# PHARMACOKINETICS OF CEFONICID IN LACTATING GOATS AFTER INTRAVENOUS, INTRAMUSCULAR AND SUBCUTANEOUS ADMINISTRATION, AND AFTER A LONG-ACTING FORMULATION FOR SUBCUTANEOUS ADMINISTRATION

**RUNNING HEAD: PHARMACOKINETICS OF CEFONICID IN GOATS**

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**ABSTRACT**

The single-dose disposition kinetics of cefonicid were determined in clinically normal lactating goats (n=6) after intravenous (IV), intramuscular (IM) and subcutaneous (**SC**) administration of a conventional formulation, and after subcutaneous administration of a long-acting formulation (SC-LA). Cefonicid concentrations were determined by high performance liquid chromatography with ultraviolet detection. The concentration-time data were analyzed by non-compartmental pharmacokinetic methods. Steady-state volume of distribution ($V_{ss}$) and clearance (Cl) of cefonicid after IV administration were 0.14 ± 0.03 L/kg and 0.51 ± 0.07 L/h·kg, respectively. Following IM, SC and SC-LA administration, cefonicid achieved maximum plasma concentrations of 14.46 ± 0.82, 11.98 ± 1.92 and 17.17 ± 2.45 mg/L at 0.26 ± 0.13, 0.42 ± 0.13 and 0.83 ± 0.20 hours, respectively. The absolute bioavailabilities after IM, SC and SC-LA routes were 75.34 ± 11.28 %, 71.03 ± 19.14 % and 102.84 ± 15.155 %, respectively. After cefonicid analysis from milk samples, no concentrations were found above LOQ at any sampling time. From these data, cefonicid administered at 20 mg/kg each 12 hours after SC-LA could be effective to treat bacterial infections in lactating animals not affected by mastitis problems.

**Keywords:** Cefonicid, cephalosporins, goats, long-acting formulation, pharmacokinetics.

## 1. INTRODUCTION

Cefonicid is a second-generation cephalosporin developed for using in human medicine. It has a broad spectrum of activity against gram-positive and gram-negative microorganisms as well as some anaerobic bacteria. In humans, cefonicid reaches very high serum levels after intravenous administration and has a prolonged half-life allowing for once-daily dosing (Karki et al, 1993). This fact constitutes a significant advantage in terms of cost-effectiveness compared with other β-lactams. As with all cephalosporins, cefonicid is considered a bactericidal antimicrobial because it inhibits bacterial cell wall synthesis, because of this, its pharmacokinetic/pharmacodynamic (PK /PD) parameter most associated with efficacy is the time above a threshold concentration, typically the MIC (Sadar et al., 2015). The Guidelines for the prudent use of antimicrobials in veterinary medicine (Commission to the European Parliament & the Council, 2015/C 299/04, 2015) indicate the preference of narrow-spectrum drugs and the priority of older antibiotics versus new ones. This concern extends to broad-spectrum cephalosporins. Third and fourth-generation cephalosporins are considered *critically important* for human health, therefore, their therapeutic use is limited in veterinary medicine where they are classified as *category 2* (restricted use to second choice therapy or last resort) leaving as an alternative first and second generation of cephalosporins for veterinary use.

The development of long-acting formulations for parenteral administration is an issue that has received a lot of attention in recent years (Bari, 2010). In veterinary medicine, these formulations achieve longer release times, high bioavailabilities and a reduction of the total dose than conventional formulations, which means a reduction in management and in veterinary costs. Poloxamer 407 (P407) is a polyoxyethylene polymer conglomerate that has low toxicity and excellent compatibility with other chemical compounds. In addition, it has a high capacity for solubilization of different medicines. Its consistency is modified

77 with temperature so that at 37° C it presents a gel consistency while at 4° C its consistency
78 is liquid (Zhang et al, 2002). The release from this P407 gel has been studied in active
79 biological proteins such as urease or interleukin-2 (Johnston et al, 1992), and in antibiotics
80 such as ceftiofur (Zhang et al., 2002). Cyclodextrins (CD) are cyclic oligomers of glucose
81 that can form water-soluble inclusion complexes with small molecules and portions of
82 large compounds. These biocompatible, cyclic oligosaccharides do not elicit immune
83 responses and have low toxicities in animals and humans. Cyclodextrins are used in
84 pharmaceutical applications for numerous purposes, including improving the
85 bioavailability of drug (Davis & Brewster, 2004). Therefore, systems containing both
86 components (P407 and CD) can be very interesting since they combine thermogelification
87 properties and the ability of drug carriers of both substances. Several studies using a
88 prolonged formulation of doxycycline with poloxamer and β-CD have been published in
89 rats, goats and calves. The results demonstrated excellent bioavailabilities and longer half-
90 lives than conventional formulations (Vargas-Estrada et al., 2008, 2011; Vargas et al.,
91 2008)
92 Infectious diseases in livestock is a relevant problem not only due to economic losses but
93 also for hygiene and safety aspects of products intended for human consumption.
94 However, treatment of bacterial infections in lactating animals not affected by mastitis
95 problems should be restricted to antimicrobials with scant penetration to milk in order to
96 avoid long withdrawal times. Distribution of cephalosporins to the mammary gland and the
97 consequent access to milk is limited. This fact has been demonstrated for cefquinome
98 (Littero, 2013) and ceftiofur (Doré et al., 2011) in studies carried out in goats where it was
99 shown a low elimination through the milk. Thus, the objective of this work was to study
100 the pharmacokinetic disposition of cefonicid in lactating goats after intravenous (IV),
101 intramuscular (IM) and subcutaneous (SC) administration of a conventional formulation

and after subcutaneous administration of a long-acting formulation (SC-LA).

## 2. MATERIALS AND METHODS

### 2.1. Animals

Six clinically healthy Murciano-Granadina female lactating goats weighing 37 to 42 kg and aged from 2 to 4 years were included in the study. All goats were obtained from the Veterinary Farm of the University of Murcia (Spain). For each treatment period of the crossover, goats were observed daily for general health, and clinical observations were made before injection and at 2, 10, and 24 hours post injection. Alfalfa hay and water were provided *ad libitum* together with a pelleted concentrate free of any drug. No one of them were treated with antibiotics for at least 30 days preceding the study. The experimental protocol was approved by the Bioethical Committee of the University of Murcia (Spain).

### 2.2. Drug and gel preparation

An aqueous solution (15%) of cefonicid sodium was prepared from pure substance (Santa Cruz Biothecnology, Dallas, USA) and sterilized by filtration in our laboratory.

Gel formulation was prepared on a weight basis using the cold method (Schmolka, 1972). Concentrations of P407, β-CD and cefonicid sodium reported here are expressed as weight percentage (% wt/wt). For each animal the gel was made with 31% of P407 to which 15% of β-CD was slowly added at 4°C, incorporating cefonicid sodium sufficient to yield a 29% concentration who was dissolved in the cold solution. The solution was kept refrigerated at 5ºC until a clear solution was formed.

### 2.3. Experimental design

A crossover design (2 × 2 × 1 x 1) was used in four phases. In each phase, each animal received a single IV, IM and SC injection of an aqueous solution of cefonicid at a dose of

10 mg/kg or a SC-LA administration of cefonicid at 20 mg/kg with at least a 15-day washout period.

For IV administration, the solution was injected into the left jugular vein, and blood samples (4 mL) were collected from the contralateral jugular vein into heparinized tubes. Intramuscular injection was given into the semimembranosus muscle and subcutaneous (SC and SC-LA) injections were administered under the skin of the back at a single location in the thoraco-lumbar region lateral of the midline. Blood samples were collected at 0 (pre-treatment), 0.083, 0.167, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 10, 12, 24, 36 and 48 hours postdosing. Samples were centrifuged at 1,500 × g for 15 min and the plasma taken and stored at –45°C until assayed. Milk samples for analysis were collected from homogenized milking yields collected immediately before dosing on the day of treatment administration (time 0) and at 1, 2, 4, 6, 8, 10, 12, 24, 36 and 48 hours post administration. Milk samples were collected after complete evacuation of the udder by manual stripping of each gland as completely as possible, to avoid a dilution effect during the normal milking routine (once a day). For each sample, 2 aliquots (5 mL) of milk were stored at –45°C until analysis.

Damage at administration point was assessed by observation of pain signs, control of dermal temperature, inflammatory reactions, indurations, etc.

### 2.3. Analytical method

Plasma and milk concentrations of cefonicid were measured using a modified HPLC method whit ultraviolet detector as previously reported (Phelps et al., 1986). The HPLC system was equipped with a binary pump (Waters 1525), ultraviolet detector (Waters 2489), oven heater (Waters 5CH) and autoinjector (Waters 2707). The above-mentioned

system was connected to a computer with a Software Waters Breeze 2 (Waters, Massachusetts, USA).

Cefonicid for HPLC (Carbosynth, Berkshire, UK) was used for quality control and cephalothin (Sigma-Aldrich, St. Louis, USA) was used as internal standard.

For extraction of cefonicid from plasma and milk, after addition of 500 μL of acetonitrile to 500 μL of plasma or skimmed milk (by centrifugation for 10 minutes at 15000 g and 4 ºC), 10 μL of the internal standard (cephalothin 1000000 μg/L) was added. Plasma and milk proteins were precipitated by shaking in an ultrasonic bath followed by centrifugation for 10 min at 1500 g and 750 μL of supernatant were collected and dried out using a SpeedVac Eppendorf Concentrator 5301 (Eppendorf, Hamburg, Germany). After drying, the residue was reconstituted with 100 μL of water and transferred to HPLC autosampler vials.

The HPLC separation was performed with reverse-phase Kromasil C18 column (4.6 mm × 250 mm, 5 μm particle size; Tecnokroma, Barcelona, Spain) at 23 ºC with an injection volume of 50 μL. The mobile phase was composed of a 65% phase A (aqueous solution with a 0,3 % of tetrabutylamoniohydrogen-sulphate and 0,1% phosphoric acid) and a 35 % phase B (acetonitrile). The detection was performed using a UV detector set at 267 nm and the flow rate was 1 ml/min. Cefonicid and cephalothin eluted at approximately 12 and 9.8 minutes, respectively.

**2.4. Method Validation**

This method was validated before the start of sample analysis. The selectivity of the method was demonstrated because no interfering peaks from endogenous compounds in the blank goat plasma or milk samples were observed with the same retention times as cefonicid or cephalothin in the chromatograms of blank samples.

Calibration standards and quality control samples were prepared from a pool of blank goat plasma or milk spiked with 8 concentrations of cefonicid between 750 and 25000 µg/L. Plasma and milk aliquots were stored at −45°C until assay. Aliquots of quality control samples were extracted as above and 50 µL of each was injected into the chromatographic system. Standard curves were obtained by unweighted linear regression of cefonicid and cephalothin peak areas versus known concentrations. Each point was established from an average of 5 determinations. The percentage recovery was determined by comparing the peak areas of plasma and milk blank samples spiked with 1000, 4000 and 25000 µg/L cefonicid and treated as any sample, with the peak areas of the same standards prepared in mobile phase. The assay precision was assessed by expressing the SD of repeated measurements as a percentage of the mean value (CV). Intra-day precision was estimated from six replicates of three quality controls (plasma and milk). Inter-day precision was estimated from the analysis of quality controls (plasma and milk) on three separate days. The limit of quantification (LOQ) of cefonicid in plasma and milk was the lowest concentration on the calibration curves for which the CV was <20%. The limit of detection (LOD) in goat plasma and milk was defined as the lowest concentration with a signal-to-noise ratio >3.

**2.5. Pharmacokinetic analysis**

The plasma pharmacokinetic data were derived for each animal from the plasma drug concentrations of that animal. Pharmacokinetic parameters were estimated using the WinNonlinTM software package (WinNonlin Professional version 5.1.; Pharsight Corporation, Mountain View, CA, USA). WinNonlinTM model 200 was used for extravascular administrations and model 201 for intravenous administration. Noncompartmental parameters calculated were: elimination rate constant ($\lambda_z$), the elimination half-life associated with the terminal slope ($\lambda_z$) of a semilogarithmic

concentration–time curve ($t_{½\lambda z}$), the area under the concentration-time curve using the linear trapezoidal rule with extrapolation to infinity time (AUC), mean residence time (MRT), mean absorption times (MAT), systemic clearance (Cl), apparent volume of distribution at steady state ($V_{ss}$) and apparent volume of distribution calculated by the area method ($V_z$). Peak plasma concentrations ($C_{max}$), and times to reach peak concentration ($T_{max}$) were estimated directly from the experimental data. Bioavailability (F) was calculated by the method of corresponding areas:

$$F\ (\%) = (AUC_{IM,\ SC,\ SC\text{-}LA} \cdot Dose_{IV}) \times 100 / (AUC_{IV} \cdot Dose_{IM,\ SC,\ SC\text{-}LA})$$

### 2.6. Statistical analysis

Descriptive statistical parameters as mean and standard deviation (SD) were calculated. Harmonic means were calculated for half-lives of disposition. The Wilcoxon rank sum test and Student's *t*-test were used to test parameters for significant differences ($p < 0.05$) between IV, IM, SC and SC-LA administration.

## 3. RESULTS

### 3.1 Animals

Clinical examination of all goats after each phase of the trial did not reveal any abnormalities. Local or systemic adverse reactions were not observed neither during nor after IV, IM, SC and SC-LA administration, respectively

### 3.2 Analytical method

Correlations coefficients (r) were >0.99 for calibration curves (plasma and milk). The mean percentage recovery of cefonicid from plasma and milk was 89.9 and 85.4 %, respectively. The CV for the plasma and milk intra-day precision were <2.7% and <5.5%, respectively. The CV for the plasma and milk inter-day precision were <13.5%

222 and <14.1%, respectively. The LOQ and LOD was 750 μg/L and 500 μg/L,
223 respectively, for plasma and milk.

224 **3.3 Pharmacokinetics**

225 The mean (±SD) plasma concentrations of cefonicid at the times of sample collection
226 after IV, IM, SC and SC-LA administration are plotted in Figure 1. The mean ± SD
227 pharmacokinetics parameters based on non-compartmental models for each route of
228 administration are presented in Table 1. There were significant differences ($p<0,05$)
229 between SC-LA and the other administration in every pharmacokinetic parameters,
230 except for AUC between IV and SC-LA administrations. Cefonicid was detected in
231 plasma up to 1,5; 2; 2 and 6 hours after IV, IM, SC and SC-LA administration,
232 respectively. After cefonicid analysis in milk samples, no concentrations were found
233 above LOQ at any sampling time.

234

235 **4. DISCUSSION**

236 Cephalosporins form a large group of β-lactam antibiotics which are used extensively in
237 human medicine and to a lesser extent in domestic animals. Cefonicid is a second
238 generation of cephalosporin. Third and fourth-generation cephalosporins are considered
239 *critically important* for human health, therefore, their therapeutic use is limited in animal
240 medicine, leaving as an alternative first and second generation of cephalosporins for
241 veterinary use. The pharmacokinetics of cefonicid has not been studied in animals,
242 however, there are data available in humans (Barriere et al 1982; Fourtillan et al., 1985;
243 Furlanut et al, 1989; Pitkin et al., 1981), which showed high blood levels, a high fraction
244 of the drug bound to plasma proteins and a long half-life.

245 In this study, the terminal half-life ($t_{1/2\lambda z}$) of cefonicid after IV dosing was 0.21 hours, this
246 value was shorter than those reported for several cephalosporins in goats (Ambros et al.,

2011; Attia et al., 2011). Plasma concentration–time data after IV, IM and SC showed that the drug was rapidly eliminated, with short elimination half-lives and a high clearance value of 0.51 L/h·kg. In humans, $t_{1/2\lambda z}$ values after IV ($t_{1/2\lambda z}$ = 3.5 hours, Pitkin et al., 1981) and IM ($t_{1/2\lambda z}$ = 5,66 hours, Fourtillan et al., 1985) administration were higher than those reported in this study. It can be occurred because cefonicid has a high binding to plasma proteins in humans (90-98%, Benson et al., 1993), which decreases the plasmatic clearance and prolongs the elimination half-life. Our data suggest a lower degree of binding to plasma proteins in goats, as occurred with other cephalosporins in these species.

However, elimination half-life after SC-LA dosing were 1.22 hours. The drug was eliminated from plasma at a significantly slower rate after SC-LA treatment than after other treatments, suggesting the presence of a "flip-flop" effect with this formulation, because MAT was greater than $MRT_{IV}$ (Toutain & Bousquet.Melou, 2004). In this model, the last phase of the curve is determined by the absorption rate constant and not by the apparent elimination constant, because the rate of absorption is a limiting factor for the elimination process.

The volume of distribution (0.17 and 0.14 L/kg calculated by the area method ($V_z$) and at steady-state (Vss), respectively, suggests limited penetration through biological membranes. Similar values were described for cephalexin ($V_{ss}$ = 0,13 L/kg, Ambros et al., 2011) and cefepime ($V_{ss}$ = 0,14 L/kg, Prawez et al., 2010) in goats. These low volumes of distribution could explain the limited distribution of cephalosporins to the mammary gland and the consequent scant access to milk.

Following IM and SC administration of cefonicid, the drug was rapidly absorbed with $t_{max}$ of 0.26 and 0.42 hours, respectively, reached $C_{max}$ values of 14.46 and 11.98 mg/L, respectively. Short values of $t_{max}$ have been also obtained after extravascular administration of cephalosporins to goats as cefuroxime ($t_{max\text{-}IM}$ = 0.52 h, El-Sooud et al., 2000),

cefoperazone ($t_{max-IM}$ = 0.58 h, Attia et al., 2015) and cefotaxime ($t_{max-SC}$ = 0.67 h, Atef et al., 1990). These values indicate rapid absorption of cephalosporins after extravascular administrations in these species. The absorption process after SC-LA administration was slower with a $t_{max}$ of 0.83 hours corroborated by the smaller absorption rate constant ($k_a$) and slower absorption half-life.

Cefonicid was well absorbed following IM and SC administration, with absolute bioavailabilities (F) of 75.34 and 71.03%, respectively. Similarly, high values have been obtained for other cephalosporins in goats (Atef et al., 1990; Attia et al., 2015; El-Sooud et al., 2000). After SC-LA treatment, cefonicid showed a higher bioavailability (F = 102.84%) than after the other extravascular administrations and other long-acting formulation in goats (Fernández-Varón et al., 2016). The long-acting formulation with Poloxamer 407 and β-CD improved the bioavailability of cefonicid. Importance of development of sustained-release formulations in veterinary medicine and especially in food animal species has been increasing in last years. The advantages of long-acting formulations in lactating goats include less quantity of drug used (decreasing collateral effects and accumulation in long-term treatments), increased treatment efficacy (less fluctuations of the stationary concentrations and much longer release times) and a reduction in handling (stress of the animals and veterinary costs are decreased). Disadvantage of these formulations could include increase of withdrawal times in meat and milk of livestock, longer time to achieve peak concentrations, pain at the injection site specially if administration is intramuscularly, within others.

After cefonicid analysis from milk samples, no concentrations were found above LOQ at any sampling time. Below this limit, concentrations with antimicrobial activity could be reached at mammary gland, but a more sensitive method of determination is needed. This result is in agreement with other studies in goats (Doré et al., 2011; Fernández-Varón et al.,

297 2016). As a result of its acidic nature, cefonicid sodium in the blood stream (pH 7.4) would
298 act as a weak acid with and insufficient lipid-soluble properties at this pH to penetrate
299 milk, which can also explain the limited plasma volume of distribution obtained.

300 The determination of clinical optimal dosage schedules of an antimicrobial drug depends
301 on the relationship between the pharmacokinetic and pharmacodynamic properties. β-
302 lactam antibiotics, such as cephalosporins, are bactericidal but their action may be slower
303 than other bactericidal drugs, and generally a post-antibiotic effect (PAE) is not observed.
304 Therefore, the concentration should be kept above the MIC as long as possible during the
305 dosing interval (T > MIC) for the optimal bactericidal effect (Papich, 2014). Gram-positive
306 organisms are more susceptible to β-lactams than are gram-negative bacteria and the MICs
307 are lower for gram-positive bacteria. The beta-lactam antibiotics also exhibit a post-
308 antibiotic effect (PAE) against *Staphylococcus* spp. allowing longer dose intervals for
309 infections caused by *Staphylococcus* spp. as compared to gram-negative bacteria. Previous
310 reports have determined that the maximal bactericidal efficacy for cephalosporins is
311 approached when plasma concentrations are greater than the MIC of the pathogen for 60–
312 70% of the dosing interval, whereas a bacteriostatic effect is observed when T > MIC is
313 30–40% of the dosing interval (Craig, 1998; Drusano, 2004). So, if we take into account
314 this surrogate marker and cefonicid concentrations, for microorganisms with MIC $\leq$ 0.5
315 mg/L, cefonicid would be administered each 4 hours by IM or SC route at a dose of 10
316 mg/kg and each 12 hours after SC-LA administration at dose of 20 mg/kg to reach a
317 bactericidal effect. After IM or SC administration, the low T > MIC means no practical
318 application in goats, as drug administration would be very frequent for clinical success;
319 however, a SC-LA administration could provide longer T > MICs and can get a reduction
320 in handling.

321 It is concluded, that in view of general adverse reactions were not observed in any goats,

and the favourable pharmacokinetic properties such as high bioavailability and scant milk penetration, cefonicid administered at 20 mg/kg after SC-LA could be effective to treat bacterial infections in lactating animals not affected by mastitis problems. However, further studies are needed to establish a multiple dosage and clinical efficacy against specific pathogens.

## CONFLICT OF INTEREST STATEMENT

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## AUTHOR CONTRIBUTION STATEMENT

EB, PM and EE carried out the pharmacokinetic study. EB, VH and JSG contributed to sample preparation, cefonicid quantification and pharmacokinetic analysis. PM and EE took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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462 Table 1. Pharmacokinetic parameters (mean ± SD) of cefonicid in lactating goats after
463 intravenous, intramuscular and subcutaneous administration at a single dose of 10
464 mg/kg of a conventional formulation and after subcutaneous administration of a long-
465 acting formulation at a single dose of 20 mg/kg (n=6).

| **Parameters** | **Units** | **IV** | **IM** | **SC** | **SC-LA** |
|---|---|---|---|---|---|
| $\lambda_z$ | $h^{-1}$ | 3,09 ± 0,33 | 1,55 ± 0,17 [a] | 1,30 ± 0,35 [a] | 0,58 ± 0,08 [a,b,c] |
| $t_{½\lambda z}$ * | h | 0,22 | 0,45 [a] | 0,53 [a] | 1,20 [a,b,c] |
| $V_z$ | L/kg | 0,17 ± 0,03 | - | - | - |
| $V_{ss}$ | L/kg | 0,14 ± 0,03 | - | - | - |
| AUC | mg·h/L | 19,11 ± 2,58 | 14,36 ± 2,57 [a] | 13,56 ± 4,06 [a] | 19.66 ± 3.91 ϕ [b,c] |
| MRT | h | 0,27 ± 0,05 | 0,80 ± 0,12 [a] | 0,98 ± 0,23 [a,b] | 1,98 ± 0,18 [a,b,c] |
| Cl | L/h·kg | 0,51 ± 0,07 | - | - | - |
| MAT | h | - | 0,53 ± 0,09 | 0,71 ± 0,18 [b] | 1,72 ± 0,20 [b,c] |
| $C_{max}$ | mg/L | - | 14,46 ± 0,82 | 11,98 ± 1,92 [b] | 8.58 ± 1.22 ϕ [b,c] |
| $t_{max}$ | h | - | 0,26 ± 0,13 | 0,42 ± 0,13 [b] | 0,83 ± 0,20 [b,c] |
| F | % | - | 75,34 ± 11,28 | 71,03 ± 19,14 | 102,84 ± 15,15 [b,c] |

466 * Harmonic mean
467 ϕ Normalized by dose
468 [a] Significantly different from IV ($p < 0.05$).
469 [b] Significantly different from IM ($p< 0.05$).
470 [c] Significantly different from SC ($p< 0.05$).
471
472 $\lambda_z$: elimintion rate constant; $t_{½\lambda z}$: the elimination half-life associated with the terminal
473 slope ($\lambda_z$) of a semilogarithmic concentration–time curve (harmonic mean); $V_z$:
474 apparent volume of distribution calculated by the area method; $V_{ss}$: the apparent volume
475 of distribution at steady state; AUC: the area under the plasma concentration–time curve
476 from zero to infinity; MRT: mean residence time; Cl: the total body clearance of drug
477 from plasma; MAT: Mean absorption time; $C_{max}$: the peak or maximum plasma
478 concentration following extravascular administration; $t_{max}$: the time to reach peak or
479 maximum plasma concentration following extravascular administrations; F: the fraction
480 of the administered dose systemically available (bioavailability).
481

**Figure legend**

Figure 1. Semilogarithmic plot of cefonicid plasma concentrations (mean ± SD) following a single intravenous, intramuscular and subcutaneous administration at a dose of 10 mg/kg, and after subcutaneous administration of a long-acting formulation at a dose of 20 mg/kg ($n$=6).