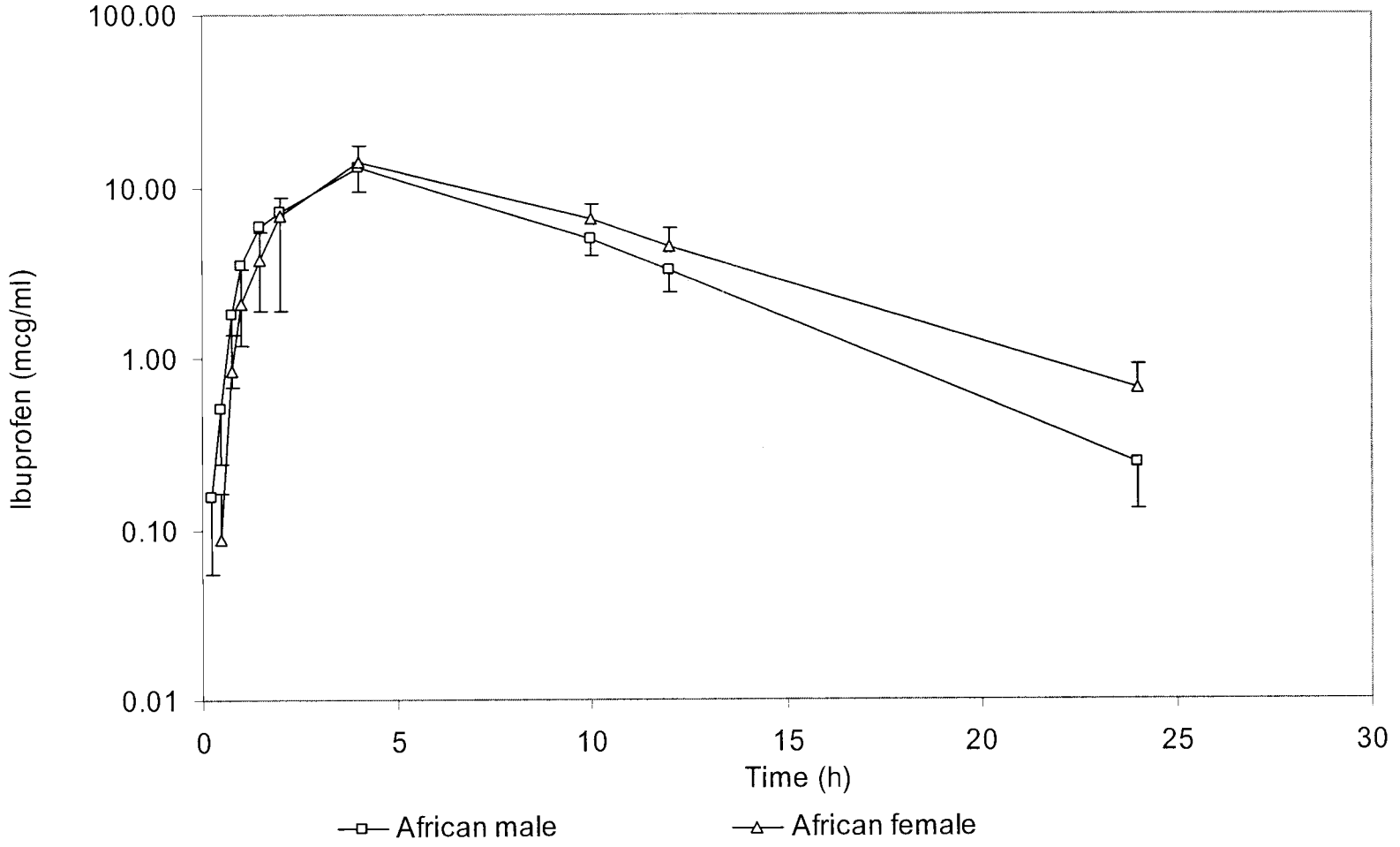

Figure 2. 17 Mean plasma concentration time profiles of ibuprofen with standard error in African Male (n=5) and Female (n=5) elephants at 4 mg/kg oral dose.

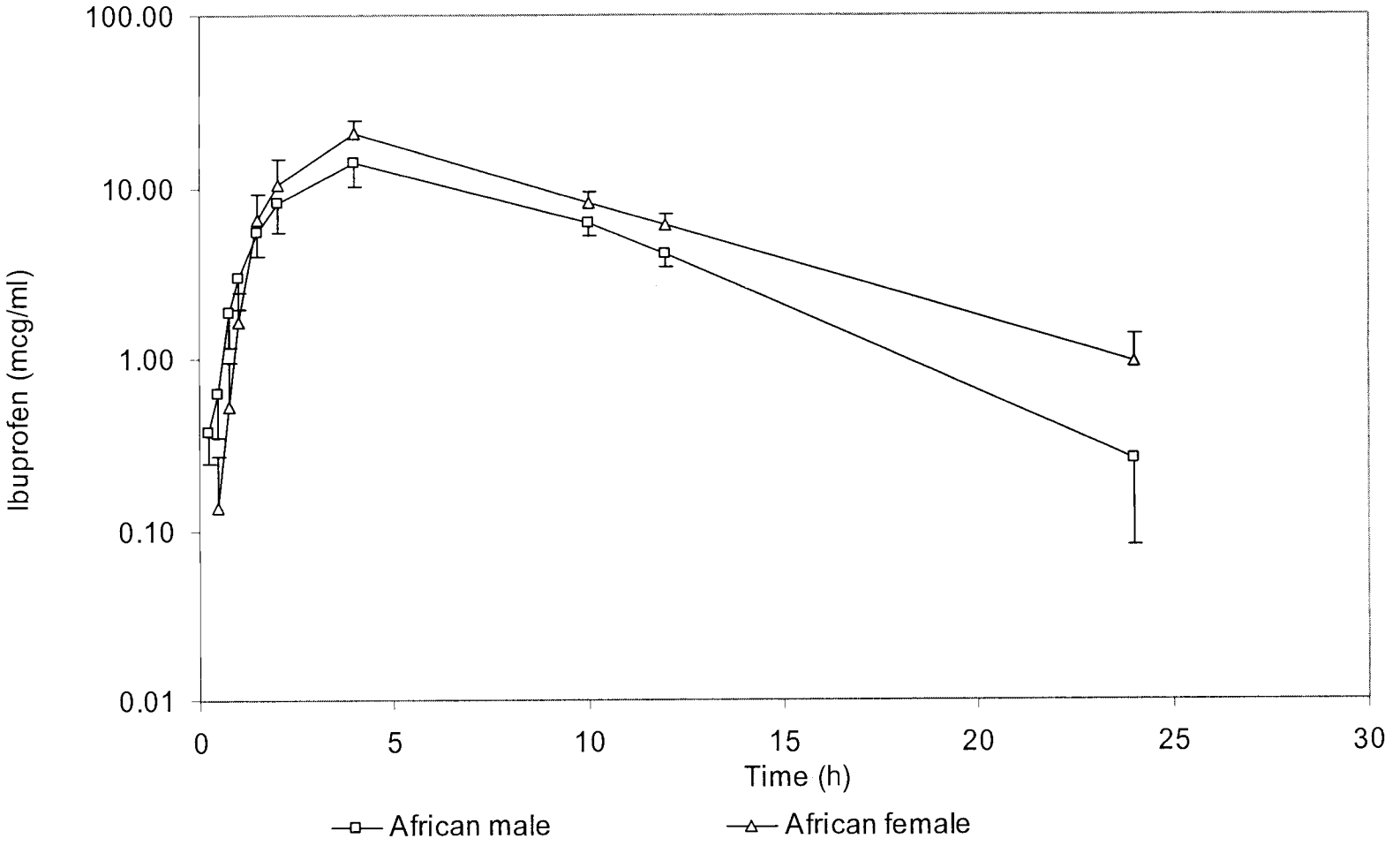

Figure 2. 18 Mean plasma concentration time profiles of ibuprofen with standard error in African Male (n=5) and Female (n=5) elephants at 5 mg/kg oral dose.

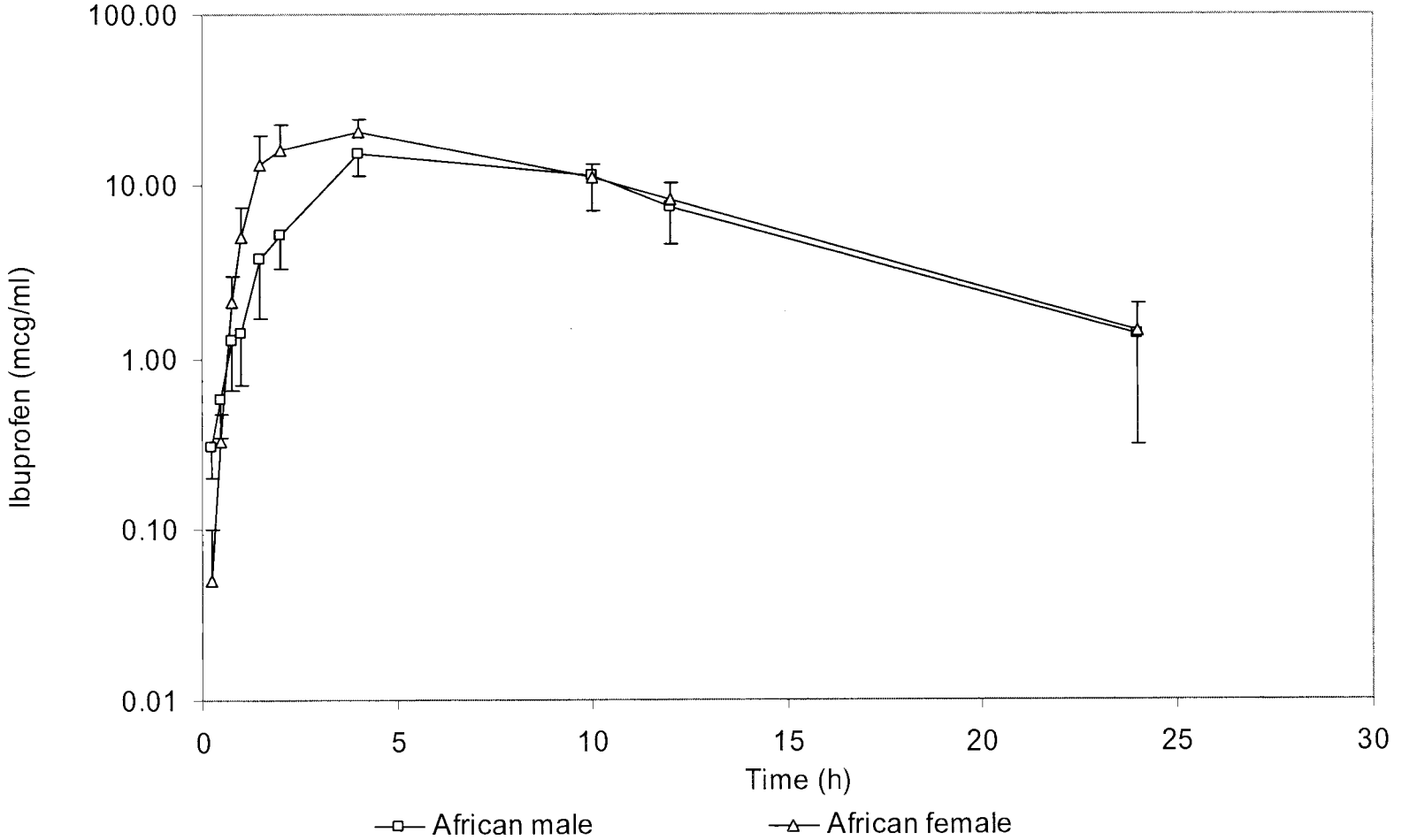

Figure 2. 19 Mean plasma concentration time profiles of ibuprofen with standard error in African Male (n=4) and Female (n=5) elephants at 6 mg/kg oral dose.

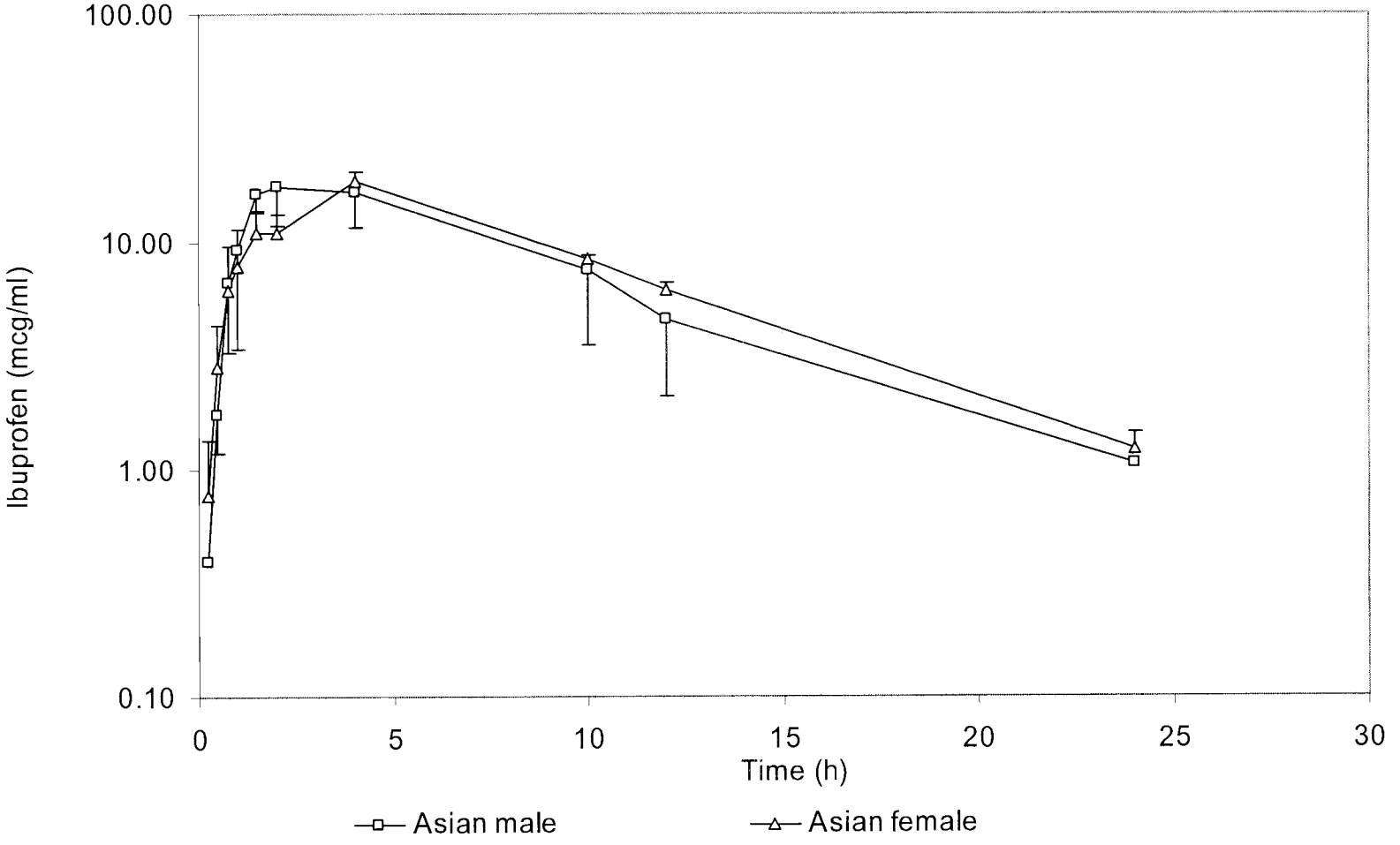

Figure 2. 20 Mean plasma concentration time profiles of ibuprofen with standard error in Asian Male (n=2) and Female (n=6) elephants at 4 mg/kg oral dose.

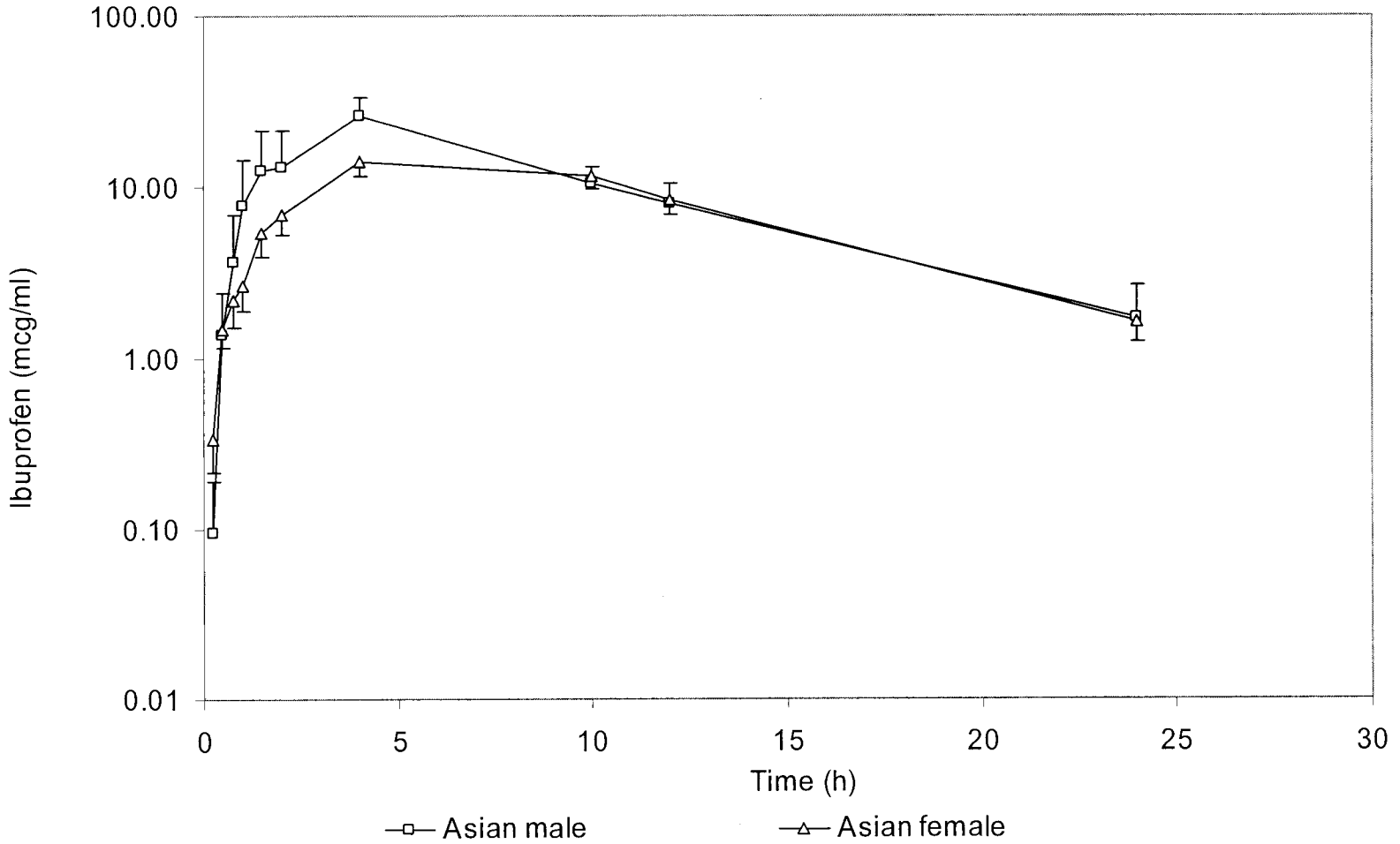

Figure 2. 21 Mean plasma concentration time profiles of ibuprofen with standard error in Asian Male (n=3) and Female (n=6) elephants at 5 mg/kg oral dose.

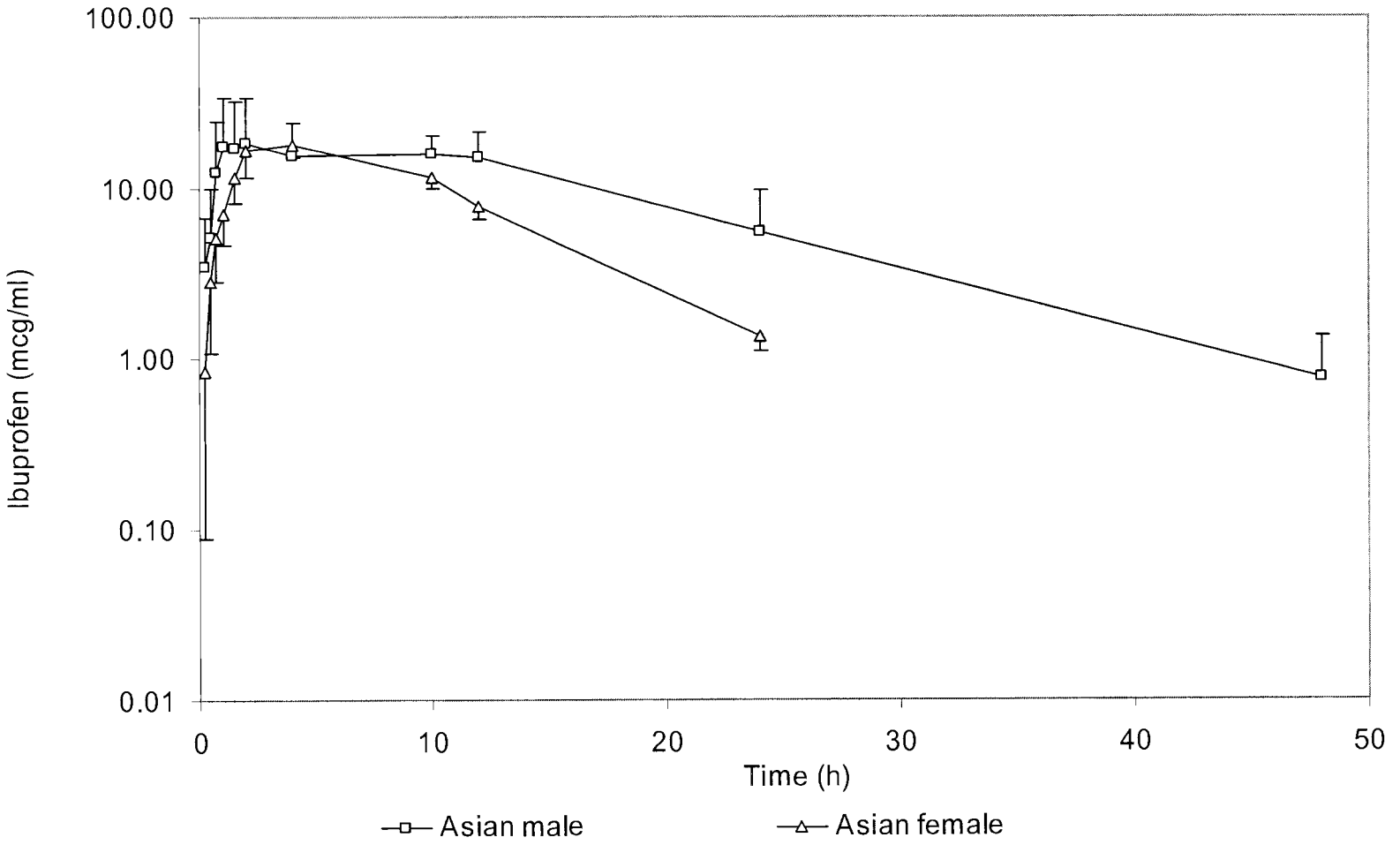

Figure 2. 22 Mean plasma concentration time profiles of ibuprofen with standard error in Asian Male (n=3) and Female (n=6) elephants at 6 mg/kg oral dose.

Multiple dose study

The plasma concentration time profiles of African elephants on repeated administration of 7 mg/kg and Asian elephants at 6 mg/kg are shown in tables 2.19 and 2.20. The plasma concentration time profiles for African and Asian elephants are shown in figure 2.23. The mean pharmacokinetic parameters at steady state are shown in table 2.21 for Asian and African elephants and the individual parameters for each African and Asian elephant are shown in table 2.22 and 2.23. By dosing the African elephants at 7 mg/kg and Asian elephants at 6 mg/kg every 12-h, the $C_{average}$ obtained was 18.05 and 18.59 mcg/ml respectively. These average concentrations achieved were comparable to the therapeutic concentrations seen in human, (range of 15-30 mcg/ml) and by dosing every 12 hours the concentrations were maintained within the range for the study period. The AUC and clearance/F for Asian elephants at a single oral dose of 6 mg/kg when compared to AUC and clearance obtained when dosing at 6 mg/kg 12 hours to steady state, no significant difference was observed by a paired t test. Similarly AUC and clearance/F for African elephants, at a single oral dose of 6 mg/kg when compared to AUC and clearance obtained when dosing at 7 mg/kg to steady state showed no significant difference by a paired t test. From the data presented it is apparent ibuprofen follows linear kinetics at doses of 4 to 6 mg/kg.

Table 2. 19 Ibuprofen plasma concentration profiles in African elephants (n=9) on repeated administration of 7 mg/kg every 12 hours.

	Ibuprofen plasma concentration (mcg/ml)										
Time (h)	Felix	Lea	Sheba	Tatto	Angus	Digger	Willie	Toby	Solomon	Mean	SD
0	0	0	0	0	0	0	0	0	0	0	0
1	0.497	1.828	1.473	5.684	1.502	2.068	0.469	0.695	0.298	1.613	1.658
2	0.543	34.519	11.099	20.917	4.444	7.429	3.818	0.756	1.059	9.398	11.450
3	0.791	31.168	14.235	21.186	9.063	13.912	15.696	1.016	1.136	12.023	10.290
4	2.047	32.841	13.410	19.993	25.780	18.432	17.151	1.411	2.271	14.815	11.139
10	4.909	17.292	12.616	11.451	30.444	6.844	12.165	22.056	11.468	14.361	7.882
11	3.509	19.007	18.444	8.230	29.904	6.854	9.271	19.021	16.024	14.474	8.230
13	2.551	10.985	10.100	6.400	20.067	5.598	11.461	12.259	18.683	10.900	5.761
14	5.295	16.761	11.474	12.728	18.650	12.609	7.681	13.656	16.580	12.826	4.319
15	4.580	14.476	6.357	13.734	16.313	18.137	7.267	10.488	13.768	11.680	4.733
16	1.205	21.917	11.799	14.395	23.818	19.056	7.741	20.019	14.244	14.910	7.257
22	5.045	27.263	37.389	17.698	79.740	9.537	16.581	14.336	17.146	24.971	22.616
23	4.166	25.571	27.929	17.048	29.776	8.144	20.010	11.394	15.460	17.722	8.926
25	5.266	19.228	25.316	12.556	35.106	4.754	17.557	8.844	9.905	15.393	10.020
26	6.225	15.037	31.500	13.324	29.693	6.280	16.823	12.226	12.038	15.905	9.058
27	5.200	25.182	26.998	15.283	26.993	13.045	11.425	15.065	11.122	16.701	7.852
28	11.553	20.152	23.944	28.863	20.871	28.428	10.663	22.110	11.301	19.765	7.107

Table 2.19 continued.

	Ibuprofen plasma concentration (mcg/ml)										
Time (h)	Felix	Lea	Sheba	Tatto	Angus	Digger	Willie	Toby	Solomon	Mean	SD
34	18.000	23.671	25.750	19.502	32.555	14.257	19.275	12.060	18.060	20.348	6.201
35	13.002	21.271	15.850	12.370	46.870	7.521	15.119	9.343	19.634	17.887	11.732
37	10.199	18.178	16.116	12.477	30.060	5.734	8.134	8.750	13.405	13.673	7.300
38	10.252	17.627	11.897	15.105	34.154	4.596	11.484	7.394	11.227	13.749	8.548
39	9.114	18.255	9.869	14.207	40.270	6.196	10.592	6.381	10.249	13.904	10.570
40	17.431	17.615	8.617	10.778	36.421	11.590	13.474	26.051	11.577	17.062	8.942
46	17.700	34.479	28.641	25.768	39.662	11.666	11.278	10.265	16.240	21.744	10.820
47	13.079	30.707	26.248	23.207	37.912	8.705	8.021	6.813	12.752	18.605	11.251
49	10.840	25.927	22.669	18.646	29.784	5.998	9.182	8.010	10.807	15.763	8.685
50	8.133	25.795	21.998	15.703	37.145	10.589	7.655	5.819	7.979	15.646	10.646
51	10.187	32.248	22.707	25.585	20.713	12.695	17.426	8.671	9.153	17.709	8.247
52	11.398	33.034	28.067	22.928	27.352	19.379	26.403	10.610	8.936	20.901	8.777
58	28.075	30.687	27.333	25.972	52.950	11.562	8.808	17.078	20.896	24.818	12.997
59	21.256	29.636	23.980	20.845	35.807	8.554	8.798	16.215	22.113	20.800	8.877

Table 2. 20 Ibuprofen plasma concentration profiles in Asian elephants (n=6) on repeated administration of 6 mg/kg every 12 hours.

	Ibuprofen plasma concentration (mcg/ml)							
Time (h)	Booper	Dixie	Limba	Peggy	Tai	Hank	Mean	SD
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1	2.442	1.825	4.630	1.170	20.450	0.776	5.216	7.585
2	35.933	37.464	12.378	4.246	16.640	9.536	19.366	14.025
3	40.043	35.413	15.630	10.549	32.584	22.686	26.151	11.716
4	31.441	31.078	23.747	17.088	29.259	29.373	26.998	5.584
10	7.482	14.541	11.558	9.859	5.633	7.357	9.405	3.266
11	6.353	11.032	10.991	7.530	9.908	6.119	8.656	2.266
13	17.282	11.989	6.216	9.147	9.532	6.362	10.088	4.134
14	25.558	13.508	8.037	10.870	8.151	6.495	12.103	7.045
15	19.139	11.534	6.787	12.302	6.432	5.099	10.215	5.253
16	20.451	14.100	8.438	12.999	7.717	6.920	11.771	5.164
22	10.437	21.689	17.393	22.819	22.665	16.863	18.644	4.795
23	9.405	21.450	17.495	18.224	19.710	11.589	16.312	4.754
25	30.680	18.193	14.166	16.956	21.807	10.674	18.746	6.949
26	29.451	42.922	13.362	13.643	36.075	21.945	26.233	12.069
27	29.599	37.654	12.270	14.966	38.492	24.322	26.217	11.114
28	36.150	41.415	15.158	19.229	35.740	22.294	28.331	10.771

Table 2.20 continued.

	Ibuprofen plasma concentration (mcg/ml)							
Time (h)	Booper	Dixie	Limba	Peggy	Tai	Hank	Mean	SD
34	11.450	17.401	26.417	17.372	15.373	8.840	16.142	6.076
35	10.416	14.541	15.016	15.867	14.363	6.317	12.753	3.674
37	6.378	16.322	10.902	13.302	13.229	8.331	11.411	3.635
38	7.066	17.285	10.827	14.339	13.976	8.358	11.975	3.906
39	9.998	17.287	8.916	12.063	13.024	12.566	12.309	2.908
40	16.946	18.858	11.779	14.620	13.557	18.591	15.725	2.864
46	11.805	29.978	15.951	17.715	31.145	8.295	19.148	9.436
47	12.616	23.060	13.205	16.836	28.430	7.395	16.924	7.658
49	14.104	21.719	14.743	18.754	35.035	25.029	21.564	7.792
50	18.199	32.475	14.767	22.363	41.921	19.278	24.834	10.322
51	27.453	41.192	19.763	23.880	46.273	18.307	29.478	11.610
52	23.435	39.737	20.347	25.315	40.199	17.230	27.711	9.886
58	20.241	17.231	15.282	16.920	20.626	8.857	16.526	4.281
59	15.453	16.076	0.000	14.690	14.239	7.931	11.398	6.311

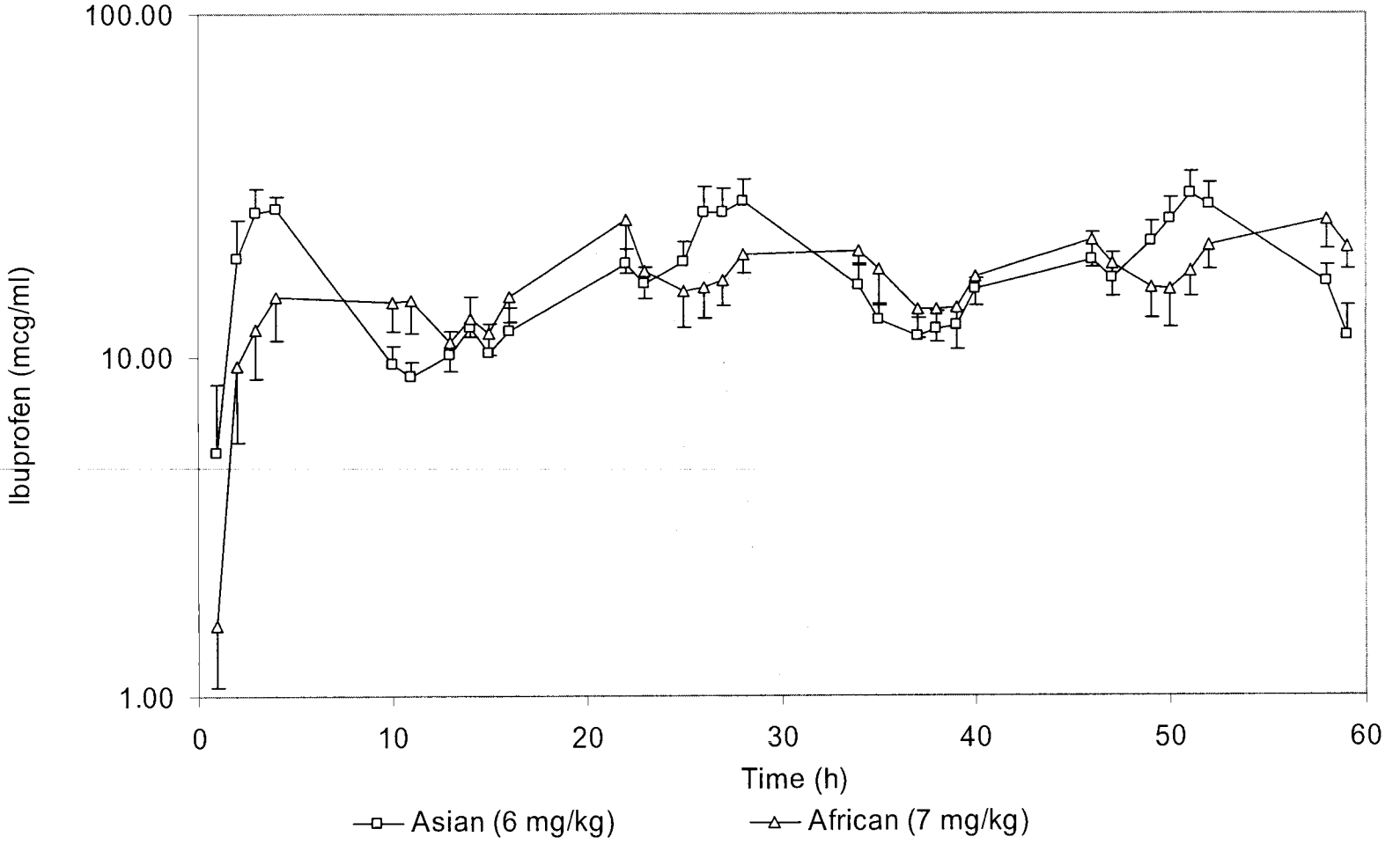

Figure 2. 23 Ibuprofen plasma concentration times profiles with standard error in African (n=9) and Asian (n=6) elephants on repeated oral administration every 12 hours.

Table 2. 21 Pharmacokinetic parameters of ibuprofen on multiple dosing in African and Asian elephants.

	African (7 mg/kg oral dose) (n=9)	Asian (6 mg/kg oral dose) (n=6)
Parameter	Mean ± SD	Mean ± SD
C_{max} (mcg/ml)	28.44 ± 10.54	30.93 ± 10.30
C_{min} (mcg/ml)	13.31 ± 7.81	13.46 ± 2.82
$C_{average}$ (mcg/ml)	18.05 ± 8.13	18.59 ± 5.54
Clearance at steady state (ml/h/kg)	37.89 ± 14.52	28.97 ± 8.60
MRT (h)	5.69 ± 0.73	4.58 ± 0.38
T_{max} (h)	7.00 ± 4.00	3.00 ± 1.10
AUC at steady state(mcg.h/ml)	216.65 ± 97.59	223.09 ± 66.48

Table 2. 22 Individual pharmacokinetic parameters of ibuprofen on multiple dosing in African elephants.

Subject	AUC_{ss} (mcg.h/ml)	C_{min} (mcg/ml)	C_{max} (mcg/ml)	$C_{average}$ (mcg/ml)	%AUCdf	Cl_{ss} (ml/h/kg)	T_{max} (h)	MRT (h)
Digger	136.77	6.00	19.38	11.40	23.27	51.18	4.00	4.98
Willie	147.84	7.66	26.40	12.32	-3.39	47.35	4.00	4.67
Solomon	138.00	7.98	22.11	11.50	27.53	50.72	11.00	6.44
Toby	123.51	5.82	17.08	10.29	27.07	56.68	10.00	6.10
Angus	402.09	20.71	52.95	33.51	16.60	17.41	10.00	6.46
Felix	172.37	8.13	28.08	14.36	32.51	40.61	10.00	6.60
Sheba	288.48	22.00	30.96	24.04	7.82	24.27	0.00	5.62
Tatto	232.09	15.70	25.97	19.34	18.53	30.16	10.00	5.30
Lea	308.76	25.79	33.03	25.73	16.67	22.67	4.00	5.07
Mean	216.66	13.31	28.44	18.05	18.51	37.89	7.00	5.69
SD	97.60	7.82	10.55	8.13	11.04	14.52	4.00	0.73

Table 2. 23 Individual pharmacokinetic parameters of ibuprofen on multiple dosing in Asian elephants.

Subject	AUC_{ss} (mcg.h/ml)	C_{min} (mcg/ml)	C_{max} (mcg/ml)	$C_{average}$ (mcg/ml)	%AUCdf	Cl_{ss} (ml/h/kg)	T_{max} (h)	MRT (h)
Peggy	209.08	14.69	25.32	17.42	-1.35	28.70	4.00	4.70
Booper	212.90	14.10	27.45	17.74	16.67	28.18	3.00	4.94
Limba	172.49	13.75	20.35	14.37	16.67	34.79	4.00	5.06
Tai	318.97	14.24	46.27	26.58	12.18	18.81	3.00	4.25
Dixie	282.65	16.08	41.19	23.55	7.20	21.23	3.00	4.37
Hank	142.46	7.93	25.03	11.87	15.57	42.12	1.00	4.15
Mean	223.09	13.47	30.93	18.59	11.16	28.97	3.00	4.58
SD	66.49	2.83	10.31	5.54	7.12	8.61	1.10	0.38

CONCLUSION

The disposition of ibuprofen after oral administration of 4, 5, and 6 mg/kg are best described by a mono-exponential equation (using WinNonlin with a weighing factor of 1/plasma concentration) although some of the elephants did not give a good fit.

Ibuprofen mean elimination half-lives were 4.73 ± 2.81, 5.11 ± 1.93, and 5.21 ± 2.53 (h), clearance/F were 35.94 ± 19.87, 33.08 ± 13.68, and 32.84 ± 15.03 (ml/h/kg), apparent volume of distribution/F were 208.82 ± 87.53, 228.96 ± 100.36, and 225.73 ± 142.94 (ml/kg), and the mean residence times were 9.05 ± 2.87, 10.11 ± 2.55, and 10.21 ± 3.64 (h), for 4, 5, and 6 mg/kg doses given orally to elephants respectively. Following oral administration of 4, 5, and 6 mg/kg, approximate linearity of AUC to dose of ibuprofen was found. However, ibuprofen plasma profiles in African and Asian elephants appear to be different but are not statistically significantly different. Firm conclusions of a species difference cannot be made due to large variability and small sample size. There was no gender differences in pharmacokinetic parameters for African and Asian elephants. Although the CL/F of ibuprofen in African females was less than the African males the difference was not statistically significant.

By dosing African elephants 7 mg/kg and Asian elephants 6 mg/kg every 12 hours the $C_{average}$ obtained was with in the therapeutic range of 15-30mcg/ml seen in humans and was maintained for the study period. No significant difference was

observed in AUC and clearance/F of any of single doses values when compared to AUC and clearance at steady state, showing ibuprofen follows linear kinetics.

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Chapter 3

Pharmacokinetics of Phenylbutazone orally given at single doses 2 mg/kg, 3 mg/kg and 4 mg/kg and on multiple dosing in Healthy Asian and African Elephants

Roopesh K. Neelkant, Ursula Bechert and J. Mark Christensen

ABSTRACT

The aim of this study was to determine pharmacokinetics and dose proportionality of phenylbutazone in the Asian and African elephants following single dose oral administration of 2, 3, and 4 mg/kg and on multiple dose administration. In this study, fifteen healthy elephants, each received all the three doses with a washout period of four weeks between each dose. Blood samples were taken over a 12 day period and analyzed for measurement of plasma phenylbutazone concentrations using an HPLC assay. Statistical comparisons were made using analysis of variance mixed effects model following necessary transformation. The means and standard deviations of peak plasma concentrations (C_{max}), the time to C_{max} (T_{max}), the area under the plasma concentration versus time curve (AUC), the apparent half life ($T_{1/2}$), the total body clearance (CL/F) after oral phenylbutazone administration, and the terminal volume of distribution (Vd/F) were calculated using noncompartmental pharmacokinetic analysis following the three doses of 2, 3 and 4 mg/kg phenylbutazone yielding values of 5.76 ± 2.66, 8.85 ± 4.52, and 11.26 ± 4.37 mcg/ml, 8.04 ± 4.55, 10.31 ± 12.05 and 11.08 ± 11.79 hours, 161.05 ± 141.95, 221.31 ± 210.48 and 296.61 ± 244.58 mcg.h/ml, 29.72 ± 20.76, 23.18 ± 19.95, and 21.61 ± 16.14 hours, 19.72 ± 16.30, 20.85 ± 11.93, and 21.80 ± 15.61 ml/h/kg and 563.3 ± 294.14, 477.34 ± 186.40, and 467.82 ± 223.33 ml/kg respectively for the pharmacokinetic parameters. Approximate linearity of phenylbutazone pharmacokinetics in elephants was found in the dose range of 2-4

mg/kg. The mean values of AUC_{0-n} and C_{max} were 161.05, 221.31, and 296.61 mcg.h/ml and 5.76, 8.85, and 11.26 mcg/ml following 2, 3, and 4 mg/kg dose, respectively. The dose normalized AUCs were lower in African elephant's than in the Asian elephant's, and the difference was statistically significant. Similarly the CL/F in Asian elephants was statistically significant lower than in African elephants. Phenylbutazone plasma concentrations were highly variable following oral administration in the three doses given. There was no gender difference in pharmacokinetic parameters in African and Asian elephants. By dosing African elephants 2 mg/kg every 24 hours the $C_{average}$ obtained was 2.89 ± 0.72 mcg/ml. The clearance at steady state is found to be 30.14 ± 6.17 ml/h/kg. By dosing the Asian elephants at 3 mg/kg every 48 hours, the $C_{average}$ obtained was 7.04 ± 0.84 mcg/ml. The clearance at steady state is found to be 9.16 ± 1.10 ml/h/kg.

INTRODUCTION

Dosages for anti-inflammatories used in elephants are customarily by consensus close to reported dosages given to equine. Phenylbutazone is given to horses at a dose 4 mg/kg every 12 hours (2-8 mg/kg range) (1). There are no reported pharmacokinetic studies with anti-inflammatories in elephants, and is an area in need of research.

Phenylbutazone is one of the most commonly used drugs for the treatment of inflammatory conditions in captive elephants. Empirical dosages of phenylbutazone administered to captive elephant's today range between 1-2 mg/kg based on scaling the equine dose up to elephants, and the median dosing frequency used by zoo veterinarians for phenylbutazone is 24 hours (1). No pharmacokinetic trials have been conducted for anti-inflammatory agents in elephants; although several studies have been published for various antibiotics (2, 3, 4, 5, 6). Results from the antibiotic studies suggest that metabolic scaling calculations for elephants are unreliable, mostly under estimating the dosage and dosing interval requirements (2, 3).

The objective of this study was to determine the pharmacokinetics of phenylbutazone in elephants after giving single doses of 2, 3, and 4 mg/kg orally and after repeated oral administration of 3 mg/kg every 48 hours in Asian elephants and 2 mg/kg every 24 hours in African elephants. From the pharmacokinetic data

obtained appropriate dosing regimens can be determined for phenylbutazone given orally in elephants.

Phenylbutazone (4-butyl-1,2-diphenyl-3,5 pyrazolidinedione) is a nonsteroidal anti-inflammatory drug with antipyretic and analgesic activity figure 3.1. In the horse it has been used for treatment of bone and joint inflammation, laminitis, and soft tissue inflammation (7). In elephants it is used to treat the musculoskeletal disorders like trauma and arthritis. Phenylbutazone belongs to enolic acid group of NSAIDs. It basically acts by inhibiting the synthesis of prostanoids, i.e., prostaglandins and thromboxanes, by blocking the enzyme cyclo-oxygenase. Phenylbutazone, meclofenamic acid, and acetylsalicylic acid have been proposed to bind irreversibly to cyclooxygenase, whereas oxyphenbutazone, an active metabolite of phenylbutazone apparently binds reversibly (8).

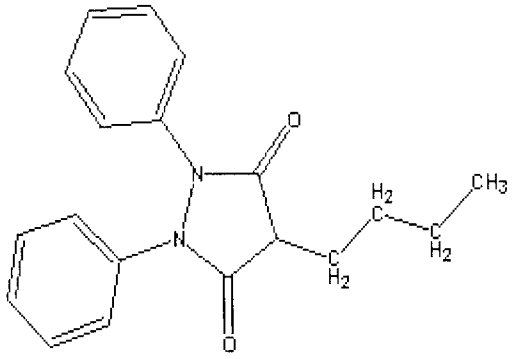

Figure 3. 1 4-n-butyl-1,2-diphenyl-3,5-pyrazolidinedione.

It has been observed in several studies that the plasma concentration and the onset of action of phenylbutazone given orally vary considerably depending on when and how they are administered (9). Studies have shown that when phenylbutazone was given to ponies orally (4.4 mg/kg body weight) and access to hay was permitted, the mean time to peak concentration in plasma was delayed 6 to 12 hours. Double peaks were seen, and it was tentatively postulated that some of the phenylbutazone administered was absorbed quickly and some might be adsorbed onto the feed and subsequently released by fermentive digestion in the large intestine and cecum (10).

The major metabolites of phenylbutazone identified in the urine are formed by oxidation and conjugation with glucuronic acid figure 3.2. Phenylbutazone is mainly metabolized to oxyphenbutazone and gamma-hydroxy-phenylbutazone, which account for 25 to 30% of administered dose over 24 hours. The active metabolite oxyphenbutazone inhibits the metabolism and thus increases the half-life of phenylbutazone. This may help to explain the dose dependent kinetics reported for phenylbutazone reported in horse (11). Plasma half lives reported have ranged from 3.5 to 10.9 hours, increasing as the drug dosage increases and with accumulation in the body (12). It has been shown that there is no correlation between urinary and plasma concentrations. This makes the urine concentrations variable and very unpredictable, and it is difficult to correlate urine concentrations to pharmacologic effect or the time of administration (13).

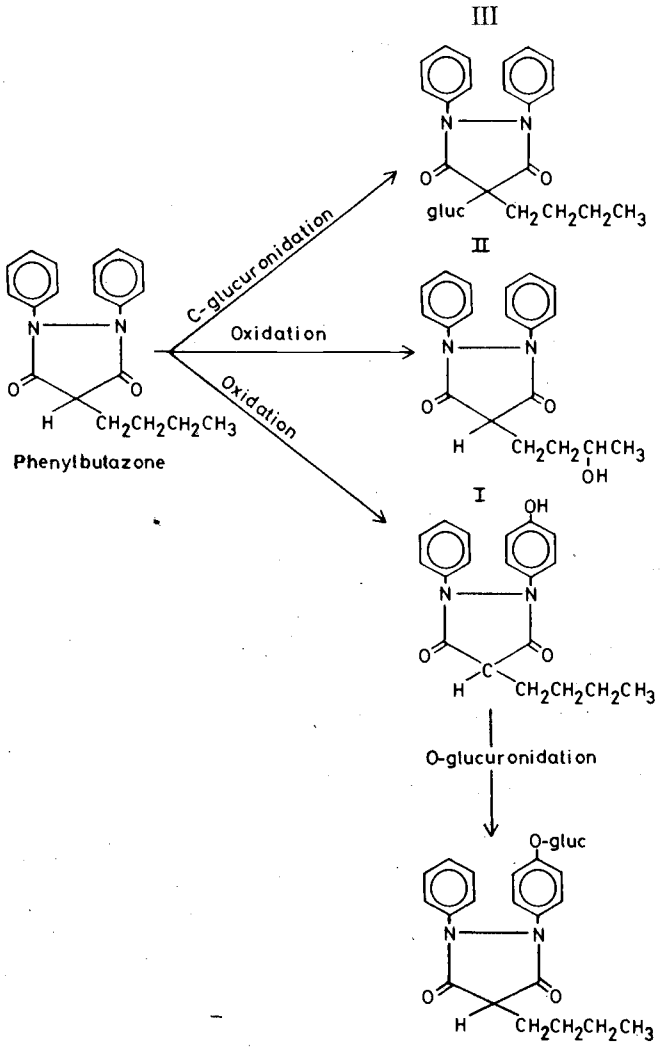

Figure 3. 2 Major metabolites of phenylbutazone in man.

I = oxypenbutazone; II = ɣ-hydroxyphenbutazone;
III = C-glucuronide of phenybutazone.

The clearance of phenylbutazone in horses was found to be age dependent, with increased clearance in younger individuals (14). Breed of the horse may also influence plasma clearance (15).

The fraction of phenylbutazone bound to plasma proteins is 98 to 99% when the drug is added in vitro to normal plasma in concentrations within the usual therapeutic range. With increasing concentrations of drug, the fraction of bound drug decreases (16). About 1% of the drug is excreted unchanged via the kidneys (22).

Reported adverse effects include oral, gastric, duodenal, and colonic ulceration and necrosis, diarrhea, renal papillary necrosis, and hematologic disturbances. The early signs are depression, anorexia, and a decline in total plasma proteins. Changes in cardiovascular, respiratory, and neural functions also have been reported, as well as blood dyscrasias and hepatotoxicity. The ulcerogenic action of NSAIDs on the gastrointestinal system could result from inhibition of prostaglandin (PGE2), which seems locally to increase mucosal blood flow. Prostaglandins also have been shown to increase mucus production, decrease gastric acid production, and aid to repair mucosal injury. It has been postulated that gastric and intestinal lesions result from microvascular injury induced by phenylbutazone rather than from a reduction in mucosal prostaglandin concentrations (17).

MATERIALS AND METHOD

Materials

Phenylbutazone was obtained from Sigma Chemical Co. (St. Louis, MO). Glacial acetic acid, acetonitrile and cyclohexane of HPLC grade were obtained from Fischer Scientific (Fair lawn, NJ). Gemfibrozil was obtained from Sigma Chemical Co. (St. Louis, MO).

Study Protocol

Fifteen healthy elephants housed in zoos throughout North America were used in the study. Ages and weights were obtained prior to the initiation of the study and ranged from 2400 to 7400 kg. In the study African male and female elephants weighed around 4400 and 3400 kg, whereas the Asian male and female elephants weighed around 4900 and 3600 kg respectively. The elephants were of standard adult age and ranged from 12 to 44 years. Phenylbutazone was given orally with food treats (e.g., apples, bread and peanut butter). Blood samples were taken from the superficial ear veins using 20 gauge butterfly catheters, which allowed for some animal movement during venipuncture. Catheter placement was generally not successful. None of the elephants received any medications for at least 4 weeks prior to the trials.

Single dose study: Pilot pharmacokinetic studies were conducted with one or two elephants using empirically derived dosing regimens and preceded both the single and multiple dose trials to ensure that proper ranges for dosage and dosing frequency would be utilized. Based on the pilot study results, the best therapeutic dose for phenylbutazone for the single dose study was determined to be dosing at 2, 3, and 4 mg/kg to each animal. Washout periods between doses were 4 weeks in duration, thereby allowing complete elimination of residual drug metabolites. Blood samples were collected at –5, 30, 60 minutes, 2, 12, 24 and 48 hours, 4, 6, 8, 10 and 12 days post administration from all the elephants. Blood samples were placed into red top tubes and centrifuged for 10 minutes at 13000 g. Serum was decanted into plastic screw-cap vials and kept frozen until time of analysis.

Multiple dose study: Each african elephant was given 2 mg/kg of oral dose every 24 hours. Blood samples were collected at 0, 1, 2, 3, 4, 10, 16, and 24 hours after each administration for Buffy and Butch and each of the elephant was dosed twice. Blood samples were collected at 0, 1, 2, 3, 4, 10, 16, 22 and 23 hours after each administration for Lea and Lois and each elephant was dosed thrice. Each Asian elephant was given 3 mg/kg every 48 hours and the blood samples were collected at 0, 3, 4, 12, 16, 28, 35, 47, 51, 60, 64, 76, 83, 95, 99, 100, 108, 112, 124, 131 and 143 hours. A four week wash out was included between single and multiple dose studies.

Determination of plasma phenylbutazone concentrations:

The HPLC system used to assay phenylbutazone consisted of an HPLC pump (Model M-600 A; Water Associates, Inc. Milford, MA) with a WISP auto sampler (Model 712, Water Associates, Inc.) and a variable wavelength detector set at 229 nm and an automated data integrator (Hewlett Packard Model 3390A, Wilmington, DE).

To 0.5 ml of serum, 100 μl of internal standard solution (25 mcg/ml of gemfibrozil) and three drops of glacial acetic acid were added and the mixture was vortexed for 30 seconds. To this solution, 0.5 ml of acetonitrile was added followed by vortexing for 30 seconds. Phenylbutazone was double extracted using 2ml cyclohexane with each extraction and vortexing for 30 seconds followed by centrifugation for 10 minutes at 4500 rpm. The supernatant was separated, and the two cyclohexane extractions were pooled, vacuum evaporated and dried. The dried samples were then reconstituted with 0.5 ml of mobile phase (40% acetonitrile and 60% 0.1M acetic acid and the pH was adjusted to 3.5) and vortexed for 1 minute followed by centrifugation for 5 minutes at 4500 rpm. Of this solution, 100 μl was injected onto an HPLC C-18 column at a flow rate of 1.5 ml per minute for the mobile phase. Calibration curves were obtained by plotting the peak areas against the corresponding concentrations of prepared phenylbutazone standard solutions. The slope, intercept, and correlation coefficient of each calibration curve were determined by linear regression analysis. The concentration of phenylbutazone in

experimental samples was calculated from the peak area of each experimental sample and the slope and intercept of the appropriate calibration curve. Correlation coefficients of calibration curves were greater than 0.99. Table 3.1 shows the calibration curve values with mean, standard deviation and percent coefficient of variation.

Table 3. 1 Calibration curve values showing mean, SD and %CV.

Concentration (mcg/ml)	Area ratio	Mean	SD	% CV
0.25	0.04	0.05	0.00	9.77
	0.05			
	0.06			
	0.05			
	0.05			
0.50	0.10	0.13	0.02	13.40
	0.14			
	0.15			
	0.13			
	0.12			
1.00	0.26	0.25	0.01	5.81
	0.26			
	0.23			
	0.25			
	0.26			
5.00	1.60	1.67	0.10	5.85
	1.81			
	1.72			
	1.60			
	1.60			
10.00	3.31	3.51	0.24	6.96
	3.89			
	3.33			
	3.40			
	3.60			
25.00	11.14	9.95	0.77	7.72
	9.17			
	9.50			
	10.22			
	9.72			
50.00	20.61	20.11	0.94	4.68
	18.67			
	20.74			
	20.88			
	19.63			

SD – Standard deviation
% CV – Percent of coefficient variation

Pharmacokinetic analysis

Plasma phenylbutazone concentration profiles were analyzed utilizing both compartmental and non-compartmental approaches, using WinNonlin pharmacokinetic-pharmacodynamic software (18). Plasma phenylbutazone concentration profiles after oral doses did not yield a proper fit with any of the compartmental models.

In noncompartmental analysis, AUCs and AUMCs were calculated using log linear trapezoidal rule method. T_{max} and C_{max} were determined from the fitted data. MRT and terminal half-lives were calculated as stated above from equations 2 and 4.

The total body clearance (Cl_T/F) was calculated according to

$$Cl_T / F = Dose / AUC \quad (1)$$

The MRT is the mean residence time and is equal to

$$MRT = AUMC / AUC \quad (2)$$

The apparent volume of distribution at steady (Vd_{ss}) was calculated according to

$$Vdss = CL_T / MRT \quad (3)$$

Half-live was calculated according to

$$\lambda_i t_{1/2} = 0.693 / \lambda_i \quad (4)$$

Following administration of phenylbutazone given at 2, 3, and 4 mg/kg, dose proportionality of phenylbutazone was determined using AUC. With respect

to the lowest (2 mg/kg) dose AUC; AUC comparisons for the three doses was estimated by the following equation.

$$R = \frac{AUC_{2,3,or4mg/kg}}{AUC_{2mg/kg}} \quad (5)$$

R is dose proportionality ratio. If dose proportionality is linear, the ratios for the three doses should not be statistically different from 1.0 : 1.5 : 2.0. In addition, a power function relationship was used to describe the relationship between AUC and dose.

$$AUC = a \bullet (Dose)^b \quad (6)$$

In the above equation, a represents the coefficient, and b represents the exponent of the power function regression. If AUC exhibits linear dose proportionality, then b should be equal to unity. The parameters a and b were estimated by linear least square regression following a log-transformation as follows:

$$\ln (AUC) = \ln (a) + b \bullet \ln (DOSE) \quad (7)$$

A similar power function relationship was also established for C_{max}. Linearity was indicated if 95% confidence interval for the exponent included the value of 1 (19).

Multiple dose study: Pharmacokinetic parameters at steady state for multiple dose study were determined using Kinetica software (20). C_{max} and C_{min} are the maximum and minimum steady state drug concentrations during a dosing interval at steady state. T_{max} is the maximum time corresponding to C_{max}. AUC_{ss} is the area under the curve during a dosing interval at steady state. AUC_{ss} was

calculated using the trapezoidal rule. Cl_{ss} is the clearance at steady state. Cl_{ss} was calculated as follows.

$$Cl_{ss} = \frac{Dose}{AUC_{ss}} \tag{8}$$

$C_{average}$ is the mean or average steady state drug concentration expressed as:

$$C_{average} = \frac{AUC_{ss}}{Tau} \tag{9}$$

% AUCdf is the percentage-area-fluctuation expressed as:

$$\%AUCdf = 100\frac{AUC_{above} + AUC_{below}}{AUC_{ss}} = 100\frac{AUCbetweenC(t)andC_{average}}{AUC_{ss}} \tag{10}$$

Statistical analysis

Following oral administration of phenylbutazone at three different doses, statistical comparisons of mean plasma concentrations at each sampling time and estimates of the pharmacokinetic parameters among the three doses were made using ANOVA. Statistical comparisons between Asian and African elephants were done using ANOVA mixed effects model:

$$\log(y_{ijkl}) = s_i + z_j + \theta_k + \gamma_l + \varepsilon_{ijkl} \tag{11}$$

If y_{ijkl} represents the measured pharmacokinetic parameter on the kth dose in the jth zoo for the ith elephant of lth species then the analysis of variance model would be as above. Where s_i is a random elephant effect, z_j is a fixed zoo effect, θ_k is the

effect of the kth dose, γ_l is the effect of species and ϵ_{ijkl} is a normally distributed random error with mean value zero. The parameter for zoo was included in the model, as the elephants included in the study were from six different zoos. The number of elephants included in the study from each zoo was different. Also the number of Asian and African elephants differed between the zoos. Species was included in the model, as one of the objectives of the experiment was to see if differences exist between African and Asian elephants. Dose was included in the model as the experiment was carried out at three different doses and the aim was to see if their was any difference in the parameters with different doses.

Statistical software SAS was used for analysis (21).

RESULTS AND DISCUSSION

Fifteen elephants were used for the study with all the elephants completing the three treatments. Demographic variables of individual elephants are presented in table 3.2. Tables 3.3, 3.4, and 3.5 show plasma concentrations of phenylbutazone in each elephant after oral administration of 2, 3, and 4 mg/kg respectively, along with the averages and standard deviations at each sampling time. Individual elephant plasma levels of phenylbutazone after oral administration are shown in figures 3.3, 3.4, and 3.5, respectively, whereas figures 3.6, 3.7, and 3.8 show mean phenylbutazone concentrations and figures 3.9, 3.10 and 3.11 show mean phenylbutazone concentrations on a semilogathmic (with standard error) time curves.

Elephant phenylbutazone concentration-time profiles following the three doses were fitted non-compartmentally using WinNonlin. The obtained mean pharmacokinetic parameters for phenylbutazone are shown in table 3.6 and the individual elephant parameters are shown in tables 3.7, 3.8 and 3.9 for the three doses, respectively. Because of the erratic results, pharmacokinetic parameters of elephants Butch and Buffy for 2 mg/kg, and Buffy and Limba for 3mg/kg and Hank, and Lois for 4 mg/kg were not included in the statistical analysis of the study. Data for these elephants is in appendix C and D.

The half lives for phenylbutazone in various animals are shown in table 3.10 (22). The mean total phenylbutazone clearance for camel, horse, donkey, beef

steer and mature Holstein bulls was 12.63, 29.3, 170.3, 3.2 and 2.1 ml/kg/h when compared to clearance/F of ~ 20 ml/kg/h in elephants (23, 24, 25, 26). The mean elimination half-life in camel, horse and beef steer was 13.44, 6.2, and 33.6 h when compared to ~ 24.0 h in elephants (23, 24, 27).

Table 3. 2 Demographic characteristics of elephants in study.

Elephant	Gender	Origin	Weight (lbs)	Age (years)	Zoo
Solomon	Male	African	6800	19	Riddles
Toby	Male	African	6800	22	Riddles
Willie	Male	African	9000	23	Riddles
Angus	Male	African	13409	24	Bowmansville
Lea	Female	African	8000	*	Kansas City
Sheba	Female	African	7856	25	Bowmansville
Hank	Male	Asian	9000	14	Riddles
Limba	Female	Asian	6976	40	Bowmansville
Peggy	Female	Asian	8000	44	Riddles
Shine	Female	Asian	8850	*	Oregon
Timba	Female	African	8940	26	Seneca park
Buffy	Female	African	6975	23	EARS
Butch	Male	African	7190	22	EARS
Caesar	Male	Asian	3400	15	Bowmansville
Booper	Female	Asian	7700	30	Riddles

* No record

Table 3. 3 Plasma concentration profiles in elephants after oral administration of 2 mg/kg of phenylbutazone.

	Phenylbutazone plasma concentration (mcg/ml)														
Time (h)	Angus	Booper	Caesar	Hank	Lea	Limba	Lois	Peggy	Sheba	Solomon	Timba	Toby	Willie	Mean	SD
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.5	0.000	0.232	0.000	0.000	0.000	0.000	0.000	0.421	8.452	0.000	0.000	0.000	0.000	0.700	2.333
1	0.000	5.829	1.380	0.336	0.147	0.000	0.000	3.899	8.282	0.000	0.000	0.000	0.000	1.529	2.730
2	0.515	5.486	5.502	1.041	1.100	1.287	1.732	7.375	7.698	1.453	0.723	0.000	0.000	2.609	2.820
4	1.831	7.494	7.574	2.024	3.274	5.885	4.798	5.714	5.839	1.449	1.191	0.310	0.978	3.720	2.594
12	1.036	8.464	2.918	10.162	3.190	1.576	3.354	8.734	4.469	3.858	3.057	3.901	5.285	4.616	2.815
24	0.673	5.451	1.911	3.257	0.000	3.117	0.889	3.725	1.628	0.769	2.434	0.175	0.981	1.924	1.603
48	0.192	3.019	2.799	3.520	0.000	1.562	0.192	2.491	0.515	0.718	0.840	0.000	0.451	1.254	1.269
96	0.000	1.402	1.064	2.115	0.000	0.316	0.000	0.929	0.000	0.000	0.000	0.000	0.000	0.448	0.703
144	0.000	1.055	0.000	1.035	0.000	0.000	0.000	0.273	0.000	0.000	0.000	0.000	0.000	0.182	0.390
192	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.135	0.000	0.000	0.000	0.000	0.000	0.010	0.037
240	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
288	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 3. 4 Plasma concentration profiles in elephants after oral administration of 3 mg/kg of phenylbutazone.

	Phenylbutazone plasma concentration (mcg/ml)														
Time (h)	Angus	Booper	Butch	Caesar	Hank	Lea	Lois	Peggy	Sheba	Solomon	Timba	Toby	Willie	Mean	SD
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.5	0.000	0.000	0.000	0.000	0.531	0.000	0.000	0.796	0.000	0.000	0.000	0.000	0.000	0.102	0.255
1	0.184	4.441	0.000	0.985	3.092	1.809	3.208	6.700	0.000	1.433	0.000	0.465	0.000	1.717	2.096
2	1.050	7.593	0.000	3.452	6.384	3.528	12.193	9.317	0.000	3.269	0.450	0.453	0.000	3.668	4.036
4	1.597	17.196	3.845	10.764	12.634	6.148	16.190	4.909	0.000	7.319	0.547	2.033	6.325	6.885	5.732
12	4.955	5.672	3.461	9.233	4.092	4.548	5.248	3.248	5.708	2.629	10.818	2.888	7.067	5.351	2.449
24	1.835	6.907	2.061	4.428	12.505	1.732	2.322	4.113	2.359	1.850	3.782	1.044	0.281	3.478	3.212
48	0.357	4.593	0.463	2.713	12.773	0.793	0.679	2.791	0.345	0.510	1.058	0.416	0.717	2.170	3.438
96	0.000	0.307	0.000	1.374	0.285	0.000	0.000	0.200	0.000	0.000	0.178	0.000	0.000	0.180	0.378
144	0.000	0.464	0.000	0.000	1.772	0.000	0.000	0.287	0.000	0.000	0.000	0.000	0.000	0.194	0.496
192	0.000	0.000	0.000	0.000	1.313	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.101	0.364
240	0.000	0.000	0.000	0.000	0.566	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.044	0.157
288	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 3. 5 Plasma concentration profiles in elephants after oral administration of 4 mg/kg of phenylbutazone.

	Phenylbutazone plasma concentration (mcg/ml)														
Time (h)	Angus	Booper	Buffy	Butch	Caesar	Lea	Limba	Peggy	Sheba	Solomon	Timba	Toby	Willie	Mean	SD
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1	0.000	0.000	0.000	0.392	0.727	0.000	1.821	0.000	0.000	0.000	0.331	0.000	0.000	0.252	0.523
2	0.000	4.819	1.264	1.123	2.950	1.581	5.705	1.640	0.000	0.000	0.253	0.000	0.000	1.487	1.911
4	1.446	19.539	6.689	7.782	16.835	7.344	9.923	5.386	7.662	5.011	11.568	0.526	1.355	7.774	5.686
12	12.461	9.625	12.657	3.853	6.802	4.409	6.718	11.477	1.907	8.383	17.199	5.872	8.143	8.424	4.219
24	9.642	9.282	4.321	1.948	2.397	2.696	2.153	6.562	1.465	3.627	4.267	1.335	1.523	3.940	2.860
48	1.019	7.905	0.566	0.246	4.317	0.779	3.837	12.607	0.000	0.842	1.205	0.367	0.612	2.639	3.752
96	0.000	-	0.000	0.000	2.605	0.197	0.878	3.573	0.000	0.000	0.553	0.000	0.000	0.650	1.191
144	0.000	1.011	0.000	0.000	0.393	0.000	0.417	2.594	0.000	0.000	0.122	0.000	0.000	0.349	0.737
192	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
240	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
288	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

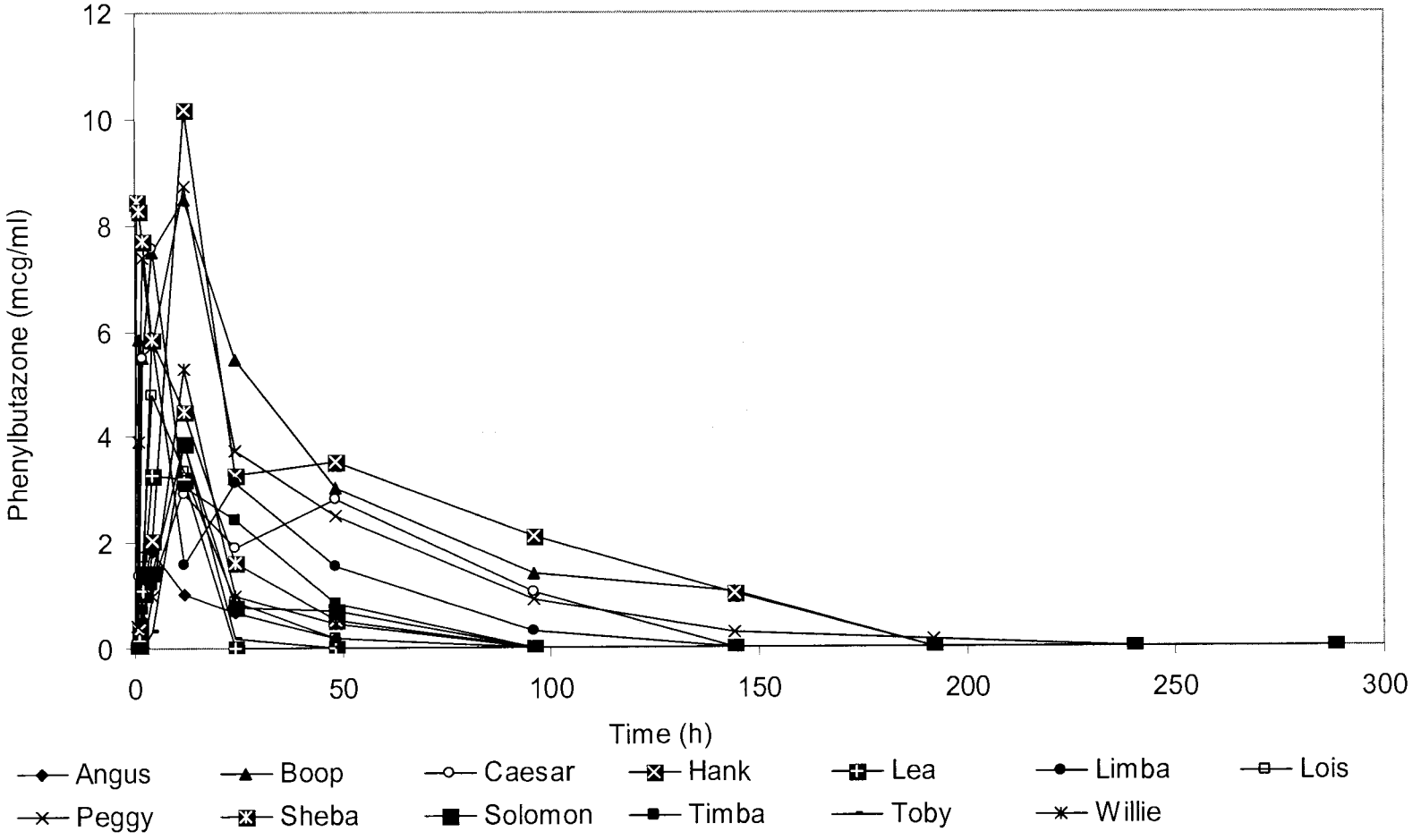

Figure 3. 3 Individual elephant plasma phenylbutazone concentration (mcg/ml) versus time (h) curves after an oral dose of 2 mg/kg.

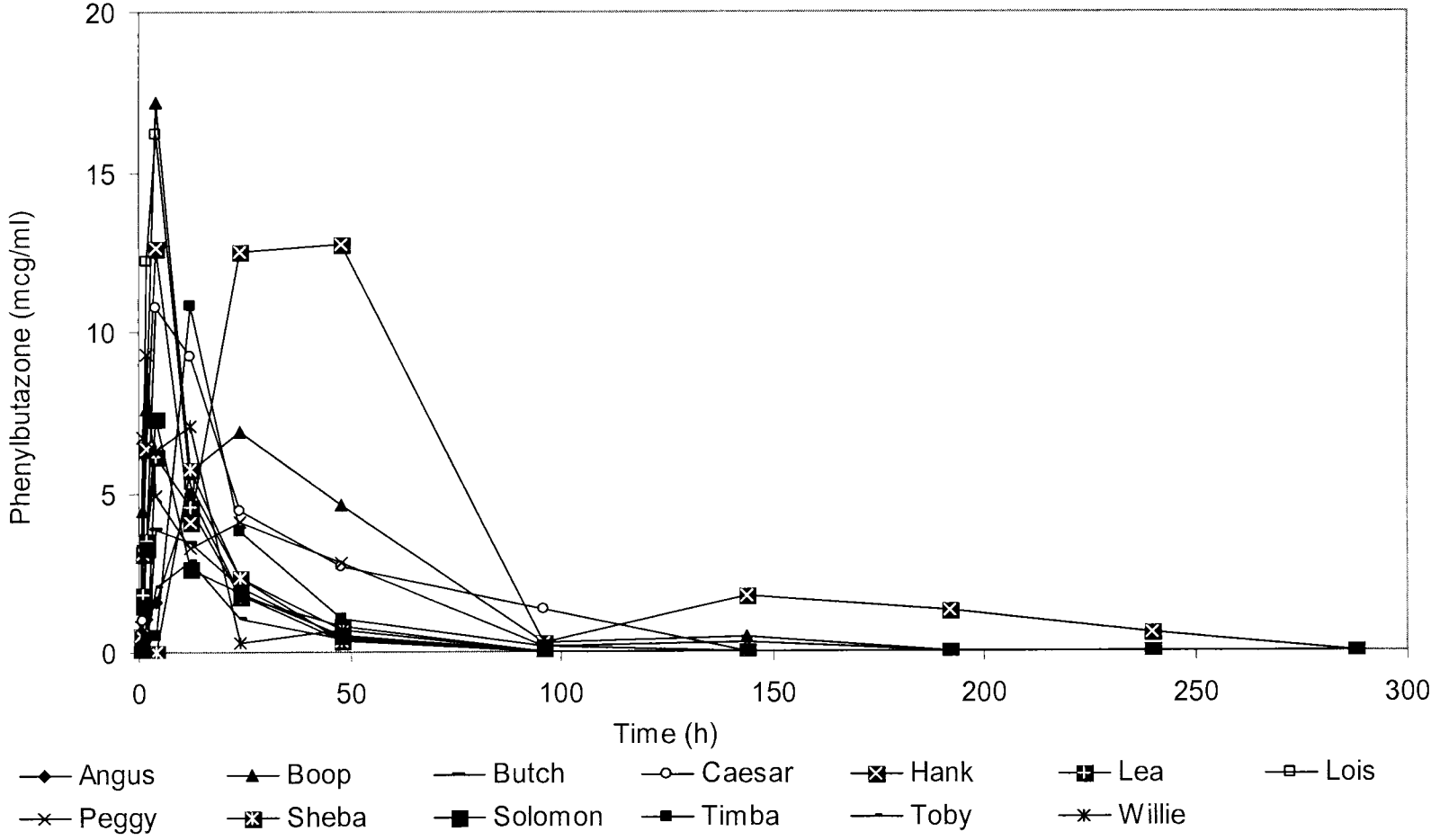

Figure 3. 4 Individual elephant plasma phenylbutazone concentration (mcg/ml) versus time (h) curves after an oral dose of 3 mg/kg.

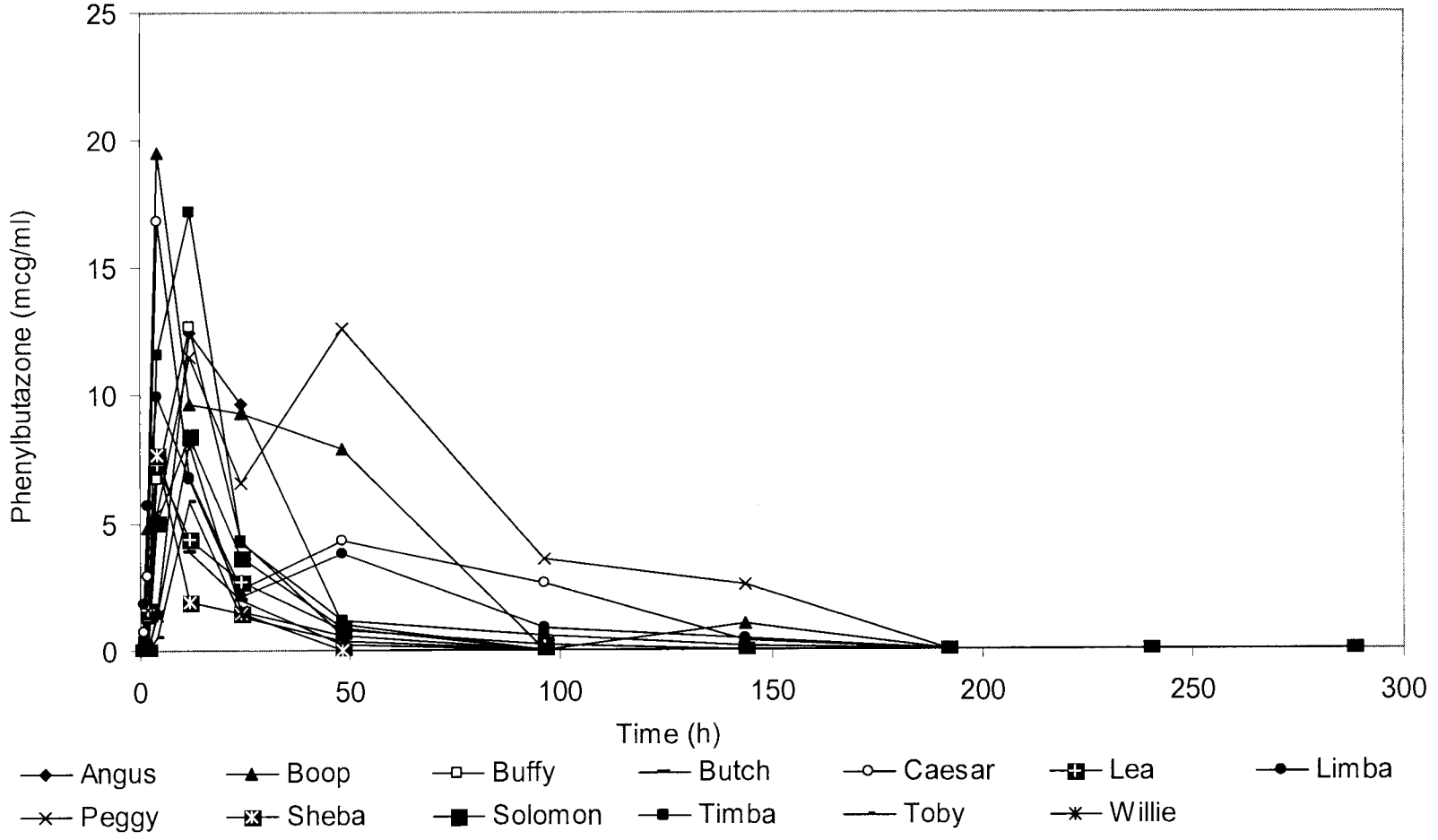

Figure 3. 5 Individual elephant plasma phenylbutazone concentration (mcg/ml) versus time (h) curves after an oral dose of 4 mg/kg.

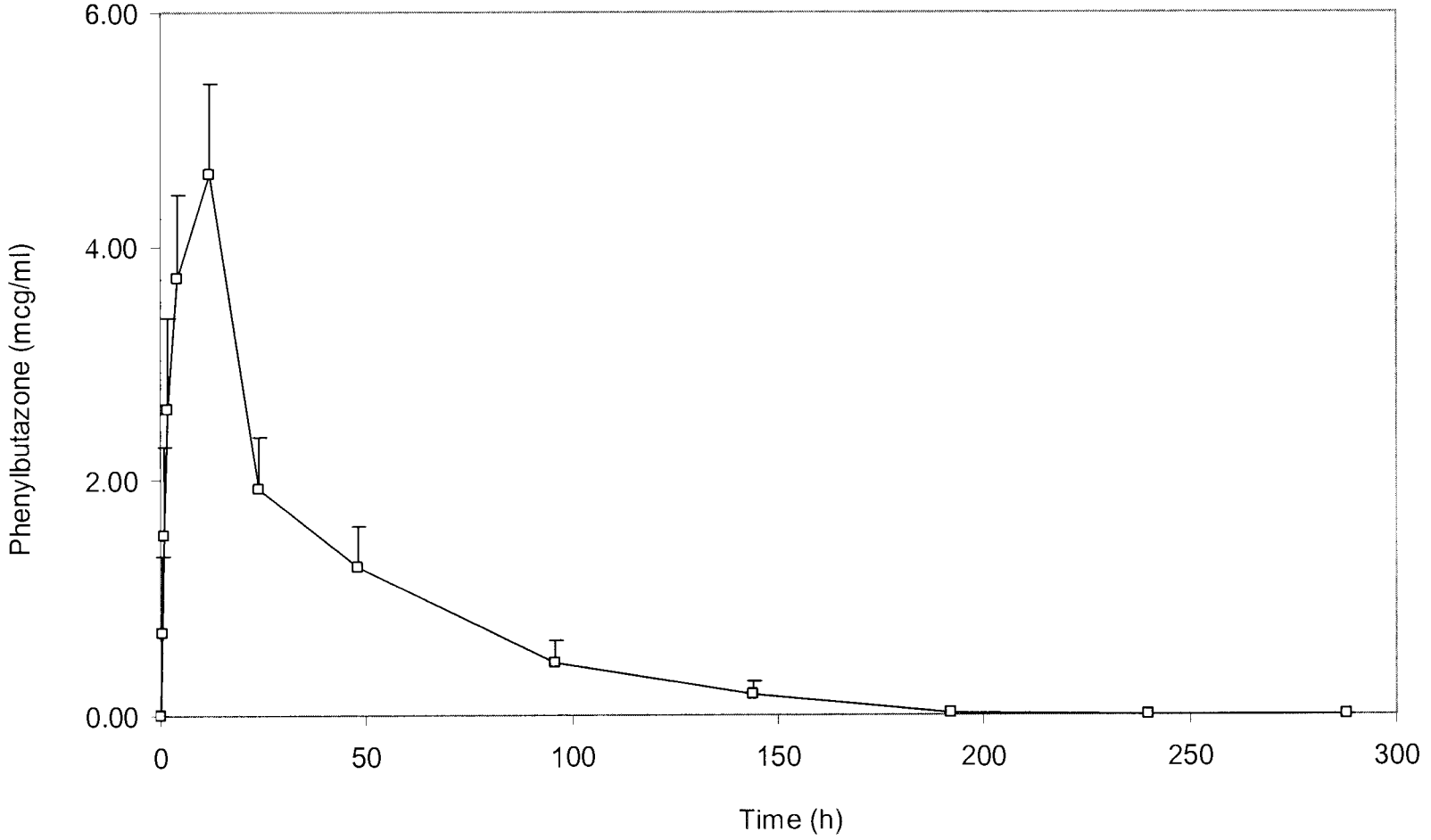

Figure 3. 6 Mean plot of concentration time profile of phenylbutazone with standard error given orally at 2 mg/kg elephants (n = 13).

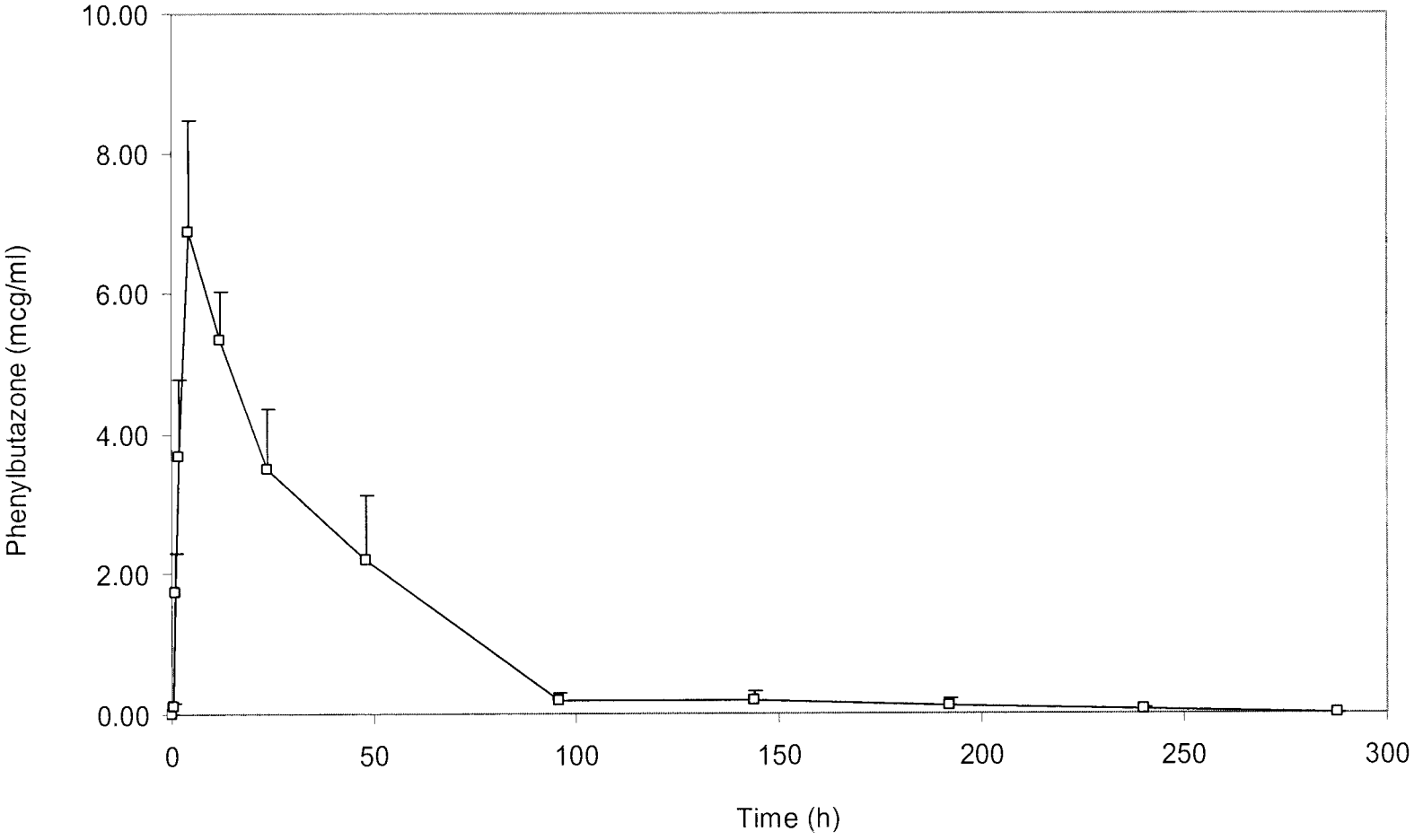

Figure 3. 7 Mean plot of concentration time profile of phenylbutazone with standard error given orally at 3 mg/kg to elephants (n = 13).

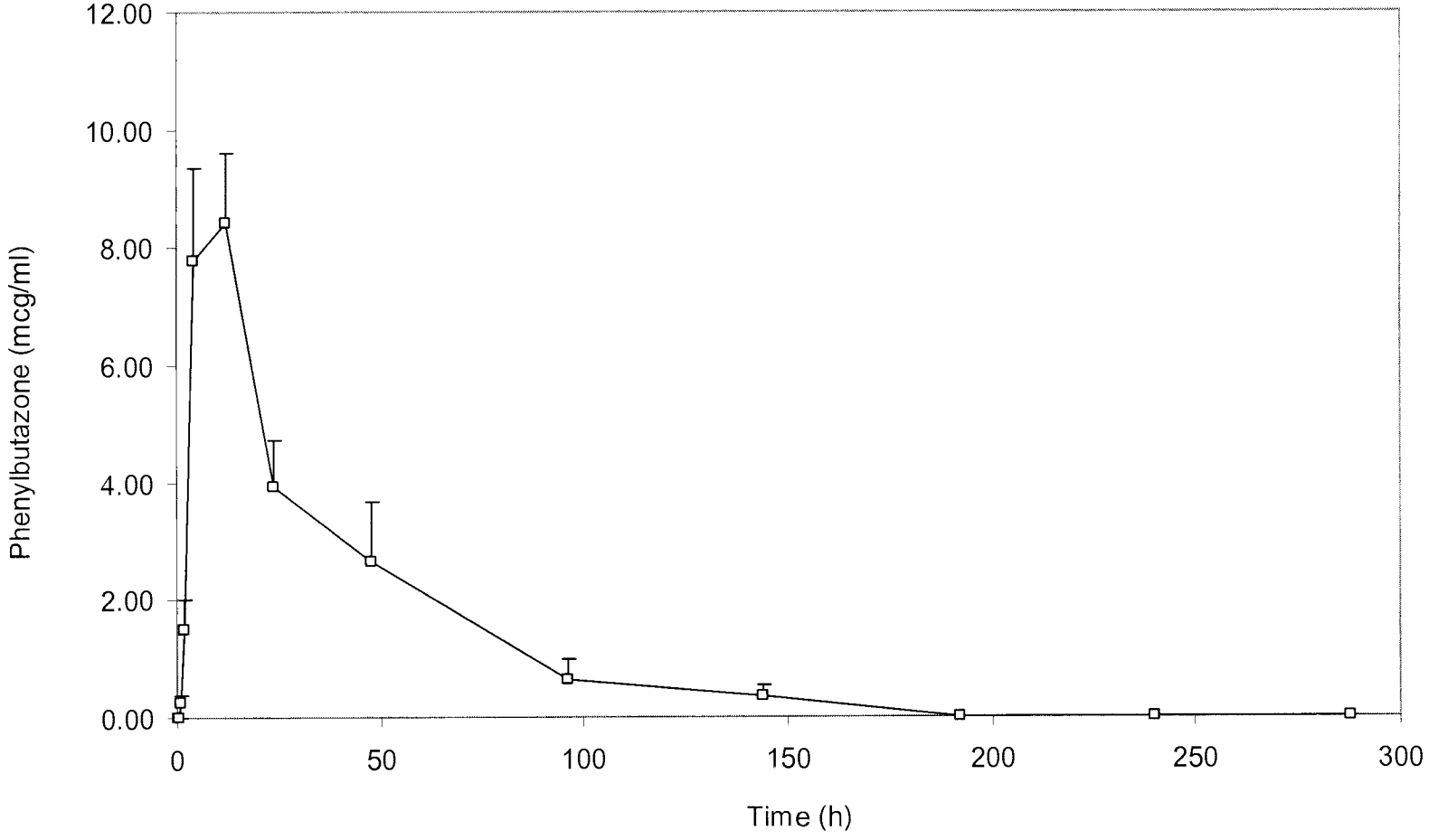

Figure 3. 8 Mean plot of concentration time profile of phenylbutazone with standard error given orally at 4 mg/kg to elephants (n = 13).

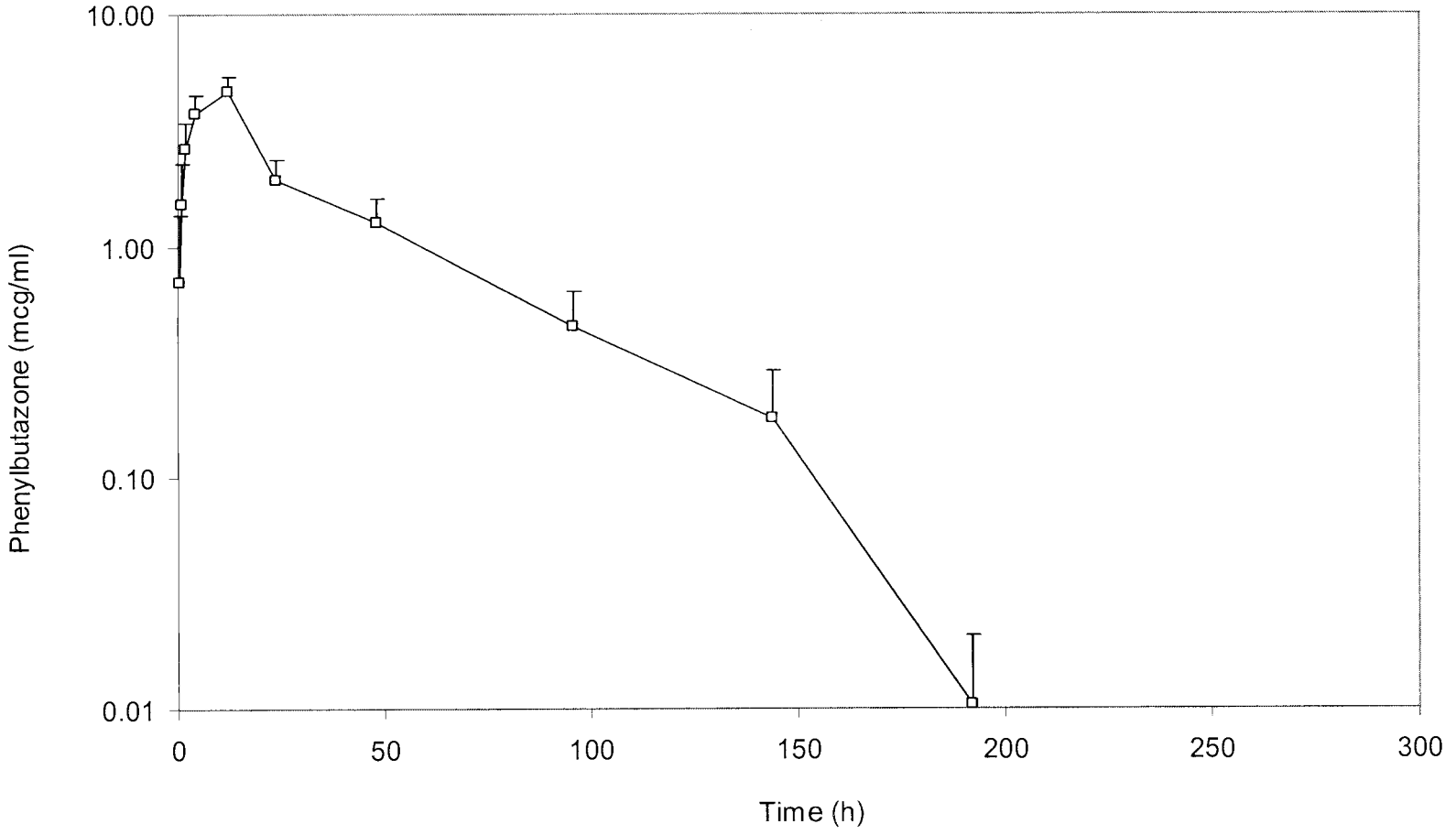

Figure 3. 9 Mean semilogarthmic plot of concentration time profile of phenylbutazone with standard error given orally at 2 mg/kg elephants (n = 13).

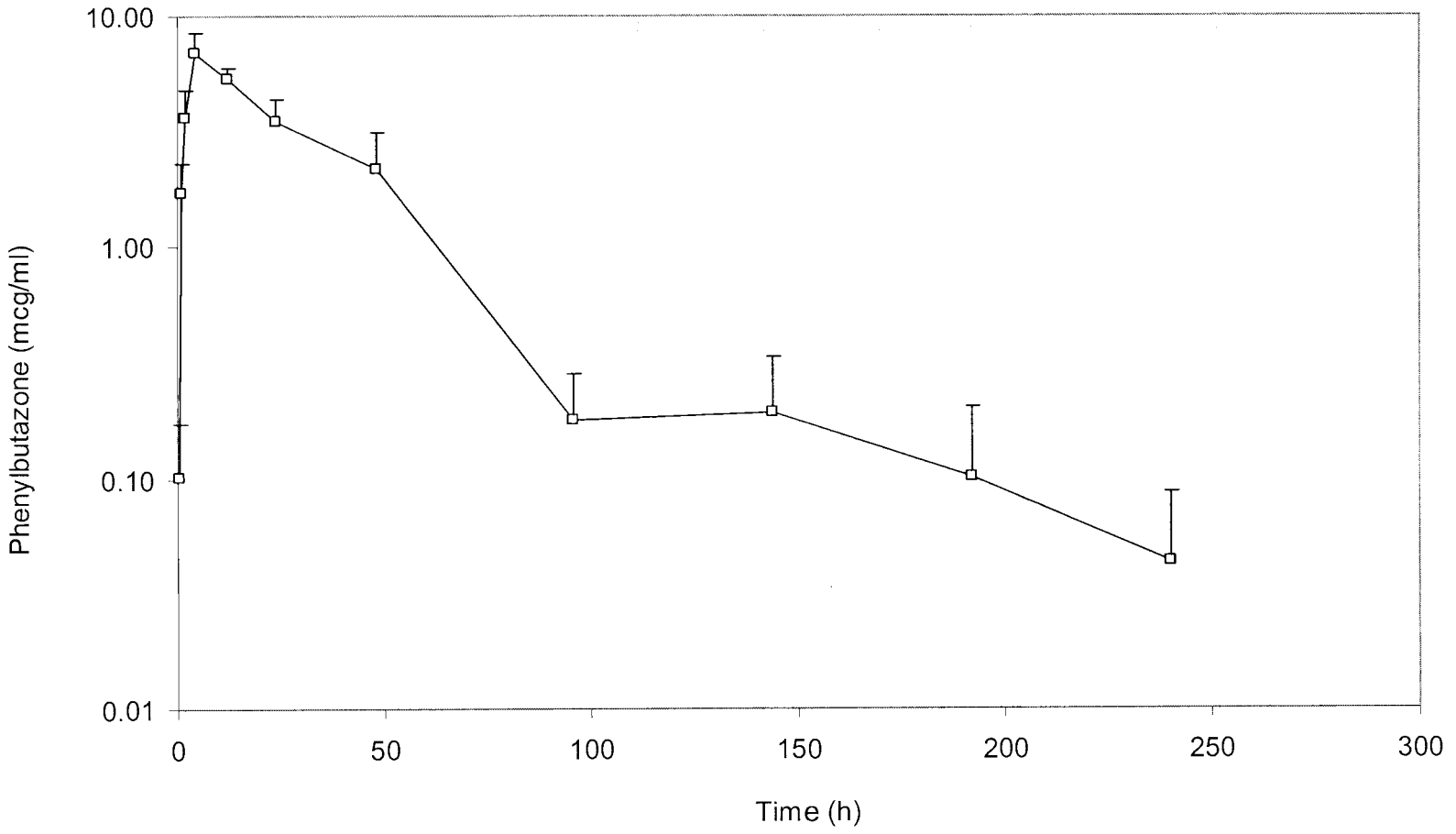

Figure 3. 10 Mean semilogarthmic plot of concentration time profile of phenylbutazone with standard error given orally at 3 mg/kg elephants (n = 13).

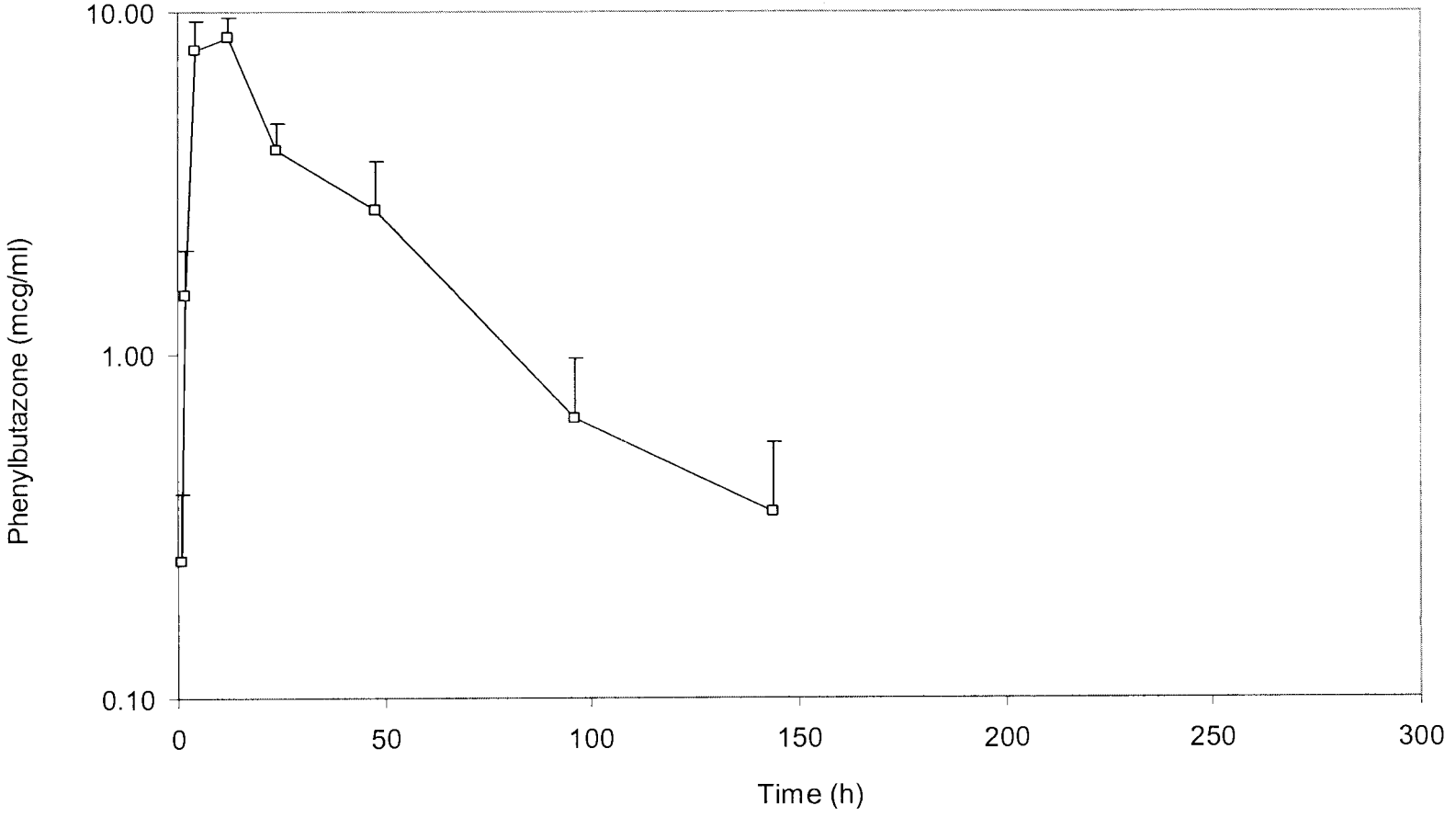

Figure 3. 11 Mean semilogarthmic plot of concentration time profile of phenylbutazone with standard error given orally at 4 mg/kg elephants (n = 13).

Table 3. 6 Phenylbutazone noncompartmental pharmacokinetic parameters after oral administration of 2, 3, and 4 mg/kg.

	2 mg/kg* (n=13)	3 mg/kg$ (n=13)	4 mg/kg% (n=13)
Parameter	Mean ± SD	Mean ± SD	Mean ± SD
C_{max} (mcg/ml)	5.76 ± 2.66	8.85 ± 4.52	11.26 ± 4.37
T_{max} (h)	8.04 ± 4.55	10.31 ± 12.05	11.08 ± 11.79
Terminal Rate Constant (1/h)	0.03 ± 0.02	0.04 ± 0.02	0.05 ± 0.03
Terminal Half-Life (h)	29.72 ± 20.76	23.18 ± 19.95	21.69 ± 16.14
Harmonic Mean Half-Life (h)	20.38	15.93	14.47
Vd_{ss} (ml/kg)	579.53 ± 299.32	517.34 ± 229.42	534 ± 237.51
Vd/F (ml/kg)	563.3 ± 294.14	477.34 ± 186.36	467.82 ± 223.33
CL/F (ml/h/kg)	19.72 ± 16.30	20.85 ± 11.93	21.8 ± 15.61
AUC_{0-n} (mcg.h/ml)	161.05 ± 141.95	221.31 ± 210.48	296.61 ± 244.58
MRT (h)	42.47 ± 25.63	31.87 ± 19.48	34.01 ± 22.44

- *Butch and Buffy – Not included
- $Buffy and Limba – Not included
- %Hank and Lois – Not included

Table 3. 7 Phenylbutazone noncompartmental pharmacokinetic parameters after oral administration of 2 mg/kg in individual elephant.

Subject	C_{max} (mcg/ml)	T_{max} (h)	AUC_{0-n} (mcg.h/ml)	K_{el} (1/h)	$T_{1/2}$ (h)	MRT (h)	CL/F (ml/h/kg)	Vd/F (ml/kg)	Vd_{ss} (ml/kg)
Hank	10.16	12	411.67	0.01	50.13	79.07	4.12	298.28	326.09
Angus	1.83	4	33.07	0.05	13.92	22.83	54.14	1086.87	1236.1
Caesar	7.57	4	227.11	0.02	44.19	66.33	6.82	434.64	452.17
Louis	4.8	4	72.84	0.08	9.16	15.33	26.59	351.54	407.63
Solomon	3.86	12	65.68	0.02	32.07	43.88	20.34	941	892.49
Timba	2.72	12	80.05	0.04	16.59	30.97	20.63	493.89	638.99
Toby	3.9	12	31.57	0.05	14.87	19.51	50.83	1090.18	991.56
Willie	5.28	12	73.06	0.03	20.68	28.67	22.62	675.02	648.57
Betty	8.46	12	424.76	0.01	83.7	100.1	3.54	427.32	354.24
Peggy	8.73	12	332.97	0.02	39.74	47.36	5.86	335.81	277.38
Lea	3.27	4	61.62	0.03	27.46	41.87	13.77	545.52	576.62
Limba	5.89	4	153.56	0.03	21.65	39.03	12.23	381.87	477.27
Sheba	8.45	0.5	125.68	0.06	12.14	17.1	14.9	260.94	254.74
Mean	5.76	8.04	161.05	0.03	29.72	42.47	19.72	563.3	579.53
SD	2.66	4.55	141.95	0.02	20.76	25.63	16.3	294.14	299.32

Table 3. 8 Phenylbutazone noncompartmental pharmacokinetic parameters after oral administration of 3 mg/kg in individual elephant.

Subject	C_{max} (mcg/ml)	T_{max} (h)	AUC_{0-n} (mcg.h/ml)	K_{el} (1/h)	$T_{1/2}$ (h)	MRT (h)	CL/F (ml/h/kg)	Vd/F (ml/kg)	Vd_{ss} (ml/kg)
Betty	17.2	4	421.13	0.02	28.83	37.22	6.93	288.23	257.99
Butch	3.84	4	91.13	0.06	12.2	22.26	30.13	530.59	670.93
Hank	12.77	48	817.71	0.01	82.06	86.35	3.37	399.17	291.15
Lea	6.15	4	119.11	0.04	16.91	24.16	21.71	529.89	524.57
Louis	16.19	4	189.72	0.05	12.67	16.43	14.82	271.07	243.56
Peggy	9.32	2	240.87	0.02	31.68	40.37	12.02	549.19	485.13
Solomon	7.32	4	101.5	0.05	14.27	20.99	26.7	549.87	560.53
Timba	10.82	12	202.04	0.04	16.63	27.39	14.56	349.17	398.78
Toby	2.89	12	60.86	0.04	16.59	25.95	42.21	1010.4	1095.53
Willie	7.07	12	97.12	0.06	11.97	17.13	28.92	499.19	495.21
Angus	4.95	12	88.88	0.07	9.58	20.54	32.02	442.53	657.72
Caesar	10.76	4	353.46	0.02	39.1	53.63	7	394.73	375.35
Sheba	5.71	12	93.45	0.08	8.85	21.83	30.64	391.39	668.93
Mean	8.85	10.31	221.31	0.04	23.18	31.87	20.85	477.34	517.34
SD	4.52	12.05	210.48	0.02	19.95	19.48	11.93	186.4	229.42

Table 3. 9 Phenylbutazone noncompartmental pharmacokinetic parameters after oral administration of 4 mg/kg in individual elephant.

Study	C_{max} (mcg/ml)	T_{max} (h)	AUC_{0-n} (mcg.h/ml)	K_{el} (1/h)	$T_{1/2}$ (h)	MRT (h)	CL/F (ml/h/kg)	Vd/F (ml/kg)	Vd_{ss} (ml/kg)
Betty	19.54	4	618.94	0.03	24.01	39.6	6.05	209.52	239.58
Buffy	12.66	12	221.5	0.09	8.05	18.14	17.54	203.84	318.13
Butch	7.78	4	107.73	0.08	8.94	16.12	36.01	464.68	580.48
Lea	7.34	4	154.89	0.03	21.21	27.87	24.85	760.28	692.51
Peggy	12.61	48	904.25	0.01	64.17	94.99	3.5	323.95	332.4
Solomon	8.38	12	172.47	0.06	10.93	21.69	21.56	339.94	467.58
Timba	17.2	12	350.57	0.03	25.24	28.2	11.26	410.2	317.66
Toby	5.87	12	80.88	0.07	9.41	20.83	46.89	636.54	976.69
Willie	8.14	12	110.72	0.03	19.95	27.49	30.4	874.66	835.7
Angus	12.46	12	281.06	0.07	9.5	23.16	13.47	184.62	312.05
Caesar	16.84	4	460.43	0.02	34.19	56.66	8.22	405.38	465.62
Limba	9.92	4	331.63	0.02	36.46	51.75	11.33	596.19	586.52
Sheba	7.66	4	60.88	0.08	8.89	15.63	52.36	671.86	818.3
Mean	11.26	11.08	296.61	0.05	21.61	34.01	21.8	467.82	534.09
SD	4.37	11.79	244.58	0.03	16.14	22.44	15.61	223.33	237.51

Table 3. 10 Half-lives of phenylbutazone in different species (22).

Species	Half-life (h)
Homo	72
Goat	14.5 ♂ 19.0 ♀
Rat	6
Dog	6 2.5
Swine	2-6
Baboon	5 3.5
Horse	4
Rabbit	3

Phenylbutazone pharmacokinetics and dose proportionality following the oral single doses

Figures 3.12 and 3.13 compare the individual AUC_{0-n} and C_{max} following oral phenylbutazone administration of 2, 3, and 4 mg/kg doses, respectively. The relationship between AUC versus dose and C_{max} versus dose, using the power model, are also presented in figures 3.12 and 3.13. Figures 3.14 and 3.15 show the mean AUC_{0-n} and C_{max} for the three oral doses of 2, 3, and 4 mg/kg.

Approximate linear dose proportional increases were noted in mean AUC and C_{max} which was supported by lack of statistically significant deviation from linearity in the dose normalized AUC and C_{max} (p-values: 0.94 and 0.99). Likewise, there was no significant difference in dose independent pharmacokinetic parameters MRT and CL/F (p-values of 0.28 and 0.87). Additionally, with the power model the exponent of dose for AUC_{0-n} [$57.15 \bullet Dose^{0.97}$] and C_{max} [$2.50 \bullet Dose^{1.03}$] were not significantly different from unity and 95% confidence interval of these exponents included 1; AUC (0.05, 1.90) and C_{max} (0.50, 1.60). The mean proportionality ratios of 1.0: 1.37: 1.83 for AUC and 1.0: 1.54: 1.95 for C_{max} were not statistically different from the expected ratio of 1.0: 1.5: 2.0. Statistical comparisons of dose-normalized plasma concentrations were also not significantly different following the three doses.

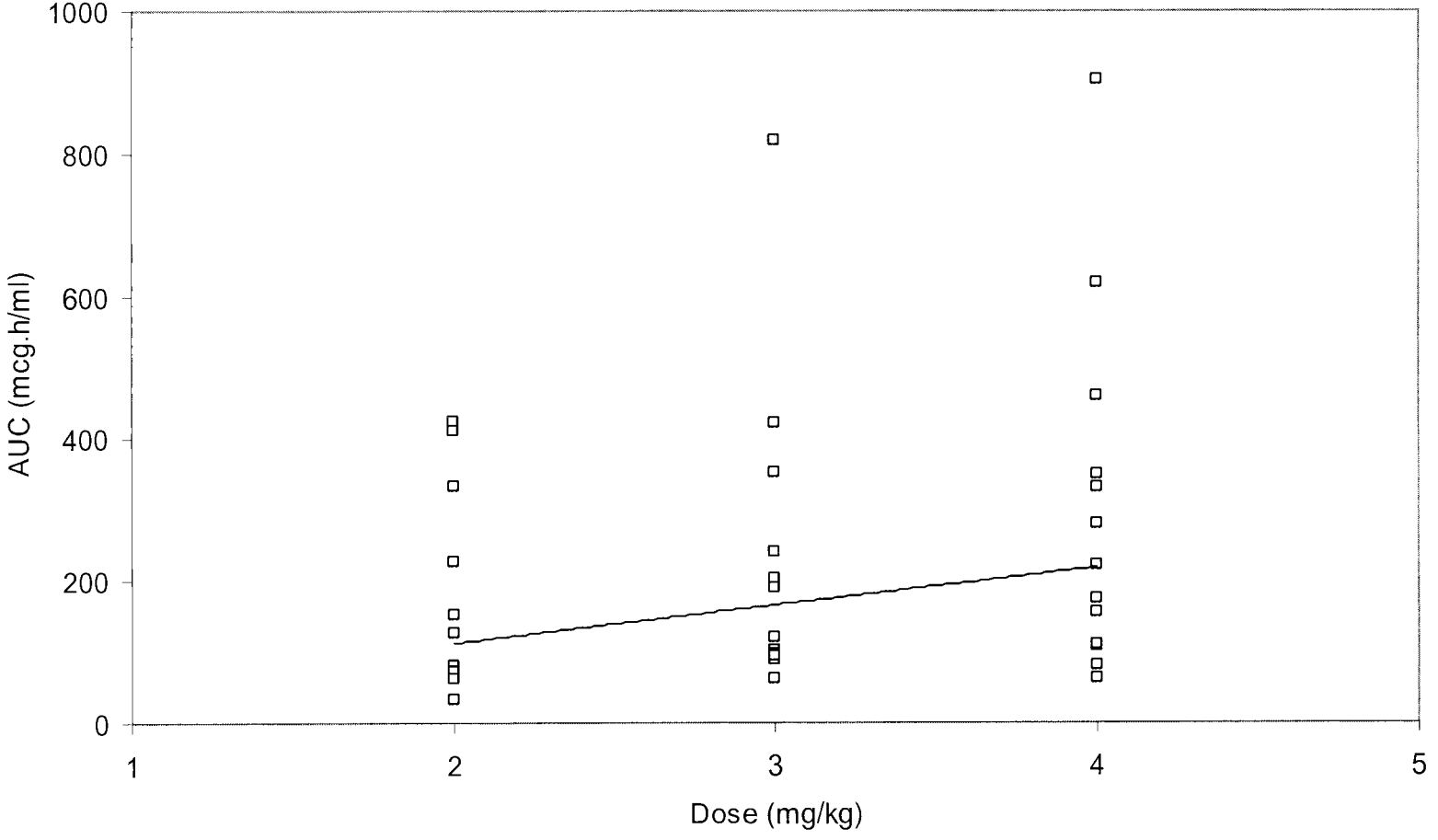

Figure 3. 12 Phenylbutazone plasma AUC_{0-n} versus dose following single doses of 2, 3, and 4 mg/kg with fitted power function.

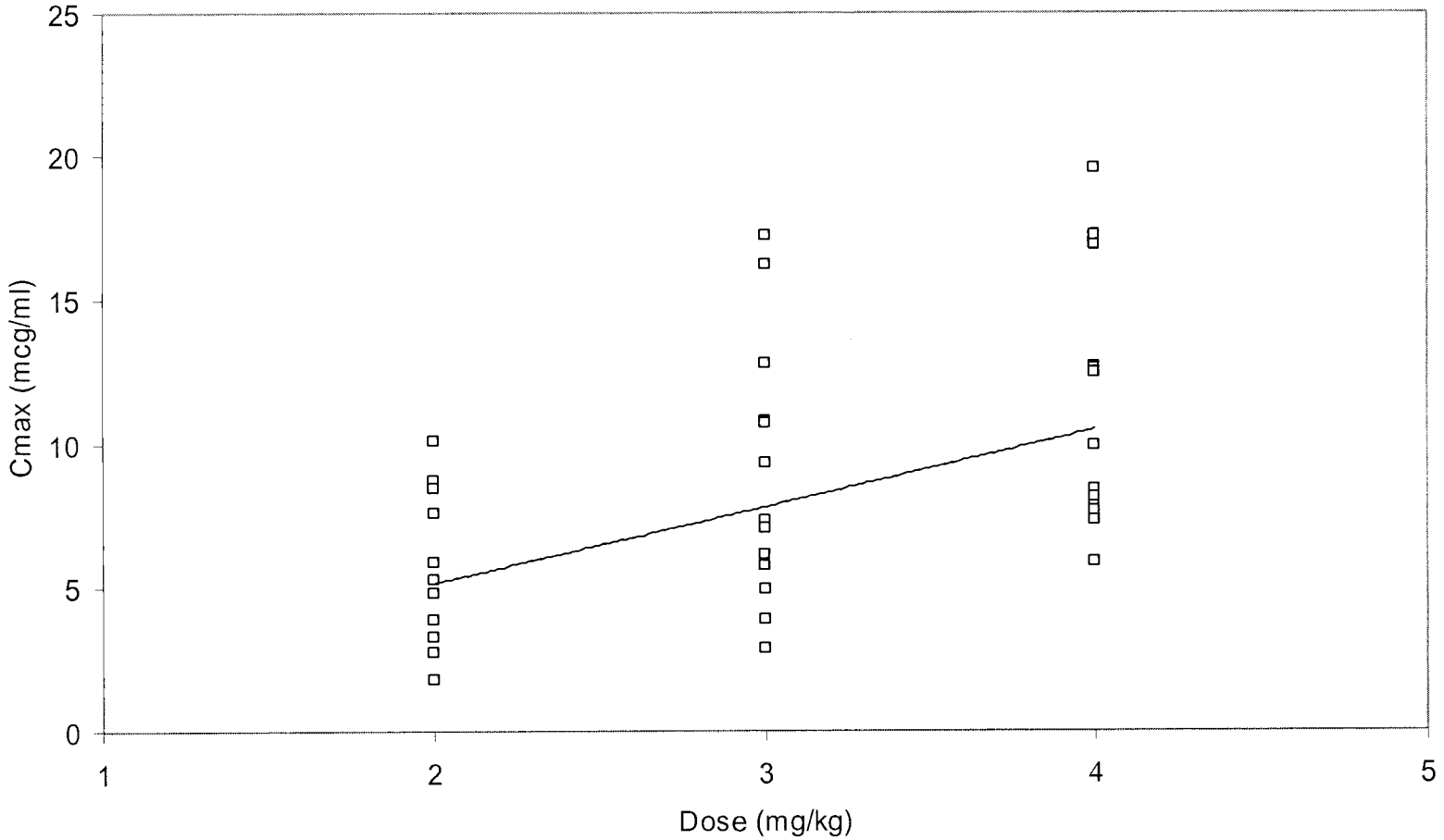

Figure 3. 13 Phenylbutazone plasma C_{max} versus dose following single oral doses of 2, 3, and 4 mg/kg with fitted power function.

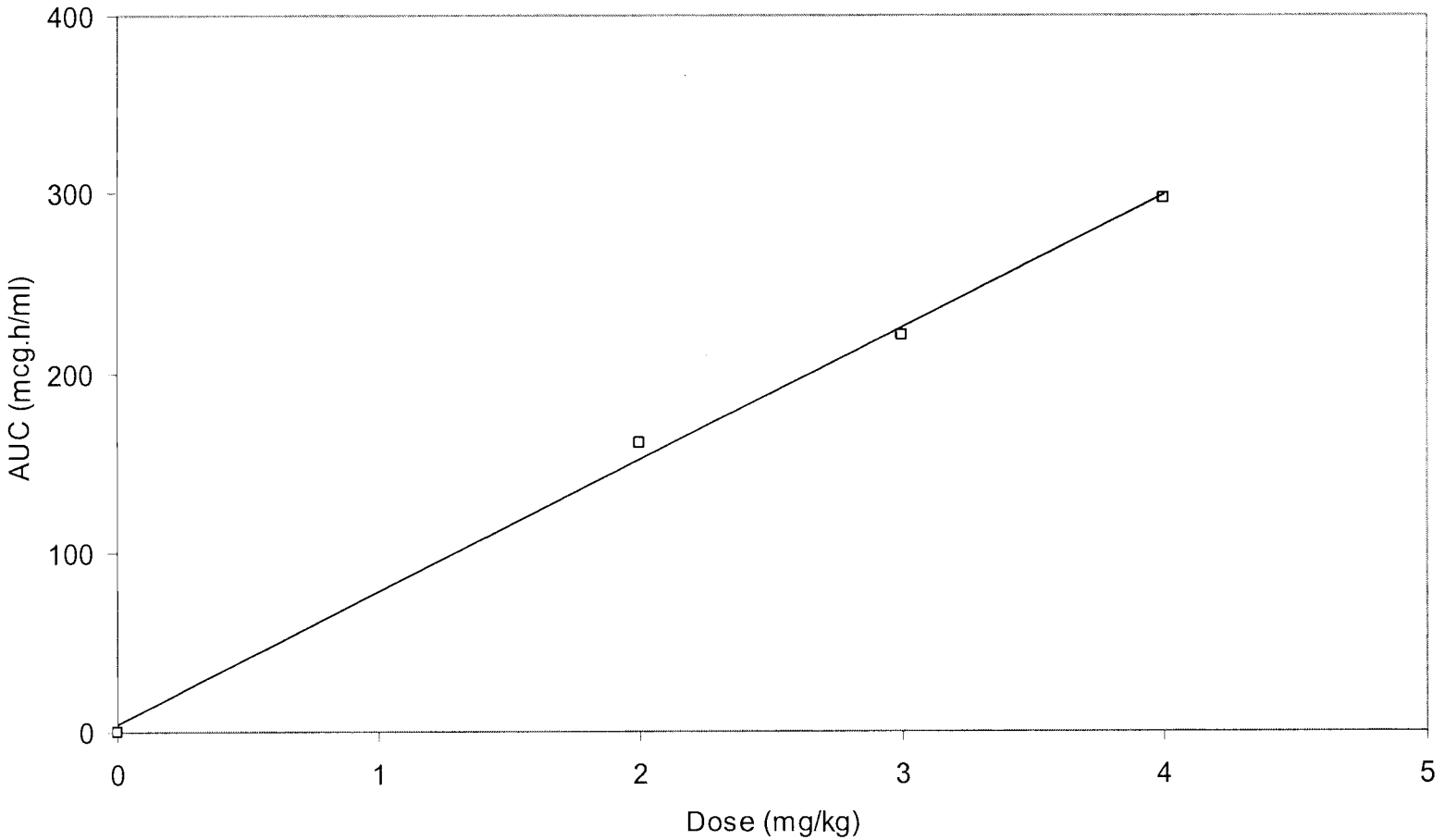

Figure 3. 14 Phenylbutazone mean AUC_{0-n} versus dose following single oral doses of 2, 3, and 4 mg/kg.

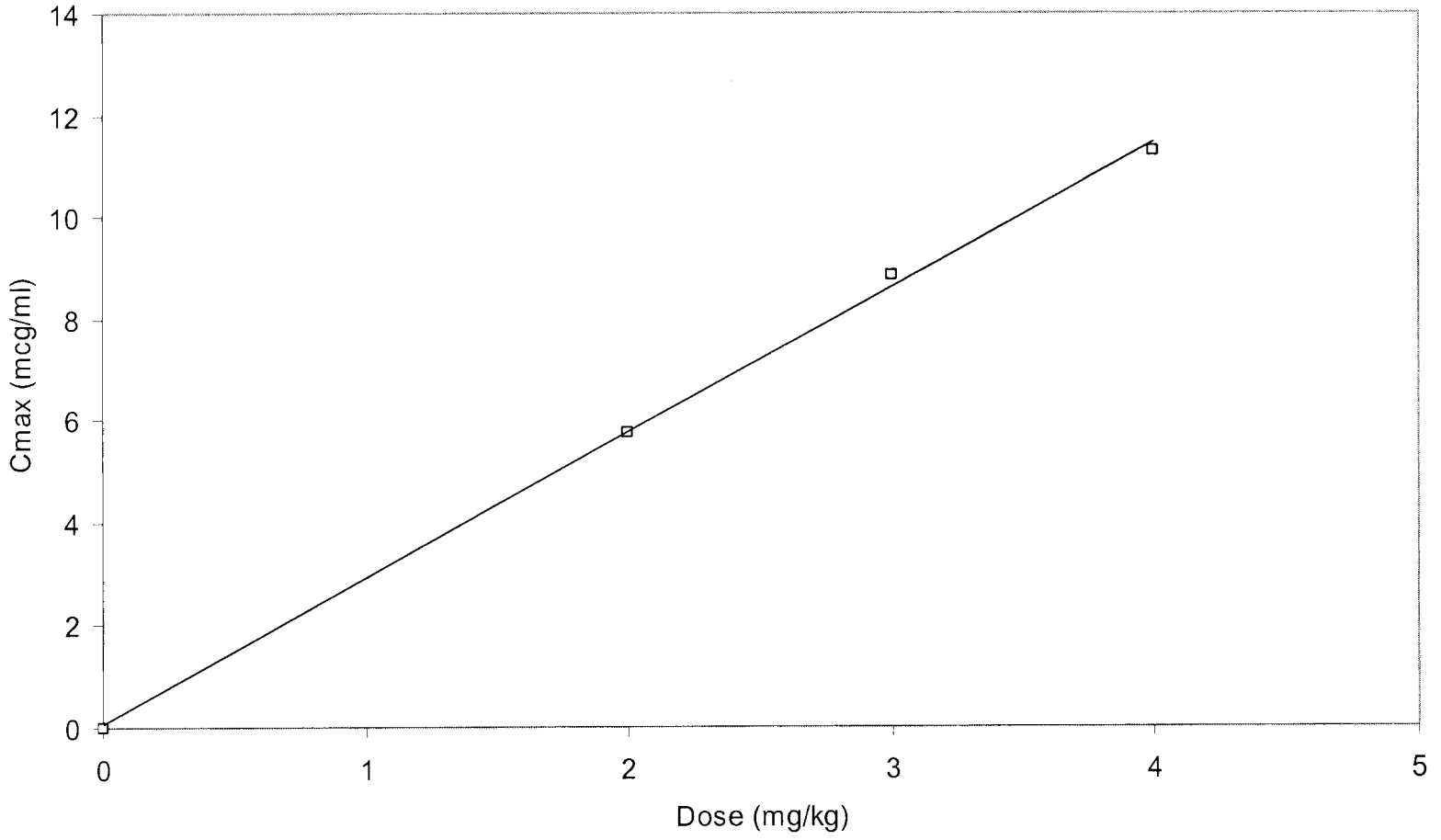

Figure 3. 15 Phenylbutazone mean C_{max} versus dose following single oral doses of 2, 3, and 4 mg/kg.

Comparison of phenylbutazone pharmacokinetics between Asian and African elephants

Summary of the statistical comparison between doses and species differences on pharmacokinetic parameters following the three doses is shown in table 3.11. The plasma concentration time profiles for African and Asian elephants for the three doses are shown in figures 3.16, 3.17 and 3.18 and there semilogarthmic plots are shown in figures 3.19, 3.20 and 3.21 respectively. Dose–dependent parameters such as AUC_{0-n} and C_{max} were dose normalized prior to comparison. Effects of dose, zoo and species groups were included in the ANOVA mixed model. The CL/F and AUC_{0-n} are statistically different between Asian and African elephants as can be seen in the table 3.11.

In humans phenylbutazone is metabolized by CYP 450 enzyme CYP 2C8/9. Phenylbutazone has been found to be a inhibitor of other drugs metabolized by CYP 2C8/9. Phenylbutazone is also reported to be an inducer of the family CYP 3A. Genetic polymorphism is seen with CYP2C9 (28, 29). Asian elephants might have lower CYP 2C8/9 enzyme levels compared to African elephants. This might explain the differences in clearance/F between Asian and African elephants. No firm conclusion can be made on differences in species due to large intra and intersubject variability and small sample size. The large intersubject variability and small sample size highly affects the power of hypothesis test. The result suggests the need of additional study of larger sample size in each species group to confirm their effects.

Plasma half lives are similar in subjects with identical genotypes and significantly greater differences were observed with dizygotic twins in humans. Phenylbutazone metabolism in man is under polygenic control (30).

Double peaks were observed for the Asian elephants indicating that phenylbutazone might be undergoing enterohepatic cycling or have an initial rapid absorption phase followed by a delayed second slower absorption phase. In horses when phenylbutazone was given along with the hay it was found that the plasma concentration profiles showed double peaks and the reason for this was attributed to phenylbutazone binding to the hay and on entering the cecum it undergoes degradation releasing the drug (10).

Table 3. 11 Pharmacokinetic parameters of phenylbutazone given at 2, 3, and 4 mg/kg doses orally to African and Asian elephants.

	2 mg/kg		3 mg/kg		4 mg/kg		ANOVA
Parameter	African (n=8)	Asian (n=5)	African (n=9)	Asian (n=4)	African (n=9)	Asian (n=4)	P-value*
AUC (mcg.h/ml)	67.94 ± 29.55	310.01 ± 117.66	115.98 ± 47.84	458.29 ± 250.88	171.19 ± 96.86	578.81 ± 246.74	0.0001
CL/F (ml/h/kg)	27..98 ±15.69	6.51 ± 3.45	26.86 ± 8.76	7.33 ± 3.55	28.26 ± 14.45	7.27 ± 3.32	0.0003
Terminal half-life (h)	18.36 ± 7.88	47.88 ±22.67	13.3 ± 3.01	45.42 ± 24.81	13.57 ± 6.61	39.71 ± 17.18	0.0001
C_{max}	4.26 ± 2.02	8.16 ± 1.58	7.22 ± 4.06	12.51 ± 3.43	9.72 ± 3.62	14.73 ± 4.29	0.0071
T_{max}	7.56 ± 4.88	8.80 ± 4.38	8.44 ± 2.16	14.50 ± 22.35	9.33 ± 4.00	15.00 ± 22.00	0.243
Vd_{ss}	705.84 ± 319.49	377.43 ± 84.76	590.64 ± 235.49	352.41 ± 101.34	591.01 ± 253.21	406.03 ± 151.94	0.0138
MRT (h)	27.52 ± 10.89	66.38 ± 24.54	21.85 ± 3.66	54.4 ± 22.46	22.13 ± 4.95	60.75 ± 23.92	0.0001

* P-value for showing difference between African and Asian elephants.

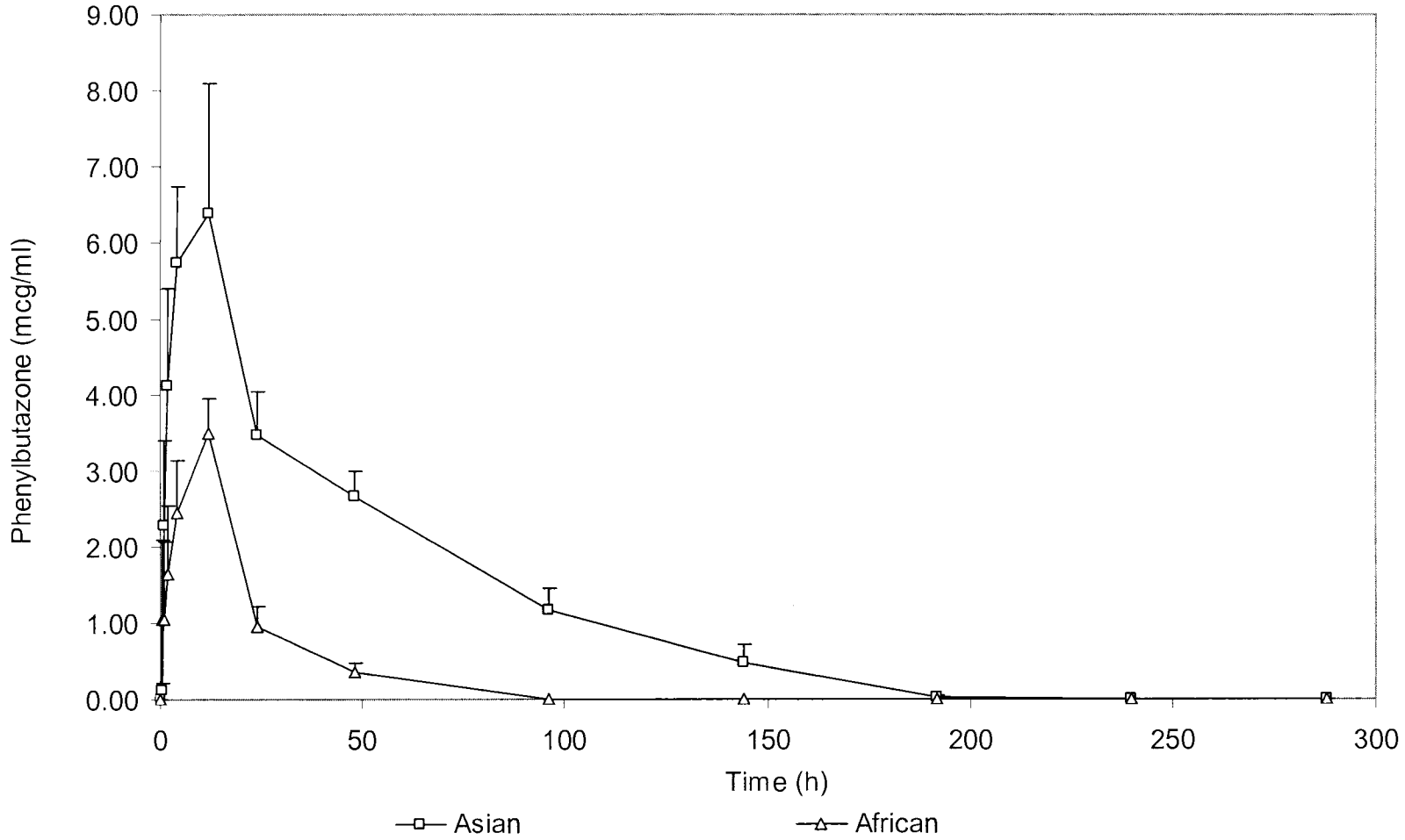

Figure 3. 16 Mean phenylbutazone plasma concentration time profiles with standard errors for African (n=8) and Asian (n=5) elephants at an oral dose of 2 mg/kg.

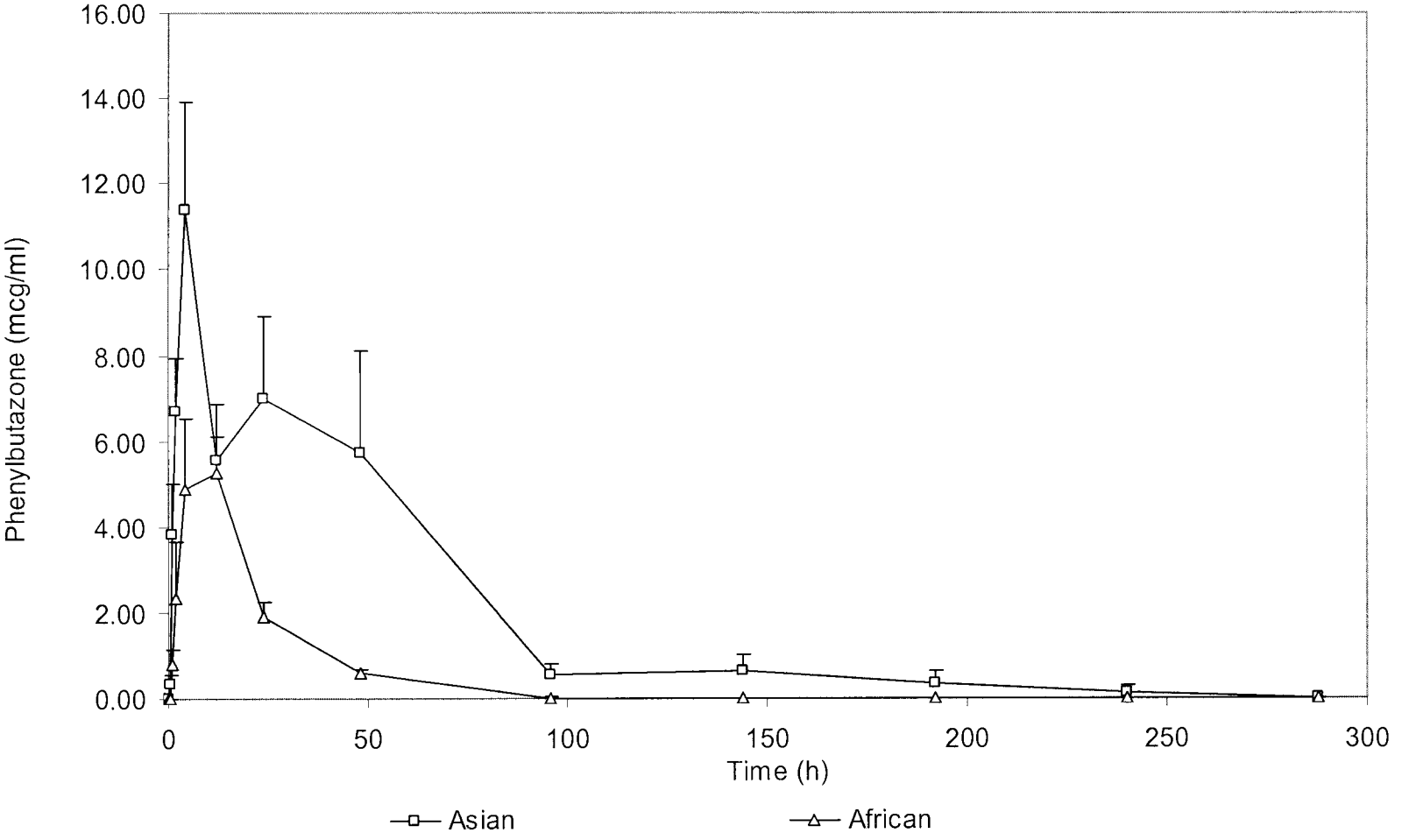

Figure 3. 17 Mean phenylbutazone plasma concentration time profiles with standard error for African (n=9) and Asian (n=4) elephants at an oral dose of 3 mg/kg.

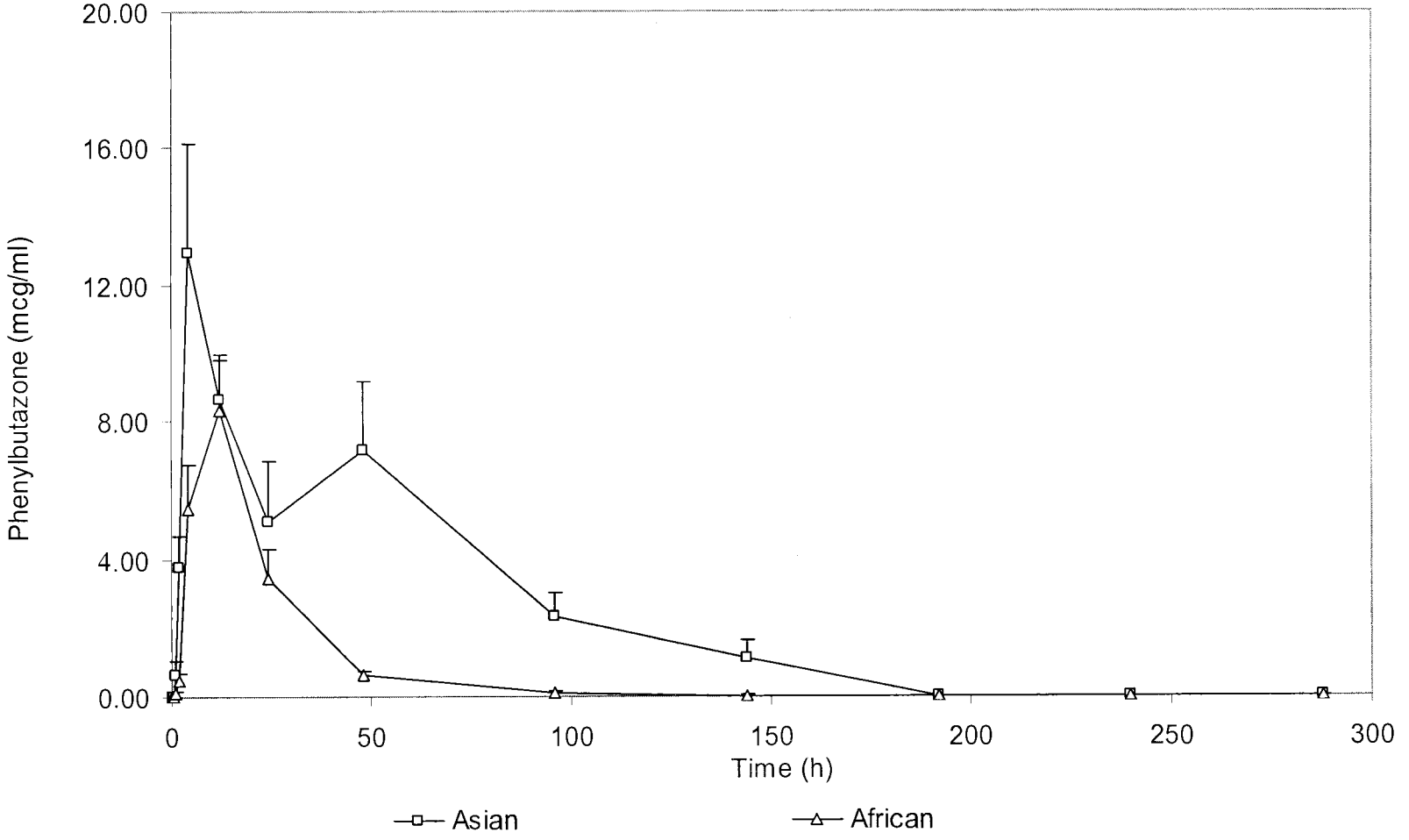

Figure 3. 18 Mean phenylbutazone plasma concentration time profiles with standard error for African (n=9) and Asian (n=4) elephants at an oral dose of 4 mg/kg.

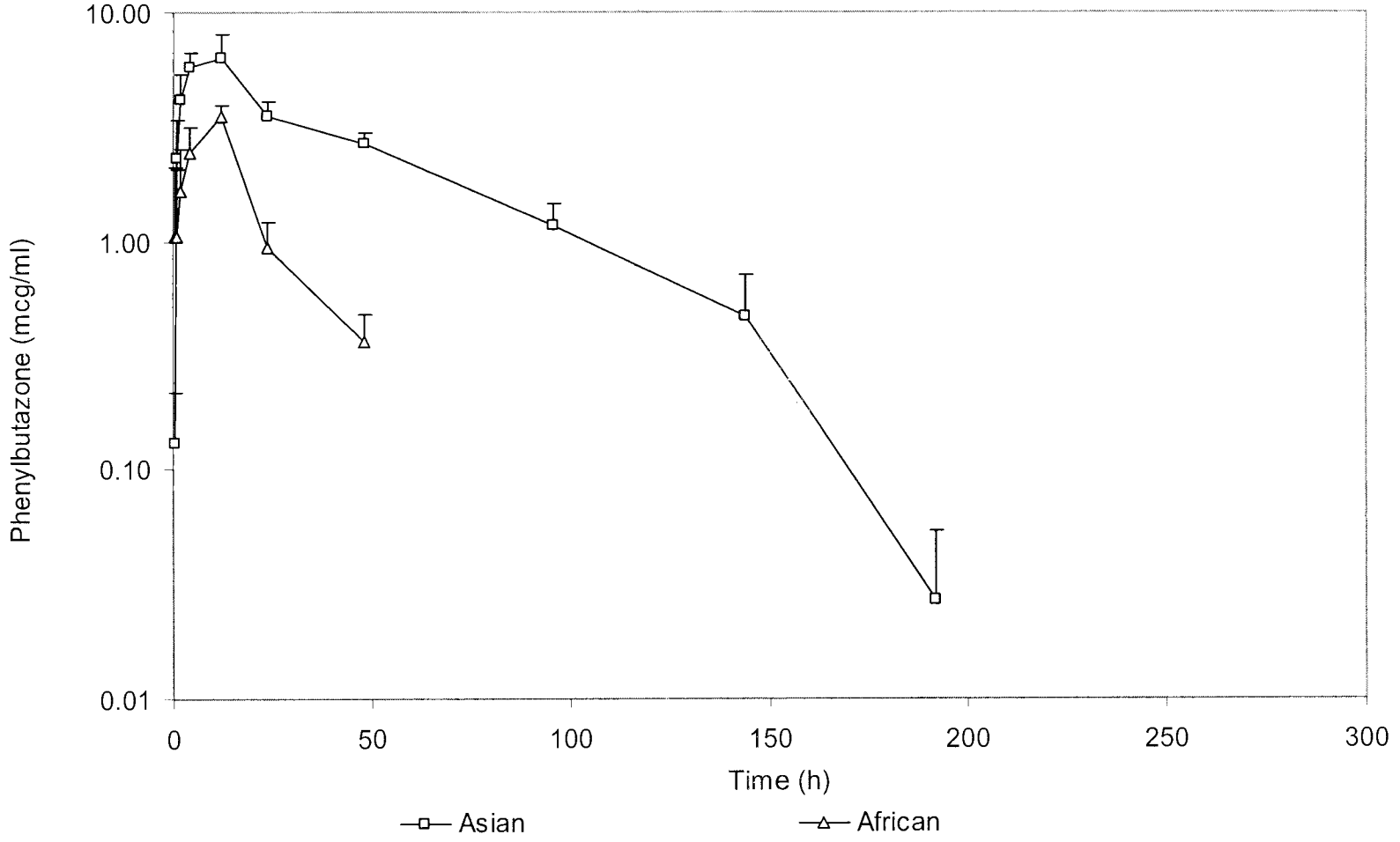

Figure 3. 19 Mean semilogarthmic plot of phenylbutazone plasma concentration time profiles with standard errors for African (n=8) and Asian (n=5) elephants at an oral dose of 2 mg/kg.

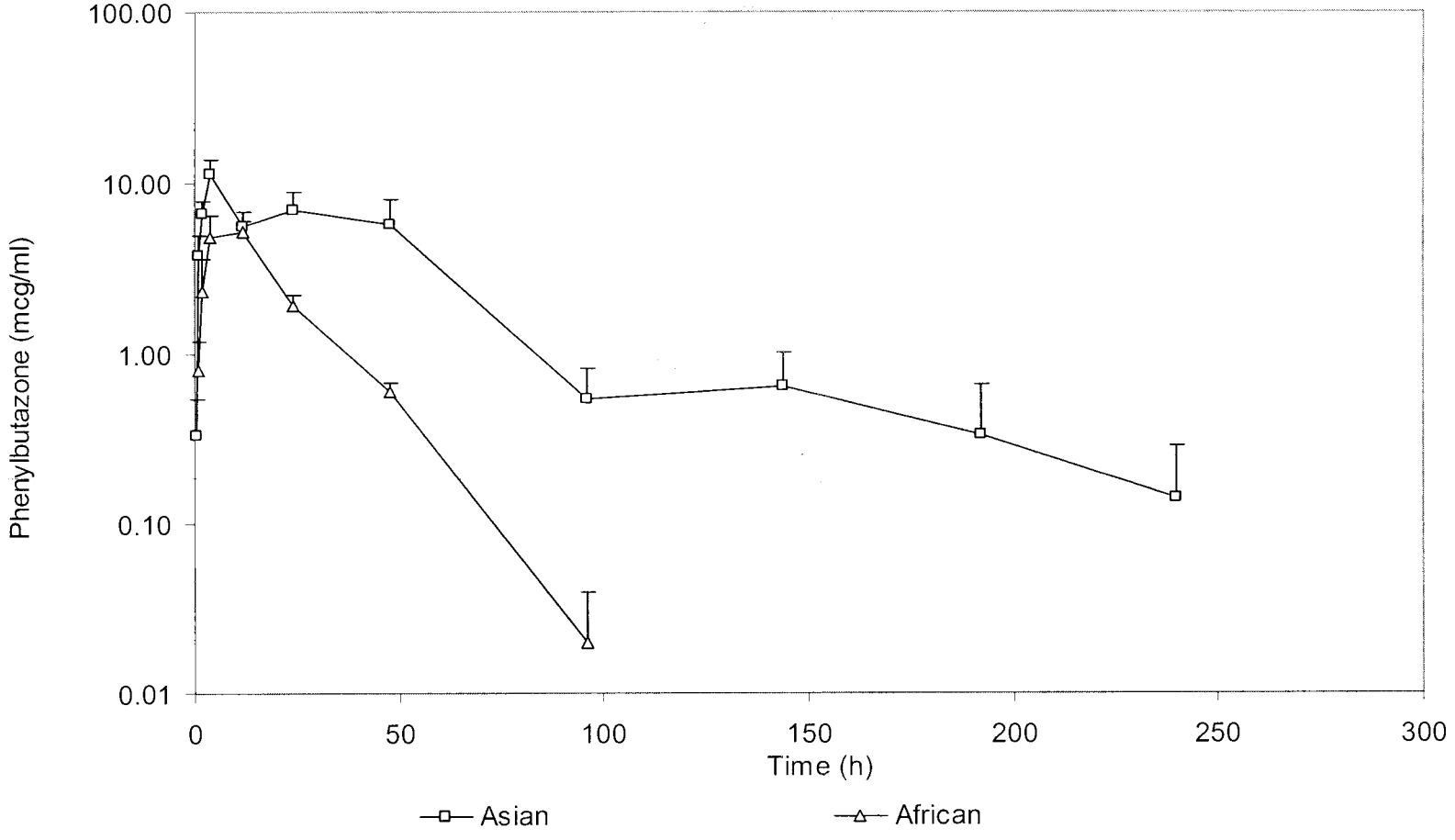

Figure 3. 20 Mean semilogarthmic plot of phenylbutazone plasma concentration time profiles with standard errors for African (n=9) and Asian (n=4) elephants at an oral dose of 3 mg/kg.

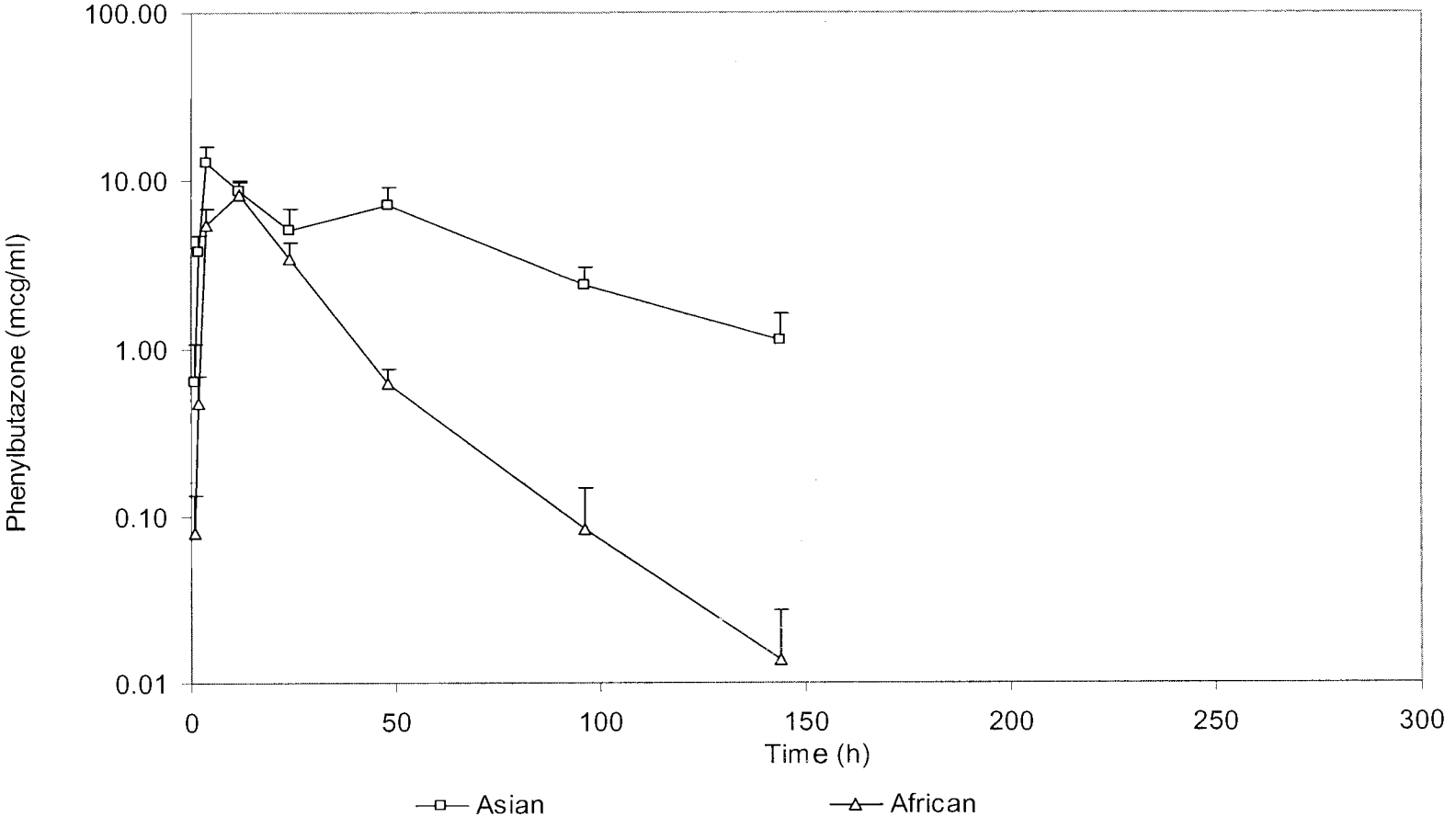

Figure 3. 21 Mean semilogarthmic plot of phenylbutazone plasma concentration time profiles with standard errors for African (n=9) and Asian (n=4) elephants at an oral dose of 4 mg/kg.

Multiple dose study

The plasma concentration time profiles of African elephants on repeated administration of 2 mg/kg every 24 h are shown in tables 3.12. The pharmacokinetic parameters of African elephants at steady state are shown in table 3.13. The plasma concentration time profiles for African elephant are shown in figure 3.22 and the mean plasma concentration time profile with standard error are shown in figure 3.23. By dosing the African elephants at 2 mg/kg every 24-h, the $C_{average}$ obtained was 2.89 ± 0.72 mcg/ml. The clearance at steady state is found to be 30.14 ± 6.17 ml/h/kg. The plasma concentration time profiles for Asian elephant are shown in figure 3.24 and the mean plasma concentration time profile with standard error are shown in figure 3.25. The plasma concentration time profiles of Asian elephants on repeated administration of 3 mg/kg every 48 h are shown in tables 3.14. The pharmacokinetic parameters of Asian elephants at steady state are shown in table 3.15. By dosing the Asian elephants at 3 mg/kg every 48-h, the $C_{average}$ obtained was 7.04 ± 0.84 mcg/ml. The clearance at steady state is found to be 9.16 ± 1.10 ml/h/kg.

Table 3. 12 Phenylbutazone plasma concentration profiles in African elephants (n=7) on repeated administration of 2 mg/kg every 24 hours.

	Phenylbutazone plasma concentration (mcg/ml)								
Time (h)	Lea	Lois	Buffy	Butch	Solomon	Willie	Toby	Mean	SD
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.00	0.00	0.47	0.00	0.00	0.00	0.00	0.00	0.07	0.18
2.00	3.25	0.00	1.16	0.00	0.00	0.00	0.00	0.63	1.23
3.00	3.85	9.39	2.21	0.00	0.53	0.44	0.00	2.35	3.41
4.00	2.55	*	5.55	1.45	0.44	0.37	0.00	1.73	2.09
10.00	4.03	6.31	3.31	3.02	3.46	1.37	0.00	3.07	2.00
16.00	1.73	2.51	1.45	2.24	2.23	1.20	0.73	1.73	0.64
22.00	1.39	2.58	*	*	*	*	*	1.98	0.84
23.00	0.91	1.84	*	*	0.69	0.83	7.93	2.44	3.10
24.00	*	*	1.27	1.11	*	*	*	1.19	0.11
25.00	1.57	2.39	0.82	0.71	1.14	0.39	2.40	1.35	0.81
26.00	2.13	2.08	1.81	0.89	0.85	0.86	2.84	1.64	0.78
27.00	2.45	*	1.66	1.21	1.07	0.17	1.46	1.34	0.75
28.00	3.24	5.62	2.73	1.66	0.68	1.25	1.69	2.41	1.66
34.00	3.56	1.75	4.44	5.32	5.00	4.14	2.50	3.81	1.31
40.00	3.71	2.05	1.26	1.20	3.44	1.13	7.32	2.87	2.23
46.00	1.67	4.66	*	*	*	*	*	3.17	2.11
47.00	1.55	3.22	*	*	1.34	1.56	4.10	2.35	1.23
48.00	*	*	2.50	2.86	*	*	*	2.68	0.25
49.00	1.30	2.13	*	*	1.41	1.12	*	1.49	0.45
50.00	*	*	*	*	1.33	0.60	*	0.97	0.51
51.00	1.59	3.98	*	*	2.80	1.79	*	2.54	1.09
52.00	1.74	6.73	*	*	2.65	3.37	*	3.62	2.18
58.00	4.21	8.89	*	*	2.53	3.98	*	4.90	2.76
64.00	3.92	1.97	*	*	3.98	2.48	*	3.09	1.02
70.00	1.86	2.30	*	*	*	*	*	2.08	0.31
71.00	1.16	1.65	*	*	1.46	1.25	*	1.38	0.22

* Sample not collected at this time point

Table 3. 13 Individual pharmacokinetic parameters of phenylbutazone on multiple dosing of 2 mg/kg every 24 hours in African elephants.

Subject	AUC_{ss} (mcg.h/ml)	C_{min} (mcg/ml)	C_{max} (mcg/ml)	T_{max} (h)	$C_{average}$ (mcg/ml)	%AUCdf	Cl_{ss} (ml/h/kg)	MRT (h)
Solomon	58.86	1.33	3.98	16.00	2.45	10.50	33.98	11.32
Willie	58.24	0.60	3.98	10.00	2.43	33.13	34.34	10.27
Toby	87.20	1.46	7.32	16.00	3.63	39.98	22.93	13.69
Buffy	57.98	0.82	4.44	10.00	2.42	8.62	34.49	11.89
Butch	57.98	0.71	5.32	10.00	2.42	11.59	34.49	12.84
Lea	64.84	1.16	4.21	10.00	2.70	21.94	30.84	11.60
Lois	100.65	1.65	8.89	10.00	4.19	37.11	19.87	9.03
Mean	69.39	1.10	5.45	11.71	2.89	23.27	30.14	11.52
SD	17.37	0.40	1.92	2.93	0.72	13.44	6.17	1.55

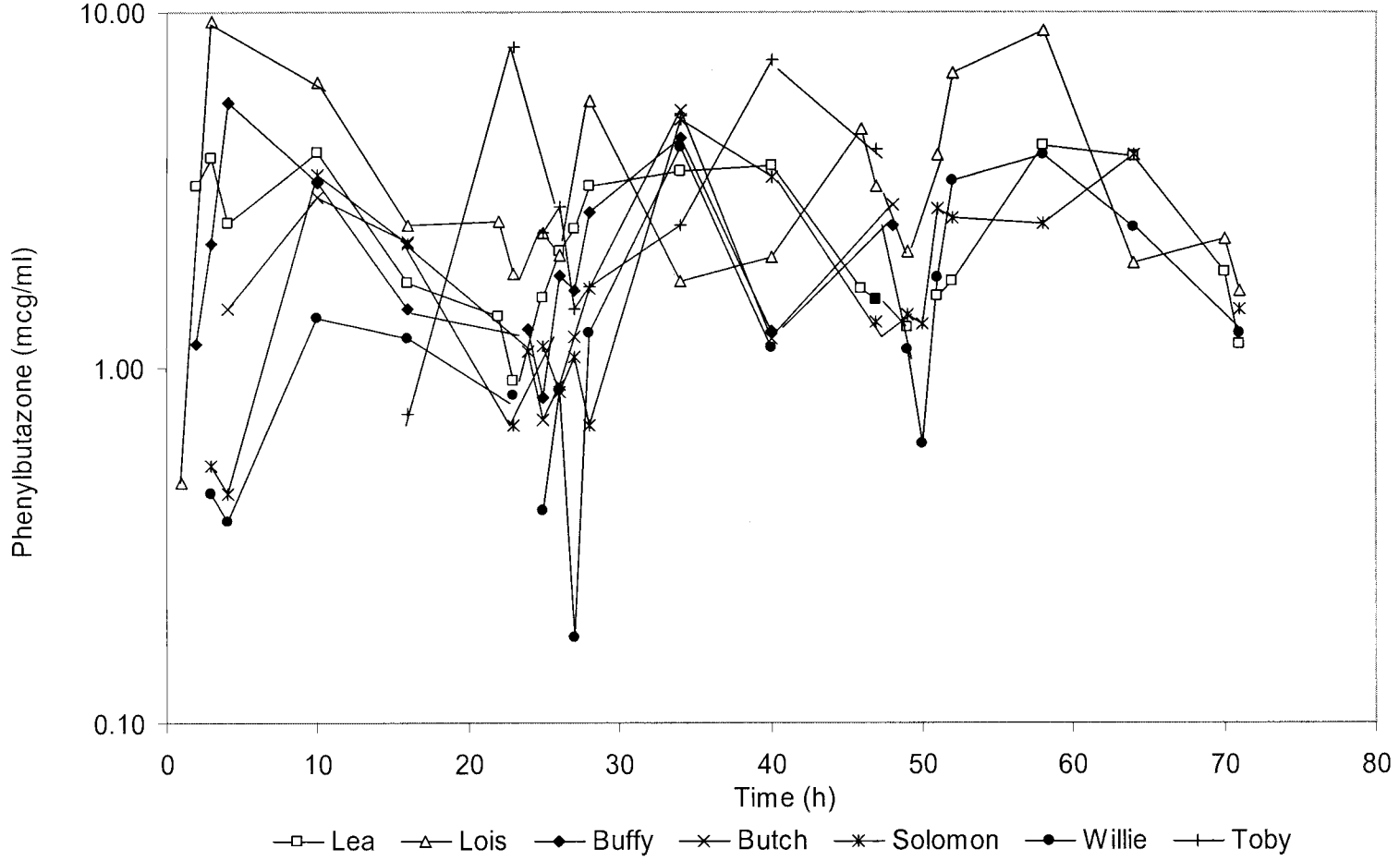

Figure 3. 22 Phenylbutazone plasma concentration times profiles of African (n=7) elephants on repeated oral administration of 2 mg/kg every 24 hours.

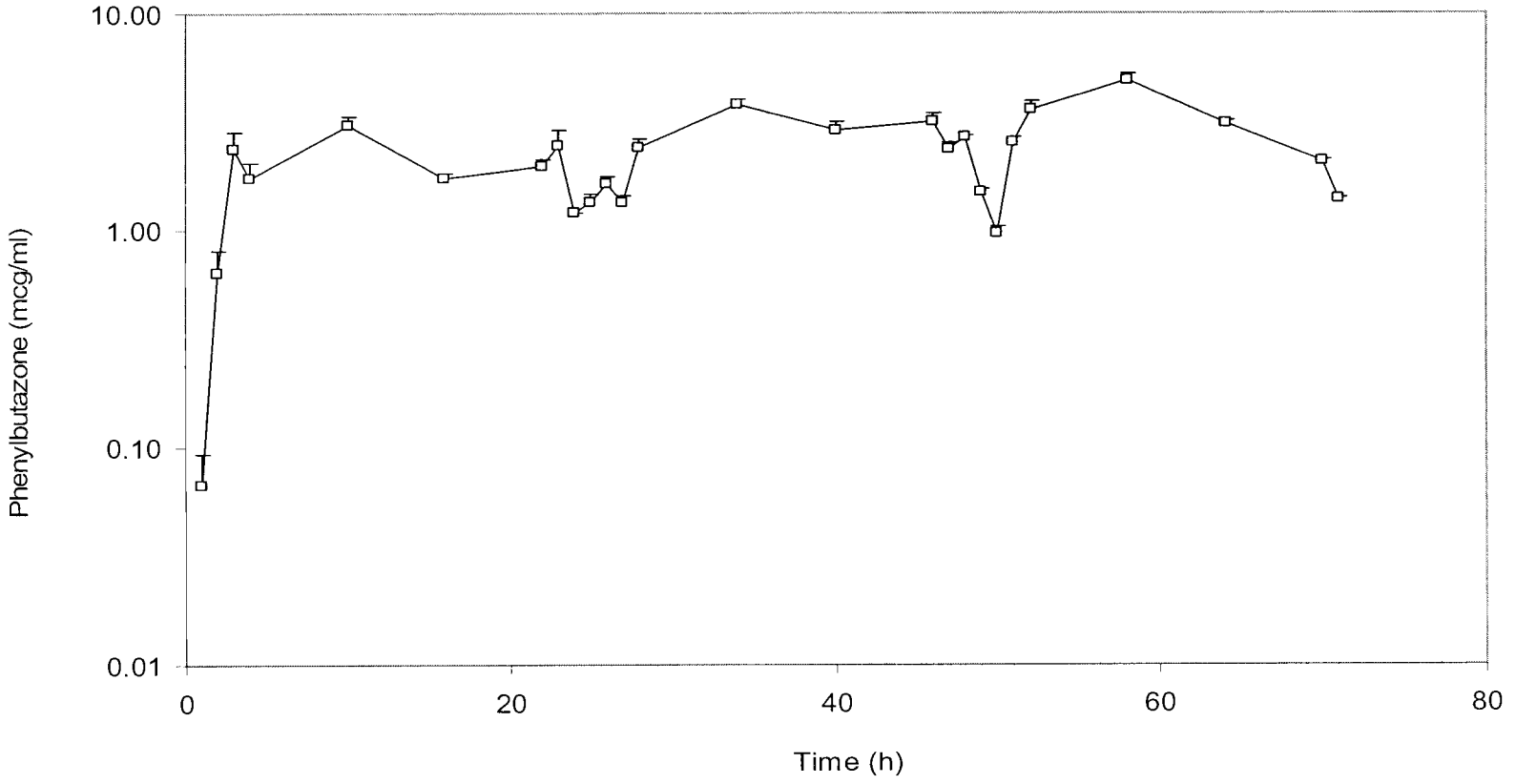

Figure 3. 23 Mean phenylbutazone plasma concentration times profiles with standard error in African (n=7) elephants on repeated oral administration of 2 mg/kg every 24 hours.

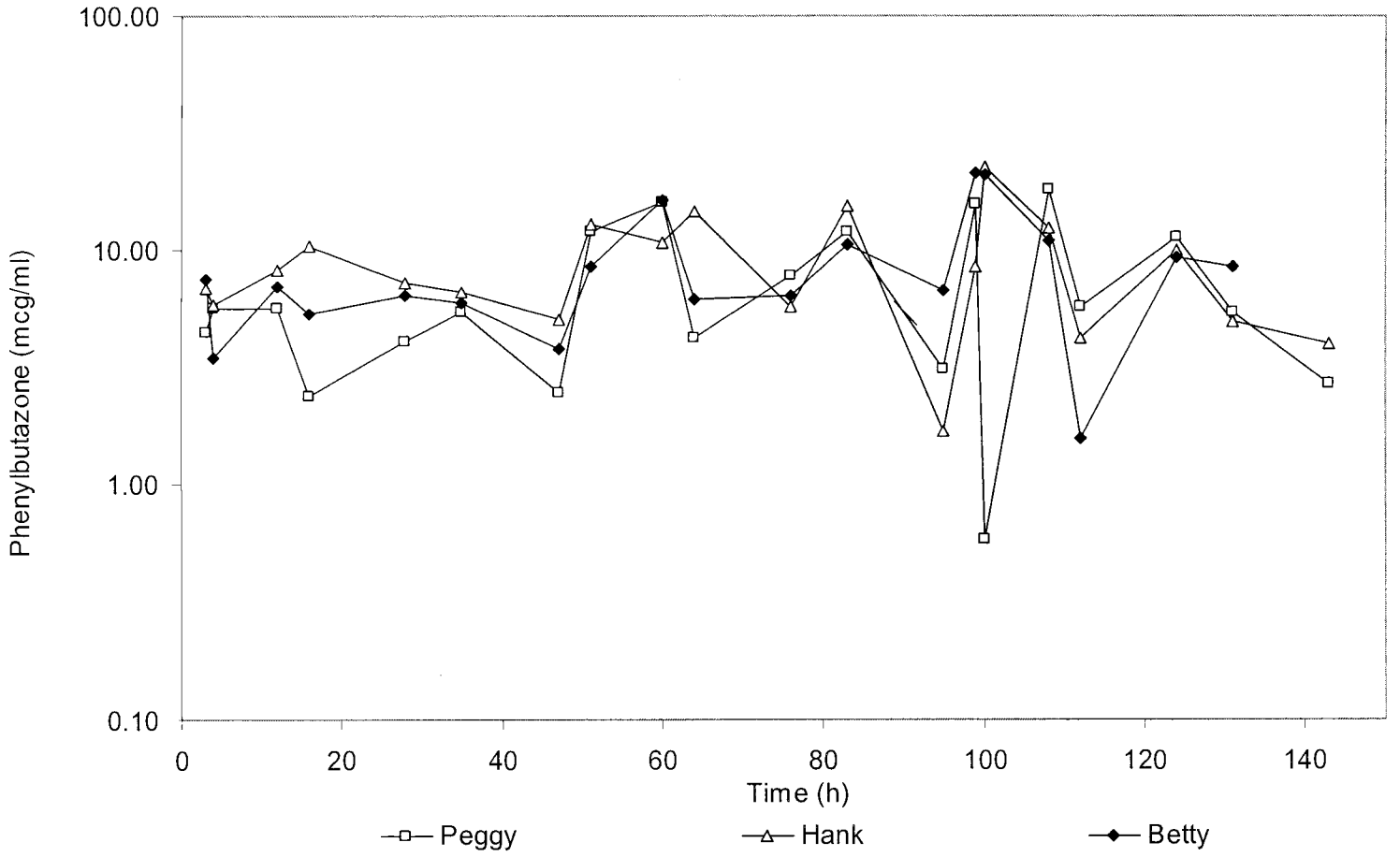

Figure 3. 24 Phenylbutazone plasma concentration times profiles of Asian (n=3) elephants on repeated oral administration of 3 mg/kg every 48 hours.

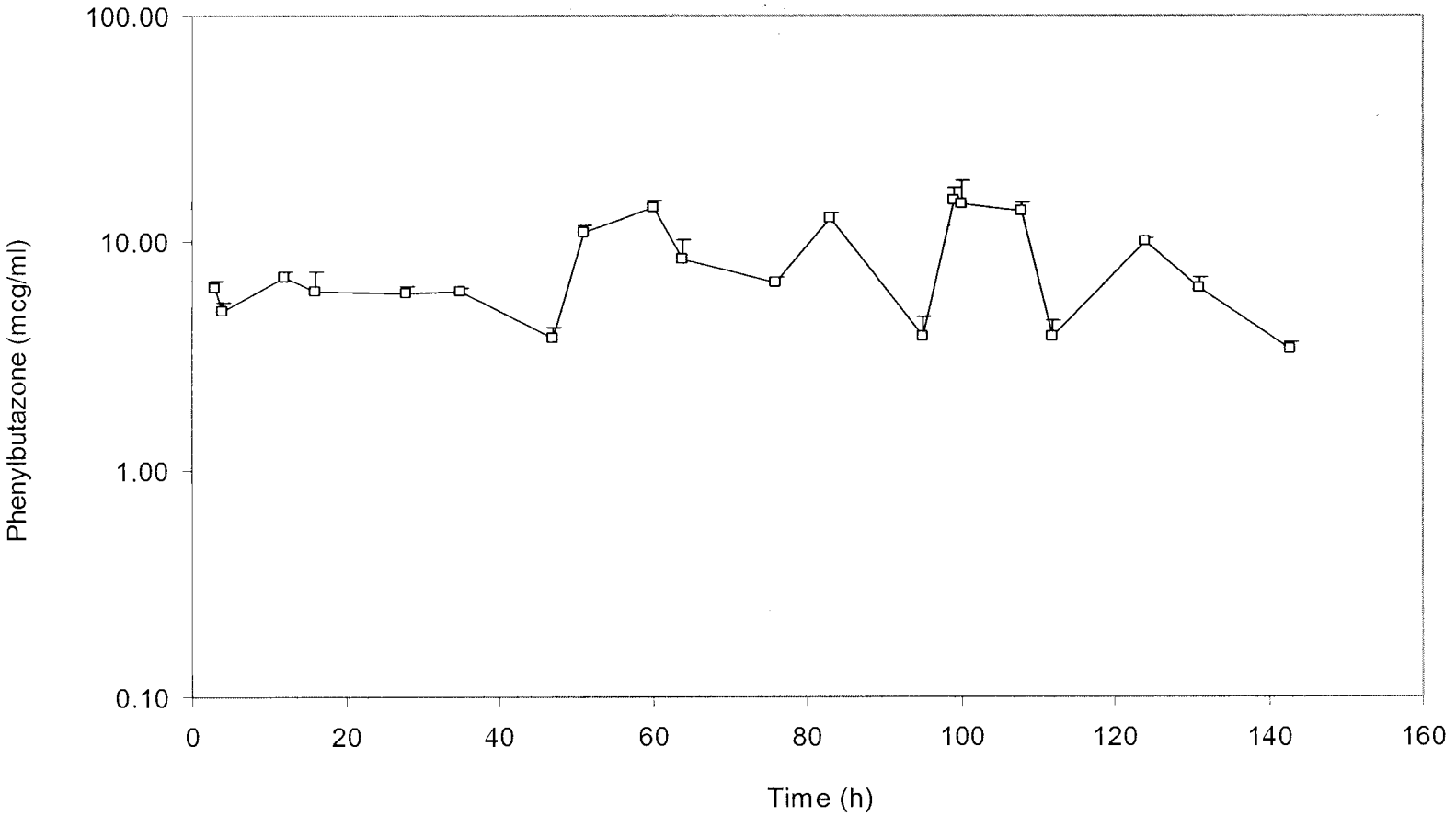

Figure 3. 23 Mean phenylbutazone plasma concentration times profiles with standard error in Asian (n=3) elephants on repeated oral administration of 3 mg/kg every 48 hours.

Table 3. 14 Phenylbutazone plasma concentration profiles in Asian elephants (n=3) on repeated administration of 3 mg/kg every 48 hours.

	Phenylbutazone plasma concentration (mcg/ml)				
Time (h)	Peggy	Hank	Betty	Mean	SD
0.00	0.00	0.00	0.00	0.00	0.00
3.00	4.46	6.90	7.45	6.27	1.59
4.00	5.62	5.78	3.44	4.95	1.30
12.00	5.65	8.28	6.94	6.95	1.31
16.00	2.37	10.35	5.34	6.02	4.04
28.00	4.03	7.26	6.35	5.88	1.66
35.00	5.42	6.67	5.97	6.02	0.63
47.00	2.46	5.05	3.77	3.76	1.30
51.00	11.91	12.79	8.44	11.05	2.30
60.00	15.92	10.81	16.32	14.35	3.07
64.00	4.24	14.63	6.18	8.35	5.52
76.00	7.78	5.77	6.41	6.65	1.03
83.00	11.97	15.46	10.48	12.64	2.55
95.00	3.10	1.69	6.75	3.85	2.61
99.00	15.74	8.56	21.42	15.24	6.45
100.00	0.58	22.51	21.07	14.72	12.27
108.00	18.19	12.31	10.91	13.80	3.86
112.00	5.71	4.23	1.57	3.83	2.10
124.00	11.24	9.94	9.25	10.15	1.01
131.00	5.47	4.97	8.52	6.32	1.92
143.00	2.70	4.00	*	3.35	0.91

* Sample not collected at this time point

Table 3. 15 Individual pharmacokinetic parameters of phenylbutazone on multiple dosing of 3 mg/kg every 48 hours in Asian elephants.

Study	AUC_{ss} (mcg.h/ml)	C_{min} (mcg/ml)	C_{max} (mcg/ml)	T_{max} (h)	$C_{average}$ (mcg/ml)	%AUCdf	Cl_{ss} (ml/h/kg)	MRT (h)
Peggy	331.16	0.58	18.19	14.00	7.05	15.27	9.06	19.86
Hank	369.70	4.00	22.51	6.00	7.87	31.77	8.11	16.80
Betty	291.13	1.57	21.42	5.00	6.19	35.70	10.30	13.93
Mean	330.66	2.05	20.71	8.33	7.04	27.58	9.16	16.86
SD	39.29	1.76	2.25	4.93	0.84	10.84	1.10	2.97

CONCLUSION

The disposition of phenylbutazone after oral administration of 2, 3, and 4 mg/kg could not be described by any exponential function.

Phenylbutazone mean elimination half-lives in elephants were 29.72 ± 20.76, 23.18 ± 19.95, and 21.61 ± 16.14 (h), clearance/F were 19.72 ± 16.30, 20.85 ± 11.93, and 21.80 ± 15.61 (ml/h/kg), apparent volume of distribution/F were 563.30 ± 294.14, 477.34 ± 186.40, and 467.82 ± 223.33 (ml/kg), and the mean residence times were 42.47 ± 25.63, 31.87 ± 19.48, and 34.01 ± 22.44 (h), for 2, 3, and 4 mg/kg doses given orally to elephants respectively. Following oral administration of 2, 3, and 4 mg/kg, approximate linearity in AUC_{0-n} and C_{max} of phenylbutazone was found in elephants. Although, phenylbutazone plasma profiles in African and Asian elephants are statistically significantly different. Firm conclusion of a species effect on the difference in pharmacokinetic parameters cannot be made due to large variability and small sample size. There was no gender difference seen in African and Asian elephants.

By dosing African elephants 2 mg/kg every 24 hours the $C_{average}$ obtained was 2.89 ± 0.72 mcg/ml. The clearance at steady state is found to be 30.14 ± 6.17 ml/h/kg. And for Asian elephants dosed at 3 mg/kg every 48 hours the $C_{average}$ and clearance at steady state are 7.04 ± 0.84 mcg/ml and 9.16 ± 1.10 ml/h/kg respectively. By dosing the Asian elephants 3 mg/kg every 48 hours the average plasma concentrations achieved were above the minimum therapeutic concentration

of 5 mcg/ml, whereas the African elephants should be dosed at 3mg/kg every 24 hours to achieve the minimum therapeutic concentrations of phenylbutazone.

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Chapter 6. Conclusion

Pharmacokinetics of ibuprofen was determined in African and Asian elephants. Approximate linearity was observed in AUC for ibuprofen single oral doses in the range of 4-6 mg/kg. Although the plasma profiles of African and Asian elephants appear to be different but they are not statistically significantly different. The clearance /F of Asian elephant is slower when compared to the clearance/F of African elephants but the difference is not statistically significantly different. By dosing African elephants 7 mg/kg and Asian elephants 6 mg/kg every 12 hours, the concentrations were in the therapeutic range of 15-30 mcg/ml as seen in humans. Clearance and AUC did not change on multiple dosing showing ibuprofen follows linear kinetics.

Pharmacokinetics of phenylbutazone was determined in African and Asian elephants. Approximate linearity was observed in AUC and Cmax for phenylbutazone single oral doses in the range of 2-4 mg/kg. The clearance/F of Asian elephant is slower when compared to the clearance/F of African elephants and the difference is significant statistically. Although firm conclusions of differences between Asian and African elephants cannot be made due to large variability and small sample size. By dosing African elephants 2 mg/kg every 24 hours the $C_{average}$ obtained was 2.89 ± 0.72 mcg/ml and the clearance at steady state was found to be 30.14 ± 6.17 ml/h/kg. By dosing the Asian elephants at 3 mg/kg

every 48 hours, the $C_{average}$ obtained was 7.04 ± 0.84 mcg/ml and the clearance at steady state was found to be 9.16 ± 1.10 ml/h/kg.

The price of ibuprofen for a 3000 kg elephant dosed at 7 mg/kg every 12 hours per day is approximately $30.00, whereas the price of phenylbutazone for a similar weight elephant dosed at 3 mg/kg every 24 hours per day is approximately $6.0. The use of phenylbutazone is more economical than ibuprofen in elephants.

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APPENDICES

Appendix A

Ibuprofen plasma concentration time profile.
(Hugo, Packy, Smokey and Toby)

Subject	Hugo			Packy	Smokey	Toby
Time (h)	Dose 4 mg/kg	Dose 5 mg/kg	Dose 6 mg/kg	Dose 4 mg/kg	Dose 4 mg/kg	Dose 6 mg/kg
0	0.00	0.00	0.00	0.00	0.00	0.00
0.25	0.00	0.00	0.72	0.00	0.39	0.48
0.5	0.00	0.00	0.57	0.32	0.98	0.37
0.75	0.00	2.66	0.63	0.62	7.71	0.52
1	0.39	3.80	0.66	0.84	15.07	0.67
1.5	5.62	5.39	1.53	1.78	21.79	1.54
2	19.56	9.17	2.27	5.11	16.40	2.23
4	14.93	1.32	24.97	33.97	16.27	5.91
10	4.87	0.05	20.56	20.18	9.81	6.92
12	3.52	*	23.55	18.96	7.36	2.40
24	0.56	*	*	10.25	2.35	0.00
48	0.00	*	*	4.25	0.00	0.00

* Sample not drawn.

Appendix B

Ibuprofen pharmacokinetic parmeters.
(Hugo, Packy, Smokey and Toby)

Subject	Dose	C_{max} (mcg/ml)	T_{max} (h)	AUC (mcg.h/ml)	K_{el} (1/h)	$T_{1/2}$ (h)	MRT (h)	CL/F (ml/h/kg)	Vd_{ss} (ml/kg)
Hugo	4	19.56	2.00	127.37	0.15	4.51	7.70	31.40	241.81
Packy	4	33.97	4.00	673.20	0.04	16.78	25.88	5.94	153.79
Smokey	4	21.79	1.50	225.98	0.10	7.10	11.28	17.70	199.73
Hugo	5	9.17	2.00	17.62	0.59	1.18	2.64	283.84	750.16
Toby	6	6.92	10.00	57.09	-	-	7.04	105.09	739.58
Hugo	6	24.97	4.00	1888.31	0.01	52.96	79.41	3.18	252.31

Appendix C

Phenylbutazone plasma concentration time profile.
(Buffy, Butch, Limba, Hank and Lois)

Subject	Buffy		Butch	Limba	Hank	Lois
Time (h)	Dose 2 mg/kg	Dose 3 mg/kg	Dose 2 mg/kg	Dose 3 mg/kg	Dose 4 mg/kg	Dose 4 mg/kg
0	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.00	0.00	0.00	1.73	0.00	0.00
1	0.00	1.67	0.00	3.84	0.00	0.00
2	0.00	8.58	0.00	6.46	0.00	1.40
4	1.09	1.43	0.00	10.72	4.56	6.08
12	0.00	0.00	0.00	9.98	11.87	3.14
24	0.00	0.00	0.00	5.44	0.59	0.60
48	0.00	0.00	0.00	5.66	1.51	0.00
96	0.00	0.00	0.00	3.67	0.20	0.00
144	0.00	0.00	0.00	1.34	0.00	0.00
192	0.00	0.00	0.00	0.81	0.00	0.00
240	0.00	0.00	0.00	0.37	0.00	0.00
288	0.00	0.00	0.00	0.00	0.00	0.00

Appendix D

Phenylbutazone pharmacokinetic parameters.
(Buffy, Butch, Limba, Hank and Lois)

Study	Dose	C_{max} (mcg/ml)	T_{max} (h)	AUC (mcg.h/ml)	K_{el} (1/h)	$T_{1/2}$ (h)	MRT (h)	CL/F (ml/h/kg)	Vd_{ss} (ml/kg)
Butch	2	0.00	0.00	0.00	-	-	-	-	-
Buffy	2	1.09	4.00	1.09	-	-	4.00	1834.89	7339.57
Buffy	3	8.58	2.00	25.40	0.17	4.01	5.81	118.10	686.72
Limba	3	10.72	4.00	764.40	0.01	47.77	75.37	3.92	295.80
Hank	4	11.87	12.00	274.07	-	-	98.15	14.59	1432.46
Lois	4	6.08	4.00	67.69	0.12	5.91	11.51	59.09	680.02