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# Nitrofurantoin plasma- and urine exposure in eight healthy beagle dogs following standard nitrofurantoin dosing regimen

Carl Ekstrand [a,*], Peter Michanek [a], Matilda Salomonsson [b], Cecilia Tegner [c], Lena Pelander [d]

[a] Swedish University of Agricultural Sciences, Department of Biomedical Sciences and Veterinary Public Health, Div. of Pharmacology and Toxicology, Uppsala, Sweden
[b] National Veterinary Institute, Department of Chemistry, Environment and Feed Hygiene, Uppsala, Sweden
[c] Swedish University of Agricultural Sciences, University Animal Hospital (UDS), Uppsala, Sweden
[d] Swedish University of Agricultural Sciences, Department of Clinical Sciences, Div. of Companion Animal Internal Medicine, Uppsala, Sweden

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ABSTRACT

Bacterial cystitis is common in dogs and is usually treated with antibiotics. Nitrofurantoin is used for treatment of bacterial cystitis in humans and might provide a feasible treatment option in dogs. The aim of this study was to investigate the nitrofurantoin plasma concentration-time course and potential adverse effects in dogs. Nitrofurantoin (4.4–5.0 mg/kg) was administered orally to eight healthy beagles every 8 h for five days before repeated plasma and urine samples were collected. An additional four beagles served as untreated controls. The nitrofurantoin plasma and urine concentrations were measured using ultra high precision liquid chromatography coupled to tandem mass-spectrometry and further analysed using a non-compartmental pharmacokinetic model. In plasma, the median $C_{max}$ was 2.1 μg/mL, $t_{max}$ was 2 h, the terminal rate constant was 0.9 per h and the terminal half-life was 0.8 h. In urine, median $C_{max}$ was 56 μg/mL, $t_{max}$ was 1 h and the terminal half-life was 4.3 h. No adverse effects were observed clinically or in haematology or biochemistry. The data presented in this study combined with *in vitro* sensitivity data from common urine pathogens and the lack of observed adverse effects suggest that nitrofurantoin in a standard dosing regimen could be effective in sporadic bacterial cystitis treatment in dogs. Further clinical studies are highly warranted to verify the effectiveness in clinical cases.

## 1. Introduction

Bacterial cystitis commonly occurs in dogs. Clinical signs of bacterial cystitis include pollakiuria, haematuria and dysuria and episodes are classified as either sporadic (sporadic bacterial cystitis, SBC) or recurrent (Weese et al., 2019). The most common causative agents involved in bacterial infection of the lower urinary tract are *Escherichia spp., Proteus spp., Staphylococcus spp.*, and *Enterococcus spp.* (Hall et al., 2013; Hernando et al., 2021; Roberts et al., 2019). Antimicrobial drug (AMD) therapy is often used in cases of SBC (Murphy et al., 2012; Weese et al., 2019; Weese et al., 2021). Due to the variety of infecting bacteria, extended spectrum penicillins *e.g.* amoxicillin (AMX) with or without clavulanic acid (CA) or potentiated sulphonamides are used for empiric treatment, pending bacterial identification and results of antimicrobial susceptibility testing (Rantala et al., 2004; Weese et al., 2021).

Of *Escherichia coli* (*E. coli*) isolates from canine urine, 16–85% were classified as resistant to amoxicillin and similar antimicrobial drugs (Anonymous, 2021; Chang et al., 2015; KuKanich et al., 2020; Moyaert et al., 2017; Roberts et al., 2019; Yousefi and Torkan, 2017; Yu et al., 2020). If AMX was combined with CA, 2–50% of *E. coli* isolates were resistant (KuKanich et al., 2020, Moyaert et al., 2017, Roberts et al., 2019, Yousefi and Torkan, 2017, Yu et al., 2020). In comparison, 7–65% of *E. coli* were resistant to potentiated sulphonamides (Anonymous, 2021; Boothe et al., 2012; Chang et al., 2015; Roberts et al., 2019; Yousefi and Torkan, 2017). Nitrofurantoin (NFN) is an AMD used for the treatment of SBC in humans (Huttner et al., 2015), and it does not appear to promote antimicrobial resistance (Chew et al., 2019; Huttner et al., 2015). One argument against the use of NFN in dogs is that its short half-life demand frequent dosing, which could impede compliance and successful therapy (Maaland and Guardabassi, 2011). In addition, concern regarding safety of its use in dogs has been raised. There are sparse peer-reviewed data supporting the use of NFN in dogs with SBC without comorbidities. The NFN pharmacokinetics and excretion into urine were studied using only four dogs in two different studies >25 years ago (Conklin et al., 1969; Niazi et al., 1983). A more recent study including 14 dogs reported tolerable gastrointestinal adverse effects

* Corresponding author.
*E-mail address:* carl.ekstrand@slu.se (C. Ekstrand).

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(diarrhoea) of NFN in one dog (Leuin et al., 2021). The limited available information regarding the use of NFN in dogs with SBC motivates further studies. The aim of this study was to investigate plasma and urine exposure of NFN after administration of standard doses *per os* to dogs. Another aim was to investigate the potential adverse effects of using NFN in dogs.

## 2. Material and methods

### 2.1. Animals

Twelve healthy beagle dogs were included in this study. Eight dogs (four males and four females) were included in the treatment group (Group N). Four dogs (two males and two females) were included as untreated controls (Group C). In Group N, the median (range) age was 5 years (3–10 years) and weight was 12.9 kg (11.8–16.0 kg). All dogs were intact although two males were treated with deslorelin acetate implants (Suprelorin, Virbac Danmark A/S, Kolding, Denmark). In Group C, median (range) age was 2.5 years (2–3) and weight 14.3 kg (12.3–15.7). All dogs were free from systemic disease and were part of a university owned group used for teaching and research. The dogs were fed and housed (in groups of two to six dogs for social and welfare reasons) in accordance with their normal routine and water was available *ad libitum* throughout the study.

### 2.2. Experimental design

Microcrystalline NFN (Furadantin, Meda AB, Solna, Sweden) tablets (4.4–5.0 mg/kg) were administered every 8 h for five days (15 doses) *per os* in food (Table 1). Blood for NFN analysis was collected from the cephalic vein into EDTA-coated tubes before the study period (denoted PRE), before the last administered dose (denoted 0 h) and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h after the last administered dose. At the time points 0–12 h, blood samples were collected by means of an intra venous catheter. The remaining blood samples were collected by direct venepuncture. Tubes were centrifuged at 1500*g* and plasma was frozen at −70 °C pending analysis. Urine for NFN analysis was collected by spontaneous urination before the first dose (PRE), before the last administered dose (0 h) and at 2.25, 5, 8.25, 12.25 and 24 h after the last administered dose. Urine was frozen immediately after collection at −20 °C pending analysis.

Blood and urine were collected from all dogs for haematology, serum biochemistry and urine analysis at times PRE and 24 h.

Ethical permission to perform the study was provided by the Animal Ethics Committee, Uppsala, Sweden (5.8.18–15,533/2018).

**Table 1**
Characteristics of the studied dogs and administered doses.

| Dog | Sex | Age (years) | Weight (kg) | Dose (mg) |
|---|---|---|---|---|
| Treatment group | | | | |
| 1 | Male | 10 | 13.6 | 65 |
| 2 | Male | 7 | 12.7 | 60 |
| 3 | Female | 3 | 13.7 | 65 |
| 4 | Female | 3 | 12.3 | 60 |
| 5 | Female | 5 | 13.1 | 60 |
| 6 | Female | 3 | 12.0 | 60 |
| 7 | Male | 5 | 16.0 | 75 |
| 8 | Male | 5 | 15.9 | 75 |
| Control group | | | | |
| 1 | Male | 2 | 15.7 | – |
| 2 | Male | 2 | 14.9 | – |
| 3 | Female | 3 | 12.3 | – |
| 4 | Female | 3 | 11.8 | – |

### 2.3. Quantification of nitrofurantoin in plasma and urine

Chemicals: Reference material of NFN and internal standard NFN-13C3 were purchased from Toronto Research Chemicals (North York, ON, Canada). Formic acid and methanol were obtained from Merck Millipore (Merck KGaA, Dramstadt, Germany). The water was purified with a MilliQ purification system from Merck Millipore (Merck KGaA, Dramstadt, Germany). All other chemicals used were of analytical grade or better.

Instrumentation: NFN was quantitatively analysed with an Ultra High Precision Liquid Chromatography Tandem Mass-spectrometry (UHPLC-MS/MS) system composed of an Acquity UPLC coupled to a Xevo TQ-Sμ tandem quadrupole mass spectrometer (Waters Corporation, MA, USA) with an electrospray interface. The separation was performed with a chromatographic system consisting of a C18 guard column (Waters Corp.) and an Acquity BEH C18 column (2.1 mm × 50 mm, 1.7 μm) (Waters Corp.), both at 65 °C. The mobile phase consisted of A) 0.1% formic acid in water and B) methanol, and it was delivered as a gradient. The gradient started at 5% B to 1.00 min, then continued with a linear increase over time to 90% B at 5.00 min, and 90% B at 5.50 min. At 5.55 min the gradient was switched to 5% B and held constant for 1.5 min for equilibration. The flow rate was 0.400 mL/min and the injection volume was 10 μL. The ionization was positive electrospray, and the instrument settings were capillary voltage of 3.2 kV, cone voltage 28 V, source temperature 150 °C, desolvation temperature 500 °C and desolvation gas flow 1000 L/h. NFN and NFN-13C3 were detected as $[M + H]+$. The mass spectrometric analysis mode was selected reaction monitoring (SRM). The SRM transitions used for the quantitative analysis were $m/z$ 239 > 95 (collision energy 12 eV) for NFN and m/z 242 > 169 (collision energy 14 eV) for NFN-13C3. The dwell time was 0.025 s and the results were evaluated using the software TargetLynx (Waters Corp.).

Sample preparation: The urine samples were centrifuged at 2862*g* for 5 min. The sample preparation of urine (study samples, calibrators, or quality control samples) was performed in a 96-well plate as follows: to 100 μL of canine urine, 880 μL of MilliQ-water and 20 μL internal standard solution were added. The samples were then vortexed for 10 min and 10 μL were injected into the UHPLC-MS/MS system. The sample preparation of plasma (study samples, calibrators or quality control samples) was performed in a 96-well plate as follows: to 100 μL of plasma, 100 μL MilliQ-water, 20 μL internal standard solution, and 280 μL of methanol was added for protein precipitation. The samples were then vortexed for 10 min and centrifuged at 2862*g* for 10 min. The supernatants (100 μL) were transferred to a new 96-well plate, diluted with 400 μL MilliQ-water and vortexed for 10 min before 10 μL were injected into the UHPLC-MS/MS system.

Quantification and method validation: For the quantitative analysis, calibration curves with eight calibrators (0.25–100 μg/mL in canine urine and 0.05–7.0 μg/mL in canine plasma) were used, and the calibration curves were constructed with the peak area ratio (NFN/NFN-13C3) as a function of the NFN concentration. The calibration functions were calculated by linear regression with a weighting factor of 1/x.

Four concentration levels of quality control samples were prepared in blank canine urine and blank canine plasma. The concentrations in urine were at 0.25 μg/mL (limit of quantification, LOQ), 0.50 μg/mL (quality control sample low, QCL), 18 μg/mL (quality control sample medium, QCM) and 75 μg/mL (quality control sample high, QCH)), and in plasma at 0.05 μg/mL (LOQ), 0.10 μg/mL (QCL), 1.2 μg/mL (QCM) and 5.0 μg/mL (QCH). Six replicates at each concentration level and two replicates of each calibrator were analysed, and the analysis was repeated on three different days.

### 2.4. Clinical pathology

Haemoglobin, packed cell volume, complete white blood cell count and leukocyte differential count were analysed in whole blood from

EDTA-coated tubes by an automated haematology analyser (ADVIA 2120, Siemens Healthcare GmbH, Ashburn, Germany) with canine settings.

The biochemistry analytes alanine transaminase, alkaline phosphatase, albumin, total protein, creatinine, urea, sodium, potassium and chloride were analysed in serum using an automated chemistry analyser (Architect c4000, Abbott Diagnostics, Lake Forest, IL, US) with reagents from Abbott Diagnostics.

Urine specific gravity was analysed with a handheld optical refractometer (Atago 2791 Master-URC/N, Tokyo, Japan). Urine was analysed with urine dipstick (Multistix 7, Siemens, Erlangen, Germany) for content of protein, haemoglobin/erythrocytes, glucose and ketones. Urine sediment was manually evaluated by microscopy. Urine creatinine and protein were analysed using an automated chemistry analyser (Architect c4000, Abbott Diagnostics, Lake Forest, IL, US) with reagents from Abbott Diagnostics.

### 2.5. Analyses of nitrofurantoin concentration-time course

A non-compartment analysis (NCA) based on the linear trapezoidal rule for increasing data and logarithmic trapezoidal rule for decreasing data was performed using the commercially available software PKanalix-2020R1 (Lixoft, Antony, France). The parameters derived from the NCA were the observed maximum concentration in plasma and urine ($C_{max}$), the time for $C_{max}$ ($t_{max}$), the elimination rate constant in plasma ($k$), the terminal half-life in plasma and urine ($t_{1/2z}$) and the area under the concentration-time curve ($AUC_{8h}$) for 8 h following the last administration.

### 2.6. Statistical analyses

The difference between PRE and 24 h results from the biochemistry and haematology analyses were calculated for the respective variable. The difference for each variable was then compared between Group N and Group C using the Mann–Whitney $U$ test.

## 3. Results

### 3.1. Validation of the analytical method

The linearity was evaluated with three days of calibration curves, and the $R^2$ value obtained were $\geq 0.99$ for all days. Intra- and inter-day precision expressed as relative standard deviation was for NFN in urine in the range of 1.7–14.6% and in plasma 2.4–12.6%. The accuracy in urine was in the range of 83–111% for LOQ and 93–106% for QCL/QCM/QCH, and in plasma in the range of 97–107% for LOQ/QCL/QCM/QCH. The LOQ was determined to be 0.25 µg/mL in urine and 0.05 µg/mL in plasma.

### 3.2. Nitrofurantoin plasma concentration-time course

All PRE samples were below LOQ. The median and range of plasma NFN concentrations over time before and following the last administered dose are shown in Fig. 1. Plasma concentrations after the last administered dose were above LOQ for 4 h in all eight dogs, for 6 h in five dogs, for 8 h in six dogs, and for 12 h in one dog. The median (range) $C_{max}$ was 2.1 (1.3–2.4) µg/mL, $t_{max}$ was 2 (1–3) h, the terminal rate constant ($k$) was 0.9 (0.4–1.4) per h, the terminal half-life ($t_{1/2z}$) was 0.8 (0.5–2) h, the ratio between total clearance and bioavailability ($Cl/F$) was 12,300 (10420–17,170) mL/h, the ratio between the volume of distribution during the terminal phase and bioavailability ($V/F$) was 14,870 (9070–30,980) mL and the $AUC_{8h}$ following the last administration was 5.3 (3.7–6.0) h•µg/mL. Individual parameter estimates are presented in Table 2. Blood sampling times deviated up to 10 min from protocol times. Exact sampling times are presented in the supplementary files (Table S1). Observed nitrofurantoin plasma concentration-time data are presented in supplementary files (Table S2).

### 3.3. Nitrofurantoin urine concentration-time course

All PRE samples were below LOQ. The median and range of urine NFN concentrations over time before and following the last administered dose are shown in Fig. 2. Nitrofurantoin concentrations were above LOQ for 12 h after the last dose in 12 dogs and for 24 h in one dog.

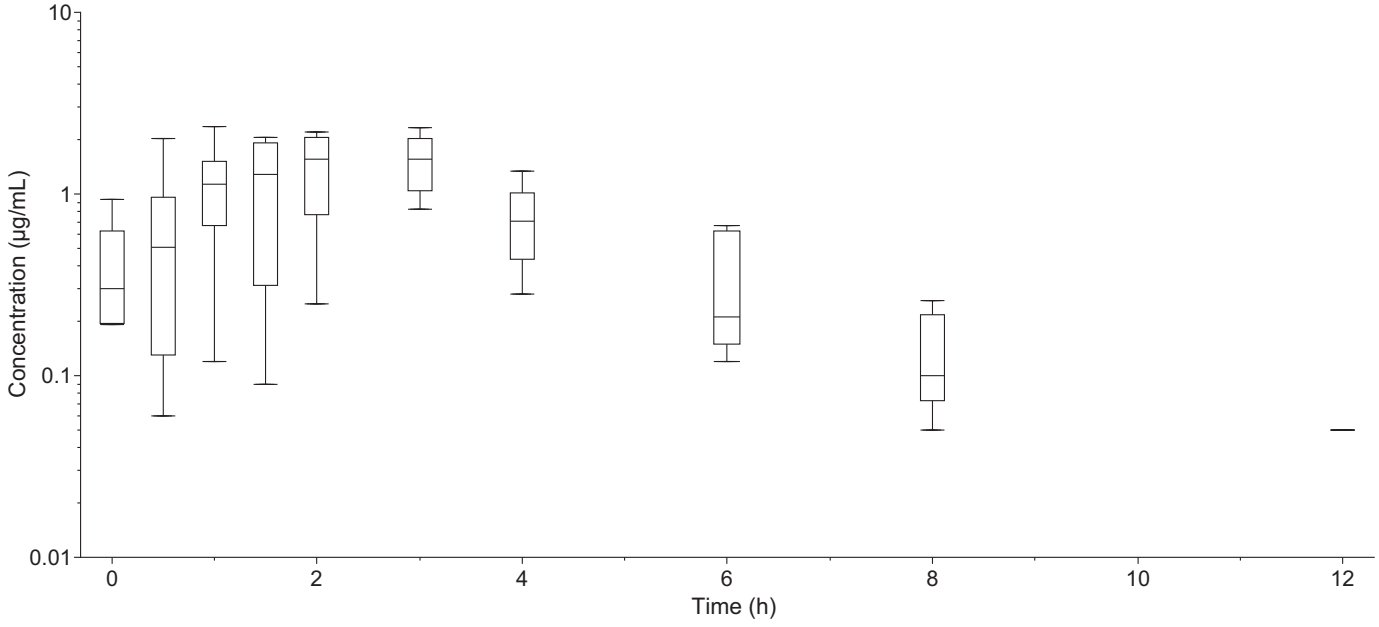


**Fig. 1.** Box plot showing observed total nitrofurantoin plasma concentrations over time following the last of 15 doses (4.4–5.0 mg/kg) administered *per os* to eight healthy beagles. Doses were administered every eight hours with the time for last administration denoted 0 h. The boxes represent the 25th to 75th interquartile range, the horizontal lines are the medians and whiskers represent the minimum to maximum concentration range.

**Table 2**
Individual parameter estimates from the non-compartment analyses in plasma and urine, respectively.

| Dog | $C_{max}$ (μg/mL) | | $T_{max}$ (h) | | $k$ (1/h) | | $t_{1/2z}$ (h) | | $Cl$/F (mL/h) | | $V$/F (mL) | | $AUC_{8h}$ (h•μg/mL) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | P | U | P | U | P | U | P | U | P | U | P | U | P | U |
| 1 | 2.3 | 32.1 | 3 | 8 | 0.8 | – | 0.9 | – | 12,140 | – | 14,810 | – | 5.3 | 167.1 |
| 2 | 1.9 | 20.5 | 2 | 5 | 1.3 | – | 0.5 | – | 15,620 | – | 12,000 | – | 3.9 | 114.3 |
| 3 | 1.3 | 57.9 | 3 | 0 | 0.7 | – | 0.9 | – | 12,180 | – | 16,570 | – | 5.3 | 318.3 |
| 4 | 2.1 | 75.9 | 3 | 0 | 1.1 | 0.1 | 0.6 | 5.1 | 16,040 | – | 14,660 | – | 3.7 | 254.4 |
| 5 | 2.4 | 82.1 | 1 | 0 | 0.7 | 0.3 | 1.0 | 2.1 | 10,420 | – | 14,930 | – | 5.7 | 253.5 |
| 6 | 2.2 | 54.1 | 2 | 2 | 0.4 | 0.2 | 2.0 | 4.3 | 10,770 | – | 30,980 | – | 5.4 | 367.5 |
| 7 | 1.9 | 71.7 | 1.5 | 2 | 0.9 | 0.2 | 0.8 | 2.8 | 17,170 | – | 18,850 | – | 4.4 | 297.4 |
| 8 | 2.1 | 31.4 | 2 | 0 | 1.4 | 0.1 | 0.5 | 5.0 | 12,400 | – | 9070 | – | 6.0 | 200.3 |

$C_{max}$ is maximum concentration, $t_{max}$ is the time for the maximum concentration, $k$ is the terminal rate constant, $t_{1/2z}$ is the terminal half-life, $Cl$/F is the ratio between total clearance and bioavailability, $V$/F is the ratio between the volume of distribution during the terminal phase and bioavailability, $AUC_{8h}$ is the area under the curve for 8 h following the last administration, P is plasma and U is urine.

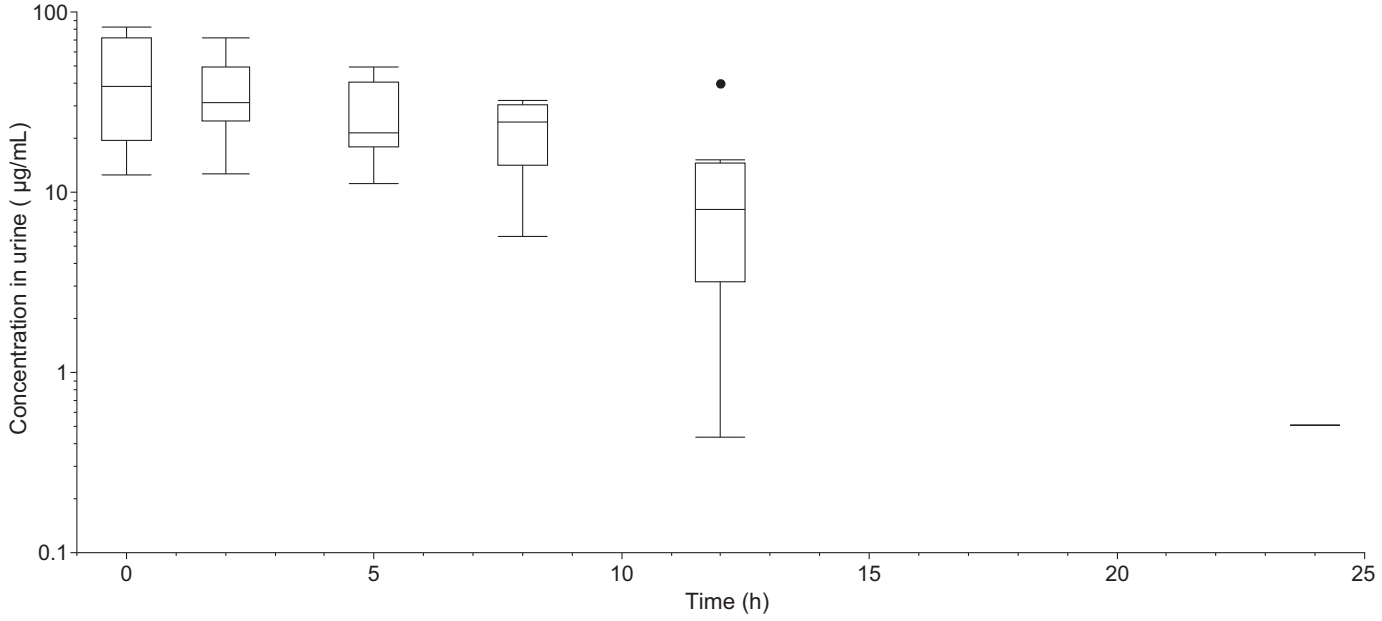


**Fig. 2.** Box plot showing observed total median (symbols) and range (bars) nitrofurantoin urine concentrations over time following the last of 15 doses (4.4–5.0 mg/kg) administered *per os* to eight healthy beagles. Doses were administered every eight hours with the time for last administration denoted 0 h. The boxes represent the 25th to 75th interquartile range, the horizontal lines are the medians and whiskers represent the minimum to maximum concentration range. The filled circle represent an outlier (concentration >1.5 times the interquartile above the 75th quantile).

The urine NFN data allowed the calculation of the terminal half-life in only five dogs. Data from the three remaining dogs included fewer than three descending observations in the terminal phase. The median (range) parameter values from the NCA analysis were: $C_{max}$ 56 μg/mL (20.5–82.1), $t_{max}$ 1 h (0–8), $t_{1/2z}$ 4.3 h (2.1–5.1) and $AUC_{8h}$ following the last administration 253.9 h•μg/mL (114.3–367.5). Individual parameter estimates are presented in Table 2. Some urine samples were not collected according to the time protocol due to the nature of collecting free-catch samples. Exact sampling times are presented in the supplementary files (Table S3). Observed nitrofurantoin urine concentration-time data are presented in supplementary files (Table S2).

### 3.4. Observed adverse effects and clinical pathology

No adverse effects were observed clinically during the study period or the following two weeks. The median difference between the PRE and 24 h samples in serum sodium concentration was −1 mmol/L for Group N and + 2 mmol/L for Group C ($P = 0.047$). Serum sodium concentrations were within the laboratory reference interval at both time points in all dogs. There were no other significant changes in biochemistry or haematology variables between groups. Median (range) differences between PRE and 24 h administration for all analysed biochemistry and haematology variables are given in Table 3. Observed biochemistry and haematology data are reported in the supplementary files (Table S4). No obvious signs of pathology could be detected in data from urinalysis. All results from urinalysis are given in supplementary files (Table S5).

## 4. Discussion

This study is the first to report plasma and urine exposure of NFN and safety data following an empiric dosing regimen of NFN, which were the aims of the study. Nitrofurantoin concentration-time course was analysed by means of NCA in both plasma and urine from all eight dogs. A more thorough pharmacokinetic study including estimation of renal clearance would have demanded both intravenous administration and collection of total urine volume, which was not planned in this study. The low $t_{max}$ value (0.9 h) suggests rapid absorption of NFN from the gastrointestinal tract and the short half-life in plasma (0.8 h) suggests rapid elimination. Both rapid absorption from the gastrointestinal tract and rapid excretion to the urine for NFN have been described previously

**Table 3**
Difference in PRE and 24 h biochemistry and haematology variables for Group N and Group C.

| Variable | Median PRE group N | median PRE Group C | Median 24 h group N | Median 24 h group C | p-value[1] | Laboratory reference range |
|---|---|---|---|---|---|---|
| Chloride | 112 | 112 | 110 | 113 | 0.094 | 107–120 mmol/L |
| Sodium | 149 | 150 | 148 | 149 | *0.047 | 142-156 mmol/L |
| Potassium | 4.8 | 4.6 | 4.5 | 4.8 | 0.443 | 4.1–5.1 mmol/L |
| Urea | 5.5 | 8.4 | 4.5 | 6.7 | 0.808 | 2.5–8.8 mmol/L |
| ALP | 0.7 | 0.6 | 0.8 | 0.6 | 0.333 | 0–2.2 μkat/L |
| ALAT | 0.7 | 0.7 | 0.6 | 0.7 | 0.361 | 0–1.3 μkat/L |
| Creatinine | 56 | 52 | 60 | 51 | 0.797 | 46–115 μmol/L |
| Protein | 65 | 67 | 61 | 64 | 0.798 | 56–75 g/L |
| Alb | 32 | 34 | 32 | 33 | 0.434 | 27–37 g/L |
| HB | 179 | 197 | 166 | 188 | 1 | 132–199 g/L |
| HCT | 0.53 | 0.57 | 0.5 | 0.58 | 0.495 | 0.38–0.57 L/L |
| WBC | 9 | 10 | 7.3 | 8.8 | 0.461 | 5.8–16•$10^9$/L |
| Neutrophils | 6.1 | 7 | 4.4 | 6.1 | 0.202 | 3.0–11.5•$10^9$/L |
| Eosinophils | 0.2 | 0.2 | 0.3 | 0.2 | 0.329 | 0.1–1.2•$10^9$/L |
| Basophils[2] | N/A | N/A | N/A | N/A | N/A | 0–0.1•$10^9$/L |
| Lymphocytes | 2.1 | 2.3 | 2.4 | 2.1 | 0.441 | 1.4–4.8•$10^9$/L |
| Monocytes | 0.4 | 0.4 | 0.4 | 0.5 | 0.863 | 0.2–1.4•$10^9$/L |

[1] p-values for the difference in change between Group N and group C for each parameter. Calculated using Mann-Whitney U test.
[2] All samples contained <0.1•$10^9$/L basophils.
* Statistic significant difference between Group C and Group N.

in dogs (Conklin et al., 1969; Niazi et al., 1983). It has been suggested that the short plasma half-life results in a short dosing interval and that this might impede compliance (Maaland and Guardabassi, 2011). The plasma compartment is, however, less relevant than the urine compartment in SBC therapy (Wijma et al., 2019). In the present study, urine concentrations varied between 10 and 100 μg/mL in up to 8 h following the last dose. Moreover, the half-life was 4 h in urine, which is more relevant to compare with the 8 h dosing interval than the half-life in plasma.

In veterinary medicine, no NFN clinical breakpoints (CBPs) exists for any bacterial species. According to the European Committee for Antimicrobial Susceptibility Testing (EUCAST) both the epidemiological cut off value and the CBP in humans is 64 μg/mL for *E. coli* and 32 μg/mL for *Staphylococcus aureus*. Recent minimum inhibitory concentration (MIC) data for *E. coli* isolated from clinical urine samples from dogs ($n = 1038$) showed that 99.4% were susceptible to NFN at 32 μg/mL, which was the lowest studied concentration (Anonymous, 2021). Only one isolate was classified as resistant (MIC >64 μg/mL). For *Staphylococcus pseudintermedius* ($n = 2155$), 96.2% were susceptible at 16 μg/mL. In data from 2018, the *E. coli* MIC for NFN was 8 μg/mL in 42% pf the samples and 16 μg/mL in 56% of the samples (Anonymous, 2019). These susceptibility data suggest that NFN might also be effective in dogs at concentrations below the human CBP.

In the present study, an established dosing regimen that has been empirically shown to have clinical efficacy was used. Several measured urine concentrations were below the human CBP for *E. coli* within 8 h after administration. In some dogs, samples were even below 32 μg/mL. It has been reported that the antibacterial activity of NFN against *E. coli* does not increase with increasing concentrations above MIC (Fransen et al., 2016; Komp Lindgren et al., 2015). Instead, data suggest a time dependency, which together with the susceptibility data from dogs described above might explain the clinical efficacy. There are also other possible explanations for why NFN might show clinical curative effects at concentrations lower than a CBP based on MIC values that were determined *in vitro*. The nature of SBC with frequent urination provides a different environment than what is present when testing *in vitro*. Moreover, Mueller-Hinton (MH) broth is commonly used for MIC determination *in vitro*. In urine, one study reported lower NFN effective concentration ($EC_{50}$) value and static growth was achieved at lower concentrations than in MH-broth (Wijma et al., 2019). However, in another study MIC values remained unchanged, and thus there is inconsistency in the literature (Sobke et al., 2018). Variation in potency and efficacy have also been shown in urine compared with MH-broth for other AMDs, *e.g.* trimethoprim-sulfamethoxazole and fosfomycin (Abbott et al., 2020, Büyükbaba-Boral et al., 2004, Drobot et al., 1996,). Finally, NFN efficacy increases with decreasing pH (Fransen et al., 2017; Sobke et al., 2018). Acidification of urine is a clinically acceptable strategy to increase positive treatment outcomes, which also has been suggested in humans. This also warrants further investigation because the *in vitro* growth rate of *E. coli* is higher in alkaline urine than in acidic urine (Thornton et al., 2018).

Fear of adverse effects is one argument against the use of NFN in dogs (Maaland and Guardabassi, 2011). In a retrospective study, successful outcome were reported in 12/14 patients and a potential adverse effect (mild diarrhoea) was reported in only 1/14 dogs (Leuin et al., 2021). No adverse effects were observed clinically in this study despite the fact that microcrystalline NFN was used. Microcrystalline NFN appears to irritate the gastric mucosa to greater extent than macrocrystalline NFN (Paul et al., 1967; Wijma et al., 2019). Moreover, between the PRE and 24 h samples there were no clinically relevant differences between Group N and Group C in biochemistry or haematology variables or in urinalysis results. However, the safety in NFN is not well studied. The present study was limited by including only eight healthy dogs of the same breed. Consequently, prospective clinical studies on the efficacy and safety of NFN in dogs are highly warranted.

## 5. Conclusion

The data presented in this study combined with *in vitro* sensitivity data produced elsewhere from common urine pathogens in conjunction with the lack of observed adverse effects suggest that NFN in a standard nitrofurantoin dosing regimen (4.4–5.0 mg/kg) *per os* could be a relevant choice for antimicrobial treatment of SBC in dogs. Further studies are highly warranted to verify the efficacy in clinically ill dogs.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rvsc.2022.08.005.

## Declaration of Competing Interest

None of the authors have any conflicts of interests to report.

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