Plasma and Tissue Depletion of Florfenicol and Florfenicol-amine in Chickens

Arturo Anadón, Maria Aranzazu Martínez, Marta Martínez, Alba Ríos, Virginia Caballero, Irma Ares, and Maria Rosa Martínez-Larrañaga*

Department of Toxicology and Pharmacology, Faculty of Veterinary Medicine, Universidad Complutense de Madrid, 28040 Madrid, Spain

Chickens were used to investigate plasma disposition of florfenicol after single intravenous (i.v.) and oral dose (20 mg kg^{-1} body weight) and to study residue depletion of florfenicol and its major metabolite florfenicol-amine after multiple oral doses (40 mg kg^{-1} body weight, daily for 3 days). Plasma and tissue samples were analyzed using a high-performance liquid chromatography (HPLC) method. After i.v. and oral administration, plasma concentration−time curves were best described by a two-compartment open model. The mean [±standard deviation (SD)] elimination half-life ($t^1/_2\beta$) of florfenicol in plasma was 7.90 ± 0.48 and 8.34 ± 0.64 h after i.v. and oral administration, respectively. The maximum plasma concentration was 10.23 ± 1.67 μg mL^{-1}, and the interval from oral administration until maximal concentration was 0.63 ± 0.07 h. Oral bioavailability was found to be 87 ± 16%. Florfenicol was converted to florfenicol-amine. After multiple oral dose (40 mg kg^{-1} body weight, daily for 3 days), in kidney and liver, concentrations of florfenicol (119.34 ± 31.81 and 817.34 ± 91.65 μg kg^{-1}, respectively) and florfenicol-amine (60.67 ± 13.05 and 48.50 ± 13.07 μg kg^{-1}, respectively) persisted for 7 days. The prolonged presence of residues of florfenicol and florfenicol-amine in edible tissues can play an important role in human food safety, because the compounds could give rise to a possible health risk. A withdrawal time of 6 days was necessary to ensure that the residues of florfenicol were less than the maximal residue limits or tolerance established by the European Union.

KEYWORDS: Florfenicol; plasma and tissue depletion; withdrawal time; chickens for fattening

INTRODUCTION

Florfenicol is a broad-spectrum antibiotic used in veterinary medicine belonging to the family of agents that includes thiamphenicol and chloramphenicol. All three compounds act by inhibiting bacterial protein synthesis by binding to 50S and 70S subunits in the ribosome to abolish the activity of peptidyltransferase (*1*). Chloramfenicol is amphiphilic and un-ionized at physiological pH, and it can pass biological membranes to reach intracellular bacteria (*2*). As a consequence of the use of chloramphenicol in human and veterinary medicine, two important adverse phenomena have severely restricted its use of chloramphenicol, i.e., the potential fatal side effect of dose-unrelated aplastic anemia in humans and the widespread development of bacterial resistance. The most frequently encountered mechanism of bacterial resistance to chloramphenicol is enzymatic inactivation by acetylation of the drug via different types of chloramphenicol acetyltransferases (*3*). The structural modification in the design of florfenicol, substitution

of a fluorine atom for the hydroxyl group at C-3 site, prevents this enzymatic modification (*4, 5*). The C-3 primary hydroxyl group, initially thought to be essential for inhibition of protein synthesis through its affinity for the peptidyltransferase of 50S ribosomes, can be replaced with fluorine (*2*). Besides the fluoro substitution at C-3 (in florfenicol), very few other substitutions are tolerated without adverse effects on antimicrobial activity (*2*). Among them, the substitution of the nitro group (−NO$_2$), which was considered to be responsible for the dose-unrelated aplastic anemia (*6, 7*), by a sulfomethyl group (−SO$_2$CH$_3$) at the *para* position of the 1-phenyl moiety became effective in thiamphenicol and florfenicol (**Figure 1**). Florfenicol is un-ionized in a pH range from 3 to 9 (*7*) and also poorly soluble in aqueous solutions. Because of its lipophilicity, florfenicol shows a good tissue penetration. Florfenicol is active at lower concentrations than its structural analogues, thiamphenicol and chloramphenicol, against a number of bacterial pathogens *in vitro*, and even against many chloramphenicol-resistant or thiamphenicol-resistant strains involved with common infections in domestic animals, such as *Klebsiella pneumoniae, Enterobacter cloacae, Shigella dysenteriae, Salmonella typhimurium, Escherichia coli, Staphylococcus aureus, Proteus vulgaris, Haemophilus sommus, Actinobacillus pleuropneumoniae, Bor-*

* To whom correspondence should be addressed: Department of Toxicology and Pharmacology, Faculty of Veterinary Medicine, Universidad Complutense de Madrid, 28040 Madrid, Spain. Telephone: +34-91-3943834. Fax: +34-91-3943840. E-mail: mrml@vet.ucm.es.
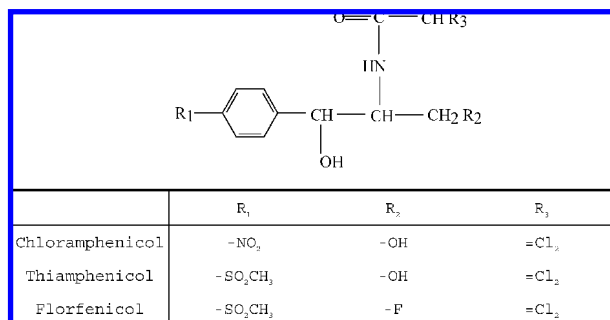
11050 *J. Agric. Food Chem.,* Vol. 56, No. 22, 2008

Anadón et al.

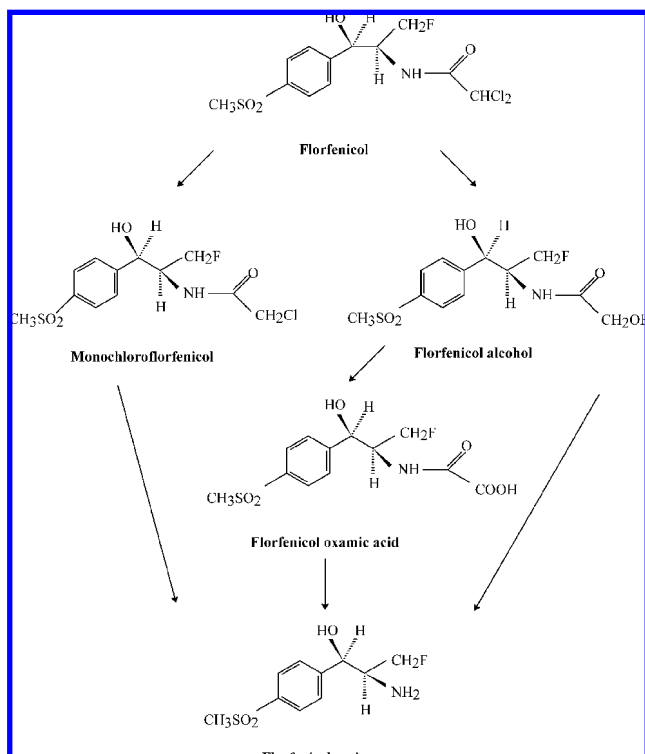**Figure 1.** Chemical structures of chloramphenicol, thiamphenicol, and florfenicol.

Figure 2. Metabolic pathways of florfenicol.

detella bronchiseptica, Streptococcus pneumoniae, and *Streptococcus suis* (*1, 4, 5, 8, 9*). Because of its distinct advantages relating to safety and efficacy over thiamphenicol and chloramphenicol, florfenicol is believed to be an ideal replacement of these two drugs.

The reports on the metabolism of florfenicol in animals *in vivo* are sparse. In calves, approximately 64% of a 20 mg kg^{-1} dose of intramuscular florfenicol is excreted as parent drug in the urine (*7, 10*). Urinary metabolites include florfenicol-amine, florfenicol alcohol, florfenicol oxamic acid, and monochloroflorfenicol (*7, 10*) (**Figure 2**). Florfenicol-amine is the longest-lived major metabolite in the liver from cattle, and therefore, it could be used as the marker residue for withdrawal calculations (*10*). A residue study was reported by the European Agency for Medicines (EMEA) on chickens given multiple oral doses of ^{14}C-florfenicol at 20 mg kg^{-1} for 3 days (*11*). The results showed that the total radioactivity administered was excreted within 7 days after the last dose. In excreta, at 7 days, the parent compound represented the major fraction (42%), florfenicol-amine (25%), florfenicol oxamic acid (5%), and florfenicol alcohol (10%), with the remaining part of radioactivity being represented by a small percentage of three unknown compounds. Monochloroflorfenicol was not detected in the

excreta (*11*). These studies revealed a metabolic fate with two bioconversion pathways, leading to a common metabolite: florfenicol-amine, (i) one minor pathway via monochloroflorfenicol and (ii) the other major pathway via florfenicol-alcohol, with or without the intermediate formation of florfenicol oxamic acid (**Figure 2**).

The major pharmacodynamic effect of florfenicol is its antimicrobial activity. Because of its broad antibacterial spectrum, florfenicol has the potential to become a valuable antibiotic in the treatment of infectious diseases in livestock and poultry. Since the product came into market in the early 1990s, efficacy has been demonstrated against many infectious diseases of domestic animals (*9, 12−17*). From a clinical point of view, florfenicol should be assayed in the plasma to compare these plasma concentrations with the minimal inhibitory concentration (MIC) of potential pathogens. Studies on the kinetic behavior of florfenicol in veal calves (*17−20*), cows (*21, 22*), horses (*23*), goats (*24*), pigs (*17, 25, 26*), and sheep (*27, 28*) are available. However, the disposition including metabolism of the florfenicol in chickens are limited, and specific pharmacokinetic data for chickens are lacking (*29−31*). Results of preliminary studies in chickens (*32, 33*) suggest a prolonged elimination of florfenicol from the body. The therapeutic use of florfenicol in poultry must be assessed not only in terms of good clinical efficacy but also considering the risk of the presence of residues in edible tissues. There is a strict legislative framework controlling the use of antimicrobial substances, with the aim of minimizing the risk to human health associated with consumption of their residues. Therefore, to ensure human food safety, the European Union (EU) has set the tolerance level for these compounds as the maximum residue limit (MRL). The MRL in all food producing species including chicken was fixed for florfenicol at 100 μg kg^{-1} in muscle, 200 μg kg^{-1} in skin + fat, 750 μg kg^{-1} in kidney, and 2500 μg kg^{-1} in liver, with the marker residue being the sum of florfenicol and its metabolites measured as florfenicol-amine (*34*).

The objectives of the present study were (i) to describe the plasma disposition of florfenicol following single intravenous (i.v.) and oral dose in healthy chickens and (ii) to evaluate the rate of depletion of florfenicol and its metabolite florfenicol-amine in edible tissues (muscle, liver, kidney, and skin + fat) of healthy chickens after multiple-dose oral administration.

MATERIALS AND METHODS

Chemicals and Reagents. Florfenicol (SCH-25298) and its metabolites florfenicol-amine (SCH-40458), florfenicol alcohol (SCH-45705), florfenicol oxamic acid (SCH-48057), and monochloroflorfenicol (SCH-49435) were provided by Schering-Plough Animal Health (Union, NJ). All chromatographic solvents used in this study were HPLC-grade. The other chemicals were of analytical grade.

Animals. The study was undertaken in accordance with the ethics requirements and authorized by the official ethical committee of our university. A total of 34 healthy Ross male chickens for fattening that were 40 days old and that each weighed 2 kg were included in the study. All chickens were obtained from a poultry breeding farm (Nutreco, SA Sada Division, Cazalegas, Toledo, Spain). Chickens were placed individually in cages in the university animal house. Chickens were allowed a 7 day acclimation period prior to the study. The animal house was maintained at 25 ± 2 °C and at 45−65% relative humidity. Antibiotic-free commercial feed and water were supplied *ad libitum*.

Experimental Design. A total of 38 chickens were randomly allotted to three groups. Group A and B animals (8 chickens per group) were used to investigate the plasma disposition of florfenicol after a single i.v. and oral administration at a dose level of 20 mg kg^{-1} body weight. Chickens of group C (*n* = 18) were used to study tissue depletion of residues of florfenicol and its metabolite florfenicol-amine. Chickens

Plasma and Tissue Depletion of Florfenicol in Chickens

J. Agric. Food Chem., Vol. 56, No. 22, 2008 **11051**

of group D ($n = 4$) did not receive any treatment and were used to determine the validation criteria of the analytical method. Chickens in group C were given serial daily oral doses of florfenicol (40 mg kg^{-1} body weight for 3 consecutive days). For groups A, B, and C, all dosages were administered between 8 and 9 a.m. One gram of florfenicol (SCH-25298) was weighed and dissolved in 5.0 mL glycerol formal (*35*) to give a stock solution of 200 mg mL^{-1}. For group A and B, the solution for i.v. and oral administration was prepared daily, taking 0.2 mL 2 kg^{-1} body weight (equivalent to 20 mg kg^{-1} body weight) of the stock solution (200 mg of florfenicol mL^{-1}) diluted with 0.3 mL of sterilized 0.9% saline solution (total volume of 0.5 mL) or diluted with 1.8 mL of sterilized 0.9% saline solution (total volume of 2 mL), respectively. For group C, the solution for oral administration was prepared daily, taking 0.4 mL 2 kg^{-1} body weight (equivalent to 40 mg kg^{-1} body weight) of the stock solution (200 mg of florfenicol mL^{-1}) diluted with 1.8 mL of sterilized 0.9% saline solution (total volume of 2 mL). Florfenicol was administered i.v. into the right brachial vein of chickens in group A or was administered orally directly into the crop of chickens of groups B and C by use of a thin plastic tube attached to a syringe. Food but not water was withheld from 12 h before until 6 h after drug administration.

Blood samples (1 mL) were collected from the left brachial vein of each chicken of groups A and B. Samples were collected into heparinized syringes through a cannula immediately before (time 0) and 10, 20, and 30 min and 1, 2, 4, 6, 8, 12, and 24 h after drug administration. Blood samples were centrifuged (1500g for 10 min), and plasma was harvested and stored frozen at −45 °C until analyzed. Florfenicol concentrations were measured in plasma samples of chickens in group A. Plasma samples of chickens in group B were assayed for florfenicol, florfenicol-amine, florfenicol alcohol, florfenicol oxamic acid, and monochloroflorfenicol.

Chickens of group C were euthanized by use of carbon dioxide at 1 ($n = 6$), 5 ($n = 6$), and 7 days ($n = 6$) h after the last dose of florfenicol. Birds were immediately exsanguinated, and tissue specimens (2 g) of kidney, liver, muscle, and skin + fat were collected separately. Each of the tissue specimens was carefully weighed and stored frozen at −45 °C until assayed for concentrations of florfenicol and florfenicol-amine.

Analytical Method and Validation. Florfenicol, florfenicol-amine, florfenicol alcohol, florfenicol oxamic acid, and monochloroflorfenicol concentrations in plasma and florfenicol and florfenicol-amine concentrations in tissues were measured using a high-performance liquid chromatography (HPLC) technique (*18*), with modifications.

Plasma Extraction. A total of 1.0 mL of plasma followed by 1.0 mL of 0.1 M phosphate buffer at pH 7.0 and 4 mL of ethyl acetate were added to each screw-capped tube, and the tube was rotated for 10 min at high speed and then centrifuged at 1500g for 10 min in a refrigerated laboratory centrifuge (RC-5B, Sorvall, Newton, CT). The organic layer (3 mL) was removed and evaporated under nitrogen. The residue was redissolved in 500 μL of mobile phase. A 20 μL aliquot was injected into the HPLC.

Tissue Extraction. Tissue samples (1 g) and 4 mL of 0.1 M phosphate buffer at pH 7.0 were placed in a test tube. The tissue was then homogenized ultrasonically (2 min at 40 W using a titanium needle probe on a Labsonic U/Braun, B. Braun Melsungen AG); 4 mL of ethyl acetate was added; and the tube was rotated at fast speed. The tube was centrifuged at 1500g for 10 min. The organic layer (3 mL) was removed and saved. A further 3 mL of ethyl acetate was added to the screw-cap tube. The tube was again rotated and centrifuged. The organic layer (3 mL) was removed and combined with the first 3 mL of organic layer, and the organic layer was evaporated under nitrogen. The residue was mixed with 500 μL of mobile phase, sonicated, and vortexed, and a 20 μL injection was made into the HPLC.

HPLC Analysis. Plasma concentrations of florfenicol, florfenicol-amine, florfenicol alcohol, florfenicol oxamic acid, and monochloroflorfenicol and tissue concentrations of florfenicol and florfenicol-amine were measured in our laboratory using a Shimadzu liquid chromatograph equipped with a system controller SCL-10A VP, two solvent delivery modules LC-10AD VP, an auto-injector SIL-10AD VP, a UV−vis photodiode array detector SPD-M10A VP set at 223 nm for monitoring the signal and at 200−400 nm for spectral information,

and a CLASS-VP version 6.1 data system. All samples were analyzed using a 5 μm particle size Dupont Zorbax C18 column (4.6 mm i.d. × 250 mm) preceded by a C18 guard column. The mobile phase was acetonitrile and water (1:2, v/v) containing 0.10% glacial acetic acid (i.e., mix 250 mL of acetonitrile with 492.5 mL of HPLC water and add 7.5 mL of 10% glacial acetic acid). The flow rate was 0.6 mL min^{-1}. Peak areas in the sample chromatograms were quantitated by an external standard technique using solutions of florfenicol, florfenicol-amine, florfenicol alcohol, florfenicol oxamic acid, and monochloroflorfenicol reference standards. The system worked at room temperature.

The analytical method was fully validated according to EU requirements for the compounds florfenicol, florfenicol alcohol, florfenicol oxamic acid, monochloroflorfenicol, and florfenicol-amine [linearity, recovery rate, accuracy, precision, trueness, quantification limit (LOQ), detection limit (LOD), and specificity] (*36*). Because the metabolites florfenicol alcohol, florfenicol oxamic acid, and monochloroflorfenicol could not be detected in the plasma from treated chickens, only the validation criteria of the analytical method for the compounds florfenicol and florfenicol-amine were presented. Drug concentrations were determined from peak areas and the use of calibration curves obtained by running plasma and tissue samples from chickens not administered florfenicol (i.e., chickens of group D) that were spiked with known concentrations of florfenicol and florfenicol-amine. For plasma and tissue specimens as determined by use of the linear least-squares regression procedure, a linear relationship existed in the calibration curve of florfenicol and florfenicol-amine over the range of 0.02−20.00 μg mL^{-1} for plasma and 20−10 000 μg kg^{-1} for tissues, which always yielded a correlation coefficient exceeding 0.9998. The overall mean recovery of florfenicol and florfenicol-amine from plasma and tissues was greater than 94 and 85%, respectively. Within-day and day-to-day precision were <5.5%. The LOQ was 0.020 μg mL^{-1} for florfenicol and florfenicol-amine in the plasma. The LOQ was 20 ng g^{-1} for florfenicol and florfenicol-amine in the different tissue matrices. The present method was comparable with those reported by De Craene et al. (*15*) and Lobell et al. (*20*) for plasma, in which the average plasma recovery rates for florfenicol in calves and cattle were 93.46 and 99.0% with the corresponding within-run precision rates of 1.22−3.70 and 1.5−5.0%, respectively.

Data Analysis. The plasma concentration versus time data were sequentially fitted to one-, two-, and multiple-compartment models, using the computer program WinNonlin (version 5.0.1; Pharsight Corporation, Mountain View, CA). The model was determined for best fit on the basis of a smaller value for the Akaike information criterion (*37*). The two-compartment model was the best fit for all chickens. This model was used to establish pharmacokinetic characteristics. Plasma curves of florfenicol after a single i.v. and oral administration and those of florfenicol-amine (the main metabolite in plasma) after a single oral administration of florfenicol were obtained for each chicken and were fitted to the following exponential equations:

$$C = A_1 e^{-\alpha t} + A_2 e^{-\beta t} \quad (\text{i.v.})$$

$$C = A_1 e^{-\alpha t} + A_2 e^{-\beta t} - A_3 e^{-K_a t} \quad (\text{oral})$$

where C is the plasma concentration of the drug, A_1, A_2, and A_3 are mathematical coefficients (i.e., A_1 and A_2 are the plasma concentrations extrapolated to time zero of the first and second elimination phases of the drug and A_3 for the absorption phase), α is the hybrid rate constant for the distribution phase, β is the hybrid rate constant for the elimination terminal phase (i.e., α and β are the slopes of the first and second elimination phases of the drug disposition), K_a is the first-order absorption rate constant, and t is the time. Absorption half-life ($t_{a\ 1/2}$), half-life of the α phase ($t_{\alpha\ 1/2}$), half-life of the β phase ($t_{\beta\ 1/2}$), distribution rate constants for transfer of the drug from the central to the peripheral compartment (K_{12}) and from the peripheral to the central compartment (K_{21}), and the elimination rate constant (K_{10}) were calculated by use of standard equations as described (*38, 39*) After i.v. and oral administration, the area under the concentration−time curves (AUC) was calculated as follows:

11052 *J. Agric. Food Chem.,* Vol. 56, No. 22, 2008

Anadón et al.

$$\text{AUC} = (A_1/\alpha) + (A_2/\beta); \text{or}$$

$$\text{AUC} = (A_1/\alpha) + (A_2/\beta) - (A_3/K_a)$$

Total plasma clearance (CL) was calculated, using the following formula:

$$\text{CL} = (\text{dose kg}^{-1})/\text{AUC}; \text{or CL} = (\text{dose kg}^{-1})(F/\text{AUC})$$

Oral bioavailability (*F*) was determined as follows:

$$F = (\text{AUC}_{\text{oral}})/(\text{AUC}_{\text{i.v.}})$$

Oral bioavailability (*F*) was calculated from the ratio between the value of AUC_{oral} for each chicken and the mean value of $\text{AUC}_{\text{i.v.}}$ for the 8 chickens used in the i.v. administration study. Complete absorption was determined on the basis of $\text{AUC}_{\text{i.v.}}$, which represents the mean AUC for the 8 chickens to which florfenicol was administered. Because of the small individual variation in $\text{AUC}_{\text{i.v.}}$ and the fact that the same 8 chickens were not available for oral and i.v. studies, the mean $\text{AUC}_{\text{i.v.}}$ rather than $\text{AUC}_{\text{i.v.}}$ for each chicken was used to estimate bioavailability after oral administration of florfenicol.

Mean residence time (MRT) was calculated as follows:

$$\text{MRT} = (A_1/\alpha^2 + A_2/\beta^2)(1/\text{AUC})$$

Apparent volume of distribution ($V_{\text{d(area)}}$) was determined as follows:

$$V_{\text{d(area)}} = (\text{dose kg}^{-1})/(\text{AUC}\beta); \text{or } V_{\text{d(area)}} = (\text{dose kg}^{-1})$$
$$[F/\text{AUC}]\beta$$

Volume of distribution in the central compartment (V_c) was determined as follows:

$$V_c = (\text{dose kg}^{-1})/A_1 + A_2; \text{or } V_c = (\text{dose kg}^{-1})(F)/A_1 + A_2$$

Volume of distribution at steady state (V_{ss}) was determined as follows:

$$V_{\text{ss}} = \text{MRT} \times \text{CL}$$

Maximum drug plasma concentration (C_{max}) after oral administration and the time at which C_{max} was achieved (T_{max}) was determined directly from the concentration versus time curve.

Mean pharmacokinetic variables were obtained by averaging the variables calculated for drug disposition after each florfenicol administration in each chicken.

The withdrawal time was estimated by linear regression analysis of log-transformed tissue concentrations and was determined at the time when the 95% upper one-side tolerance limit was below the MRL with 95% confidence. (*40*)

RESULTS

Plasma Florfenicol Disposition. Mean plasma concentrations (μg mL^{-1}, $\pm$SD) of florfenicol after i.v. administration and those of florfenicol and florfenicol-amine after a single oral administration of florfenicol are presented in **Figure 3**. The plasma concentration–time profile of florfenicol after i.v. administration and of florfenicol and florfenicol-amine after oral administration of florfenicol for each chicken were similar to the overall means. Analysis of plasma concentration versus time curves indicated a biphasic decrease after i.v. and oral administration. Good fit of the observed data for a two-compartment open model was obtained. Values of the parameters that described absorption and disposition kinetics of florfenicol in chickens are presented in **Table 1**. The kinetic parameters of florfenicol-amine after oral administration of florfenicol are summarized in **Table 2**.

After i.v. administration of florfenicol, a rapid distribution phase and a slower elimination phase, with a half-life of distribution of the α phase ($t_{\alpha\ 1/2}$) of 0.30 ± 0.05 h and a half-life of elimination of the β phase ($t_{\beta\ 1/2}$) of 7.90 ± 0.48 h, were observed (**Table 1**). The central volume of distribution was 0.45
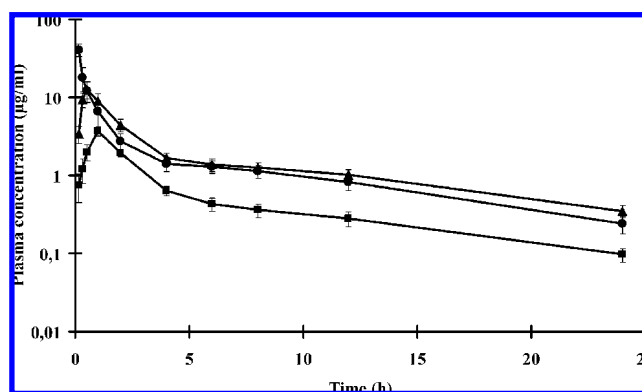

Figure 3. Mean plasma concentrations of florfenicol after a single i.v. (●) and oral (▲) administration of florfenicol (20 mg kg^{-1} body weight) and plasma concentrations of florfenicol-amine (■) after single oral administration of florfenicol (20 mg kg^{-1} body weight). Data represent mean $\pm$ SD values for 8 chickens.

Table 1. Florfenicol Kinetic Parameters for Chickens after Single i.v. and Oral Administration of Florfenicol (20 mg kg^{-1} Body Weight)[a]

parameter	i.v.	oral
A_1 (μg mL^{-1})	43.46 ± 9.04	90.09 ± 34.68
A_2 (μg mL^{-1})	2.23 ± 0.34	2.04 ± 0.42
A_3 (μg mL^{-1})	b	98.13 ± 34.53
α (h^{-1})	2.37 ± 0.40	1.48 ± 0.12
β (h^{-1})	0.088 ± 0.005	0.083 ± 0.006
K_a (h^{-1})	b	1.96 ± 0.30
$t_{\alpha\ 1/2}$ (h)	0.30 ± 0.05	0.47 ± 0.04
$t_{\beta\ 1/2}$ (h)	7.90 ± 0.48	8.34 ± 0.64
$t_{a\ 1/2}$ (h)	b	0.36 ± 0.05
V_c (L kg^{-1})	0.45 ± 0.11	0.88 ± 0.15
$V_{\text{d(area)}}$ (L kg^{-1})	2.70 ± 0.60	3.27 ± 0.75
V_{ss} (L kg^{-1})	3.16 ± 0.70	b
K_{12} (h^{-1})	1.21 ± 0.27	0.74 ± 0.10
K_{21} (h^{-1})	0.20 ± 0.03	0.20 ± 0.02
K_{10} (h^{-1})	1.04 ± 0.20	0.61 ± 0.08
AUC (mg h L^{-1})	44.38 ± 8.64	37.85 ± 5.51
F (%)	b	87 ± 16
MRT (h)	6.87 ± 1.08	b
CL (L h^{-1} kg^{-1})	0.46 ± 0.08	0.54 ± 0.08
C_{max} (μg mL^{-1})	b	10.23 ± 1.67
T_{max} (h)	b	0.63 ± 0.07

[a] Values are the mean $\pm$ SD ($n = 8$). A^1, A_2, and A_3, mathematical coefficients; α, hybrid rate constant for distribution phase; β, hybrid rate constant for terminal elimination phase; K_a, first-order absorption rate constant; $t_{a\ 1/2}$, absorption half-life; $t_{\alpha\ 1/2}$, half-life at the α phase; $t_{\beta\ 1/2}$, half-life at the β phase; $V_{\text{d(area)}}$, apparent volume of distribution; V_c, volume of distribution in the central compartment; V_{ss}, volume of distribution at steady state; K_{12}, distribution rate constant for transferring the drug from the central to the peripheral compartment; K_{21}, distribution rate for transferring the drug from the peripheral to the central compartment; K_{10}, elimination rate constant; AUC, area under the concentration–time curve; MRT, mean residence time; CL, total plasma clearance; C_{max}, maximal concentration in plasma after oral administration; T_{max}, time needed to reach C_{max}. [b] Not applicable.

± 0.11 L kg^{-1}. The apparent volume of distribution ($V_{\text{d(area)}}$) and at steady state V_{ss} and clearance (CL) values were 2.70 ± 0.60 L kg^{-1}, 3.16 ± 0.70 L kg^{-1}, and 0.46 ± 0.08 L h^{-1} kg^{-1}, respectively (**Table 1**). When administered orally, the drug was rapidly and widely absorbed. After oral administration of florfenicol (20 mg kg^{-1} body weight), drug concentrations in plasma 10 and 20 min were 3.42 ± 0.85 and 9.31 ± 1.96 μg mL^{-1}, and plasma drug concentrations exceeded 1.27 ± 0.18 and 1.02 ± 0.18 μg mL^{-1} for 8 and 12 h, respectively. The half-life of oral absorption ($t_{a\ 1/2}$) was 0.36 ± 0.05 h. Bioavailability (*F*) of florfenicol after oral administration was $87 \pm 16\%$. The maximal plasma concentration of florfenicol ($C_{\text{max}} = 10.23$

Plasma and Tissue Depletion of Florfenicol in Chickens

J. Agric. Food Chem., Vol. 56, No. 22, 2008 **11053**

Table 2. Florfenicol-amine Kinetic Parameters for Chickens after a Single Oral Administration of Florfenicol (20 mg kg^{-1} Body Weight)[a]

parameter	oral
$t_{\alpha\ 1/2}$ (h)	0.76 ± 0.16
$t_{\beta\ 1/2}$ (h)	9.00 ± 0.86
K_{12} (h^{-1})	0.41 ± 0.11
K_{21} (h^{-1})	0.16 ± 0.02
K_{10} (h^{-1})	0.44 ± 0.09
AUC (mg h L^{-1})	13.13 ± 1.38
C_{max} (μg mL^{-1})	2.41 ± 0.55
T_{max} (h)	1.16 ± 0.43

[a] Values are the mean $\pm$ SD ($n = 8$). See **Table 1** for the definitions of abbreviations.

Table 3. Tissue Concentrations of Florfenicol (FF) and Florfenicol-amine (FFA) for Chickens Orally Administered Florfenicol at the Rate of 40 mg kg^{-1} Body Weight, Daily for 3 Days[a]

tissue	time after last dose (days)	FF (μg kg^{-1})	FFA (μg kg^{-1})
kidney	1	632.84 ± 99.40	617.17 ± 72.72
	5	342.10 ± 61.89	241.00 ± 60.12
	7	119.34 ± 31.81	60.67 ± 13.05
liver	1	2388.67 ± 142.16	617.34 ± 91.77
	5	1028.17 ± 106.60	247.00 ± 56.57
	7	817.34 ± 91.65	48.50 ± 13.07
muscle	1	506.17 ± 244.55	63.17 ± 8.95
	5	72.17 ± 15.54	<LOQ
	7	<LOQ	<LOD
skin + fat	1	781.83 ± 100.24	258.50 ± 70.57
	5	138.83 ± 10.59	<LOQ
	7	<LOQ	<LOD

[a] Each value is the mean $\pm$ SD for 6 chickens. LOD = 12.5 and 13.3 μg kg^{-1} for FF and FFA, respectively, in chicken muscle. LOD = 14.2 and 16.5 μg kg^{-1} for FF and FFA, respectively, in chicken skin + fat. LOD = 11.2 and 13.3 μg kg^{-1} for FF and FFA, respectively, in chicken kidney. LOD = 11.5 and 12.3 μg kg^{-1} for FF and FFA, respectively, in chicken liver. LOQ = 20 μg kg^{-1} for both components in all tissue matrices.

$\pm$ 1.67 μg mL^{-1}) was detected 0.63 $\pm$ 0.07 h after oral administration.

A fraction of florfenicol was metabolized to florfenicol-amine after oral administration of florfenicol. This metabolite represented 35% of the parent drug plasma concentrations, as calculated by the ratio between the mean AUC for florfenicol-amine and mean AUC for florfenicol after oral administration of florfenicol. The other metabolites florfenicol alcohol, florfenicol oxamic acid, and monochloroflorfenicol could not be detected in plasma. The plasma concentration of florfenicol-amine (2.59 $\pm$ 0.34 μg mL^{-1}) peaked at 0.93 $\pm$ 0.16 h after oral administration of florfenicol. The $t_{\beta\ 1/2}$ of florfenicol-amine after oral florfenicol administration was 9.00 $\pm$ 0.86 h (**Table 2**).

Tissue Residue Depletion. Residues of florfenicol and its metabolite florfenicol-amine in tissue specimens after oral administration of florfenicol (40 mg kg^{-1} body weight, daily for 3 consecutive days) were determined. The tissue concentration–time profiles are presented in **Table 3** for kidney, liver, muscle, and skin + fat. Mean tissue concentrations of florfenicol and florfenicol-amine ranging from 2388.67 $\pm$ 142.16 to 506.17 $\pm$ 244.55 μg kg^{-1} and from 617.34 $\pm$ 91.77 to 63.17 $\pm$ 8.95 μg kg^{-1}, respectively, were measured 1 day after administration of the final dose of florfenicol (**Table 3**). The florfenicol and florfenicol-amine concentrations depleted much slower from the kidney and liver tissues than the muscle and skin + fat tissues. Florfenicol and florfenicol-amine were detected in liver (817.34 $\pm$ 91.65 and 48.50 $\pm$ 13.07 μg kg^{-1}, respectively) and kidney (119.34 $\pm$ 31.81 and 60.67 $\pm$ 13.05 μg kg^{-1}, respectively)

tissues but not in muscle and skin + fat tissues, 7 days after termination of florfenicol treatment. Marker residue concentrations (florfenicol + florfenicol-amine) were below the MRL in all samples, 5 days after the end of treatment in kidney, liver, muscle, and skin + fat.

Withdrawal Time Estimation. The mean florfenicol concentrations were below the LOQ at 7 days after cessation of medication in muscle and skin + fat. The mean fluorfenicol-amine concentrations in muscle and skin + fat were below the LOQ at 5 days after cessation of medication and below the LOD at 7 days after dosing. Linear regression analysis of the logarithmic transformed data can be considered for the calculation of the withdrawal periods. Using this approach, the withdrawal time was determined as the time when the one-sided, 95% upper tolerance limit of the regression line with 95% confidence level was below the MRL (*40*). Generally, when the majority of data from one slaughter point is below the LOD or LOQ, the whole time point should be excluded (*40*). Using this approach and considering the marker residue for the MRL (the sum of florfenicol and its metabolite florfenicol-amine), the withdrawal time for florfenicol could only be calculated for liver and kidney tissues after oral administration (40 mg kg^{-1} body weight, daily for 3 consecutive days): 3.02 and 5.59 days, respectively, resulting in a final withdrawal time of 6 days. **Figure 4** illustrates a plot of withdrawal time calculation for florfenicol in chicken kidney after oral administration (40 mg kg^{-1} body weight, daily for 3 consecutive days).

DISCUSSION

Florfenicol provides important therapy for empiric treatment of life-threatening Gram-positive and Gram-negative aerobic and anaerobic bacteria but also chlamydiae, mycoplasmas, and rickettsiae or where culture and sensitive results indicate they will be effective for treatment of severe or recurrent infections in the urinary and respiratory tracts, skin, or soft tissues. Improper use of this agent can potentially lead to bacterial resistance (*41*) and thereby remove it from the veterinarian's arsenal of antimicrobial compounds. Prudent use of highly potent antimicrobials, such as florfenicol, in veterinary medicine is strongly required to maintain the efficacy and safety of florfenicol for the future. Therefore, plasma disposition characteristics of this antibiotic should be considered in choosing dosage regimens that maximize efficacy and minimize development of bacterial drug resistance. To the best of our knowledge, the present paper is the first to report in chickens the plasma disposition of florfenicol. This study showed that plasma concentration of florfenicol after i.v. and oral administration (20 mg kg^{-1}), as well as plasma concentration of florfenicol-amine after oral administration of florfenicol (20 mg kg^{-1}), follows a two-compartment open model in all animals.

After i.v. administration of 20 mg kg^{-1}, the distribution phase of florfenicol was fast ($t_{\alpha\ 1/2} = 0.30 \pm 0.05$ h) and with a high value of volume of distribution at steady state ($V_{ss} = 3.16 \pm 0.70$ L kg^{-1}) and apparent volume of distribution ($V_{d(area)} = 2.70 \pm 0.60$ L kg^{-1}), which indicate that florfenicol is extensively distributed in extravascular tissues. The mean elimination half-life calculated after i.v. administration ($t_{\beta\ 1/2} = 7.90 \pm 0.48$ h) was much longer than those previously reported in other studies: 2.8–3 h in chickens (*31*), 2.86–4.11 h in calves (*15, 18, 19*), 2.35 h in goats (*24*), 2.63–6.72 h in pigs (*17, 26*), and 1.80 h in equines (*23*).

Florfenicol was rapidly ($t_{a\ 1/2} = 0.36 \pm 0.05$ h) and efficiently absorbed through the gastrointestinal tract in chickens, resulting in a higher maximal plasma concentration ($C_{max} = 10.23 \pm$
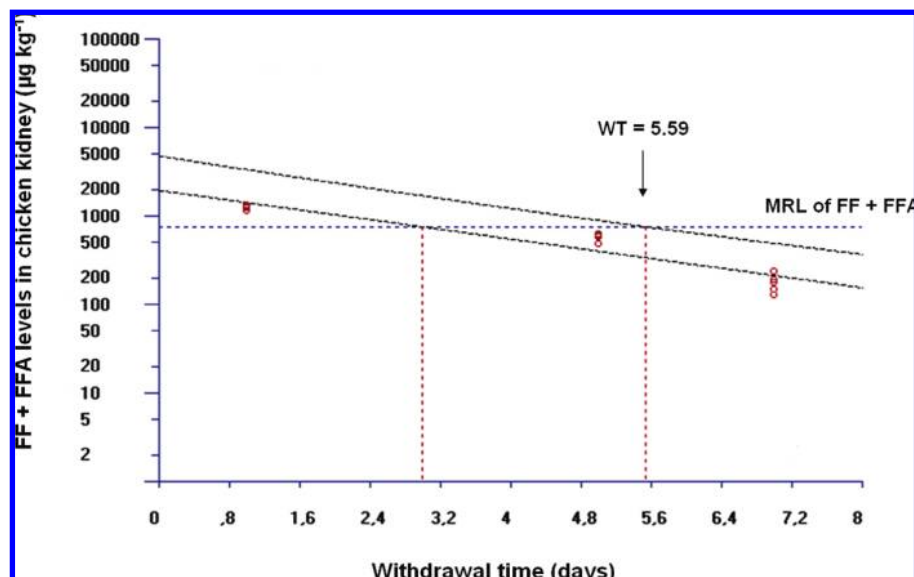
11054 *J. Agric. Food Chem.,* Vol. 56, No. 22, 2008

Anadón et al.

Figure 4. Plot of the withdrawal time calculation for florfenicol in chicken kidney at the time when the one-sided 95% upper tolerance limit is below the EU MRL for florfenicol (750 μg kg^{-1}) after oral administration of florfenicol (40 mg kg^{-1} body weight, daily for 3 days) [residue marker is the sum of florfenicol (FF) and its metabolite florfenicol-amine (FFA)].

1.67 μg mL^{-1}) and a shorter T_{max} (0.63 $\pm$ 0.07 h) to values reported previously in the literature for poultry (3.50 $\pm$ 1.13 and 6.79 $\pm$ 1.38 μg mL^{-1} at 0.9 $\pm$ 0.43 and 1.35 $\pm$ 0.43 h after oral dose of 15 and 30 mg kg^{-1}, respectively) (*31*). This may be due to the biases introduced by individual animals or pharmaceutical factors in the oral formulation. The mean maximal plasma concentration (10.23 $\pm$ 1.67 μg mL^{-1}) in this study was similar to those of studies in which florfenicol administered at the same oral dose (20 mg kg^{-1}) yield a C_{max} of 10.84 $\pm$ 2.71 μg mL^{-1} in pigs (*17*). Oral bioavailability of florfenicol was 87 $\pm$ 16% in chickens of our study, which was also into the range reported in pigs (99.57 $\pm$ 19.33%) (*17*), administering an amylum-based drug mixture (containing 15% florfenicol) diluted in saline solution. In this study, the oral bioavailability of florfenicol (87%) was higher than that of 78% reported in our preliminary study (*32*) using an analytical method with a different extraction procedure. In the current study, effective blood concentrations against microorganisms were achieved in a relatively short time (10−20 min) and were maintained up to 12 h after oral administration. Drug concentrations in plasma after 10 and 20 min were 3.42 $\pm$ 0.85 and 9.31 $\pm$ 1.96 μg mL^{-1}, and plasma drug concentrations exceeded 1.27 $\pm$ 0.18 and 1.02 $\pm$ 0.18 μg mL^{-1} for 8 and 12 h, respectively. In our study, the similar $t_{\beta\ 1/2}$ (8.34 $\pm$ 0.64) after oral dosing compared to the i.v. administration demonstrated that in chickens the plasma disposition of florfenicol after oral administration is not conditioned by the absorption process.

Florfenicol is metabolized in chickens. Florfenicol-amine is the major metabolite in ruminants (*10*). This metabolite represented 35% of the parent drug plasma concentrations. The rate of elimination of florfenicol-amine ($t_{\beta\ 1/2}$ = 9.00 $\pm$ 0.86 h) after oral florfenicol administration was not statistically different to that of florfenicol ($t_{\beta\ 1/2}$ = 8.34 $\pm$ 0.64).

Antimicrobial dosage regimen recommendations for bacteriostatic drugs are typically based on maintaining plasma concentrations above the minimum inhibitory concentration (MIC) values for the bacterial pathogen throughout the dosing interval. Both experimental and clinical studies indicate that, to obtain the optimal effects of time-dependent antibacterial drugs, such as florfenicol, plasma drug concentrations do not need to exceed the MIC several fold. It is the period for which

the plasma concentration of drug exceeds the MIC (T > MIC) that correlates best with the outcome of therapy (*42*). For drugs acting by a time-dependent mechanism, two criteria for setting dosing schedules seem to be an absolute requirement: C_{max} should exceed twice the MIC, and the plasma concentration should exceed the MIC for the whole of the interdose interval (*43*), or for at least half of the interdose interval (*44*). Florfenicol has a high potency and a broad spectrum of activity against a number of bacterial pathogens including the primary bacterial pathogens involved with chicken infections, such as *E. coli*, *Salmonella* spp., *Pasteurella multocida*, *Pasteurella hemolytica*, *Haemophilus paragallinarum*, *Haemophilus gallinarum*, and others. The MICs of florfenicol for bacteria isolates from poultry have not yet been determined. On the basis of the MIC values at which 90% of the isolates are inhibited (MIC$_{90}$) for bacteria from fish, swine, calves, cows, and humans, concentrations of florfenicol range from 0.25 to 2 μg mL^{-1}, with the majority of values at 1 μg mL^{-1} having showed high efficacy against most bacteria (*8, 9, 18, 20, 45, 46*). In the present study, single oral administration of 20 mg kg^{-1} body weight in healthy chickens produced a maximum blood concentration of 10.23 $\pm$ 1.67 μg mL^{-1}. This concentration exceeds the MICs of florfenicol for most susceptible bacteria. Florfenicol was detected in chicken plasma at concentrations higher than 1 μg mL^{-1} for approximately 12 h. Therefore, on the basis of the present results, we suggest that florfenicol should be given twice a day at a dosage of 20 mg kg^{-1} body weight to maintain therapeutic concentrations.

Tissue depletion of florfenicol and its metabolite florfenicol-amine after daily oral administration of florfenicol (40 mg kg^{-1} body weight for 3 consecutive days) was also determined. The dosage regimen of 40 mg kg^{-1} body weight for 3 consecutive days was used because the results presented here suggests from the integration of *in vitro* pharmacodynamics and *in vivo* pharmacokinetics that the drug should be administered orally at 20 mg kg^{-1} every 12 h (or alternatively at 40 mg kg^{-1} every 24 h), not only to guarantee clinical efficacy but also to minimize the selection and spread of resistant pathogens. The present work is the first to describe the residue tissue depletion of florfenicol and its major metabolite florfenicol-amine in edible chicken tissues using a validated HPLC method. Florfenicol and

Plasma and Tissue Depletion of Florfenicol in Chickens

J. Agric. Food Chem., Vol. 56, No. 22, 2008   **11055**

florfenicol-amine concentrations in kidney, liver, muscle, and skin + fat tissues were high initially and decreased over time. Concentrations of florfenicol-amine in tissues, except kidney, were so much smaller than those detected for the parent drug florfenicol. A total of 5 days after the last dose, the mean florfenicol and florfenicol-amine concentrations in all tissues were below the MRL (**Table 3**). In a preliminary tissue distribution study of florfenicol (*33*), the mean tissue concentration of florfenicol and florfenicol-amine also declined to mean values below MRL at 5 days after oral administration of 20 mg kg^{-1} for 3 consecutive days. This indicates that florfenicol is not removed from the body at a slower rate when dosed at the higher level (40 mg kg^{-1}). It can be assumed that there are no dose-dependent differences in tissue drug distribution and elimination rate between those two doses. The study of plasma concentrations in chickens after 15 and 30 mg kg^{-1} also revealed the same result (*31*). AUCs were proportional to the dose, but other parameters, such as V_c, V_{ss}, CL, $t_{\alpha\ 1/2}$, and $t_{\beta\ 1/2}$ were not dose-dependent (*31*).

From a public health viewpoint, it is important to know about the persistence of a drug and its active metabolites in edible tissues when it is administered for therapeutic purposes in food-producing animals. The next step is to define the withdrawal time necessary to ensure that the residues being monitored will fall below the established MRL or tolerance. Numerous experimental designs and a statistical approach are used to establish the withdrawal time. The European Medicines Evaluation Agency (EMEA) recommends use of a linear regression technique as the method of choice (*40*). In our study, taking into account MRLs in chickens and considering the marker residue and the sum of florfenicol and its metabolites measured as florfenicol-amine, the calculated withdrawal time was 6 days. It is important to consider this pre-slaughter withdrawal interval in light of an overall risk-benefit assessment for consumers of chicken and chicken products. This withdrawal time of 6 days allow for the concentrations that correspond to the sum of concentrations of florfenicol and florfenicol-amine in edible tissues to be less than the EU MRL (*34*).

Our study provides data for a more prudent use of florfenicol in chickens, suggesting a possible rational dosing and a withdrawal time after treatment to guarantee safety in foods for the consumers.

ACKNOWLEDGMENT

LITERATURE CITED

(1) Cannon, M.; Harford, S.; Davies, J. A comparative study on the inhibitory actions of chloramphenicol, thiamphenicol and some fluorinated derivatives. *Antimicrob. Agents Chemother.* **1990**, *26*, 307–317.

(2) Shaw, W. V. Chloramphenicol acetyltransferase, enzymology and molecular biology. *Crit. Rev. Biochem.* **1983**, *14*, 1–46.

(3) Murray, I. A.; Shaw, W. V. *O*-Acetyltransferases for chloramphenicol and other natural products. *Antimicrob. Agents Chemother.* **1997**, *41*, 1–6.

(4) Neu, H. C.; Fu, K. P. In vitro activity of chloramphenicol and thiamphenicol analogs. *Antimicrob. Agents Chemother.* **1980**, *18*, 311–316.

(5) Syriopoulou, V. P.; Harding, A. L.; Goldmann, D. A.; Smith, A. L. In vitro antibacterial activity of fluorinated analogs of chloramphenicol and thiamphenicol. *Antimicrob. Agents Chemother.* **1981**, *19*, 294–297.

(6) Yunis, A. A. Chloramphenicol: Relation of structure to activity and toxicity. *Annu. Rev. Pharmacol. Toxicol.* **1988**, *28*, 83–100.

(7) Sams, R. A. Florfenicol: Chemistry and metabolism of a novel broad-spectrum antibiotic. *Tieraerztl. Umschau* **1995**, *50*, 703–707.

(8) Graham, R.; Palmer, D.; Pratt, B. C.; Hart, C. A. In vitro activity of florfenicol. *Eur. J. Clin. Microbiol. Infect. Dis.* **1988**, *7*, 691–694.

(9) Ueda, Y.; Suenaga, I. In vitro antibacterial activity of florfenicol against *Actinobacillus pleuropneumoniae*. *J. Vet. Med. Sci.* **1955**, *57*, 363–364.

(10) Sams, R. A. Florfenicol: Chemistry and metabolism of a novel broad-spectrum antibiotic. In Proceedings of the 18th World Buiatrics Congress, Bologna, Italy, 1994; pp 13−17.

(11) Anonymous. Florfenicol (Extension to chicken)−Summary report. In EMEA/MRL/589/99-FINAL, Committee for Veterinary Medicinal Products/The European Agency for the Evaluation of Medicinal Products, Canary Wharf, London, U.K., 1999; pp 1−3.

(12) Nordmo, R.; Varma, K. J.; Sutherland, I. H.; Brokken, E. S. Florfenicol in Atlantic salmon, *Salmo salar* L.: Field evaluation of efficacy furunculosis in Norway. *J. Fish Dis.* **1994**, *17*, 239–244.

(13) Booker, C. W.; Jim, G. K.; Guichon, P. T.; Schunicht, O. C.; Thorlakson, B. E.; Lockwood, P. W. Evaluation of florfenicol for the treatment of undifferentiated fever in feedlot calves in western Canada. *Can. Vet. J.* **1997**, *38*, 555–560.

(14) Sheldon, I. M. A field trial of florfenicol for enteric disease in calves. *Cattle Pract.* **1997**, *5*, 195–198.

(15) De Craene, B. A.; Deprez, P.; D'Haese, E.; Nelis, H. J.; van den Bossche, W.; De Leenheer, A. P. Pharmacokinetics of florfenicol in cerebrospinal fluid and plasma of calves. *Antimicrob. Agents Chemother.* **1997**, *41*, 1991–1995.

(16) El-Banna, H. A. Pharmacokinetics of florfenicol in normal and *Pasteurella*-infected Muscovy ducks. *Br. Poult. Sci.* **1998**, *39*, 492–496.

(17) Liu, J.; Fung, K. F.; Chen, Z.; Zeng, Z.; Zhang, J. Pharmacokinetics of florfenicol in healthy pigs and in pigs experimentally infected with *Actinobacillus pleuropneumoniae*. *Antimicrob. Agents Chemother.* **2003**, *47*, 820–823.

(18) Varma, K. J.; Adams, P. E.; Powers, T. E.; Powers, J. D.; Lamendola, J. F. Pharmacokinetics of florfenicol in veal calves. *J. Vet. Pharmacol. Ther.* **1986**, *9*, 412–425.

(19) Adams, P. E.; Varma, K. J.; Powers, T. E.; Lamendola, J. F. Tissue concentrations and pharmacokinetics of florfenicol in male veal calves given repeated doses. *Am. J. Vet. Res.* **1987**, *48*, 1725–1732.

(20) Lobell, R. D.; Varma, K. J.; Johnson, J. C.; Sams, R. A.; Gerken, D. F.; Ashcraft, S. M. Pharmacokinetics of florfenicol following intravenous and intramuscular doses to cattle. *J. Vet. Pharmacol. Ther.* **1994**, *17*, 253–258.

(21) Bretzlaff, K. N.; Neff-Davis, C. A.; Ott, R. S.; Koritz, G. D.; Gustafsson, B. K.; Davis, L. E. Florfenicol in non-lactating dairy cows: Pharmacokinetics, binding to plasma proteins, and effects on phagocytosis by blood neutrophils. *J. Vet. Pharmacol. Ther.* **1987**, *10*, 233–240.

(22) Soback, S.; Paape, M. J.; Filep, R.; Varma, K. J. Florfenicol pharmacokinetics in lactating cows after intravenous, intramuscular and intramammary administration. *J. Vet. Pharmacol. Ther.* **1995**, *18*, 413–417.

(23) McKellar, Q. A.; Varma, K. J. Pharmacokinetics and tolerance of florfenicol in equidae. *Equine Vet. J.* **1996**, *28*, 209–213.

(24) Lavy, E.; Ziv, G.; Soback, S.; Glickman, A.; Winkler, M. Clinical pharmacology of florfenicol in lactating goats. *Acta Vet. Scand.* **1991**, *87* (Supplement), 133–136.

(25) Voorspoels, J.; D'Haese, E.; De Craene, B. A.; Vervaet, C.; De Riemaecker, D.; Deprez, P.; Nelis, H.; Remon, J. P. Pharmacokinetics of florfenicol after treatment of pigs with single oral or intramuscular doses or with medicated feed for three days. *Vet. Rec.* **1999**, *145*, 397–399.

(26) Jiang, H.; Zeng, Z.-L.; Chen, Z.-L.; Liu, J. J.; Fung, K.-F. Pharmacokinetics of florfenicol in pigs following intravenous, intramuscular or oral administration and the effects of feed intake on oral dosing. *J. Vet. Pharmacol. Ther.* **2006**, *29*, 153–156.

(27) Jianzhong, S.; Xiubo, L.; Haiyang, J.; Walter, H. H. Bioavailability and pharmacokinetics of florfenicol in healthy sheep. *J. Vet. Pharmacol. Ther.* **2004**, *27*, 163–168.

(28) Lane, U. M.; Villarroel, A.; Wetzlich, S. E.; Clifford, A.; Taylor, I.; Craigmill, A. L. Intravenous and subcutaneous pharmacokinetics of florfenicol in sheep. *J. Vet. Pharmacol. Ther.* **2004**, *27*, 191–196.

(29) Afifi, N. A.; El-Sooud, K. A. Tissue concentrations and pharmacokinetics of florfenicol in broiler chickens. *Br. Poult. Sci.* **1997**, *38*, 425–428.

(30) Shen, J.; Wu, X.; Hu, D.; Jiang, H. Pharmacokinetics of florfenicol in healthy and *Escherichia coli*-infected broiler chickens. *Res. Vet. Sci.* **2002**, *73*, 137–140.

(31) Shen, J.; Hu, D.; Wu, X.; Coats, J. R. Bioavailability and pharmacokinetics of florfenicol in broiler chickens. *J. Vet. Pharmacol. Ther.* **2003**, *26*, 337–341.

(32) Martínez-Larrañaga, M. R.; Rios, A.; Martínez, M. A.; Martínez, M.; Isea, G.; Anadón, A. Oral bioavailability of florfenicol and its metabolite florfenicol amine in chickens. In 9th International EAVPT Congress, Lisboa, Portugal, July 13−18, 2003. Abstract in *J. Vet. Pharmacol. Ther.* **2003**, *26* (Supplement 1), 115.

(33) Martínez, M. A.; Rios, A.; Martínez, M.; Caballero, V.; Ares, I.; Castellano, V.; Diaz, M. J.; Martínez-Larrañaga, M. R.; Anadón, A. Tissue distribution and depletion of florfenicol in chickens. In 10th International EAVPT Congress, Torino, Italy, Sept 17−22, 2006. Abstract in *J. Vet. Pharmacol. Ther.* **2006**, *29* (Supplement 1), 155.

(34) Anonymous. Florfenicol (extension to all food producing species)−Summary report. In EMEA/MRL/822/902-FINAL, Committee for Veterinary Medicinal Products/The European Agency for the Evaluation of Medicinal Products, Canary Wharf, London, U.K., 2002; pp 1−2.

(35) Sanderson, D. M. A note on glycerol formal as a solvent in toxicity testing. *J. Pharm. Pharmacol.* **1959**, *11*, 150–156.

(36) Anonymous. Guideline on validation of analytical procedures: Methodology (VICH GL2). In CVMP/VICH/591/98-FINAL, Committee for Veterinary Medicinal Products/The European Agency for the Evaluation of Medicinal Products, Canary Wharf, London, U.K., 1998; pp 1−10.

(37) Yamaoka, K.; Nakagawa, T.; Uno, T. Application of Akaike's information criterion (AIC) in the evaluation of linear pharmacokinetic equations. *J. Pharmacokinet. Biopharm.* **1978**, *6*, 165–175.

(38) Wagner, J. G. *Fundamentals of Clinical Pharmacokinetics*, 1st ed.; Drug Intelligence Publications: Hamilton, IL, 1975.

(39) Wagner, J. G. Linear pharmacokinetic equations allowing direct calculation of many needed pharmacokinetic parameters from the coefficients and exponents of polyexponential equations which have been fitted to the data. *J. Pharmacokinet. Biopharm.* **1976**, *4*, 443–467.

(40) Anonymous. Updated Application Software relating to Note for Guidance on Approach towards Harmonisation of Withdrawal Periods for Meat. In EMEA/CVMP/563/02, Committee for Veterinary Medicinal Products/The European Agency for the Evaluation of Medicinal Products, Canary Wharf, London, U.K., 2002; p 1.

(41) Schwartz, S.; Kehrenberg, C.; Doublet, B.; Cloeckaert, A. Molecular basis of bacterial resistance to chloramphenicol and florfenicol. *FEMS Microbiol. Rev.* **2004**, *28*, 519–542.

(42) Hyatt, J. M.; Mckinnon, P. S.; Zimmer, G. S.; Schentag, J. J. The importance of pharmacokinetic/pharmacodynamic surrogate markers to outcome. Focus on antibacterial agents. *Clin. Pharmacokinet.* **1995**, *28*, 143–160.

(43) Lees, P.; Aliabadi, F. S. Rational dosing of antimicrobial drugs: Animals versus humans. *Int. J. Antimicrob. Agents* **2002**, *19*, 269–284.

(44) Craig, M. A. Pharmacokinetic/pharmacodynamic parameters: Rationale for antibacterial dosing of mice and men. *Clin. Infect. Dis.* **1998**, *26*, 1–12.

(45) Inglis, V.; Richards, R. H. The in vitro susceptibility of *Aeromonas salmonicida* and other fish-pathogenic bacteria to 29 antimicrobial agents. *J. Fish Dis.* **1991**, *14*, 641–650.

(46) Priebe, S.; Schwarz, S. In vitro activities of florfenicol against bovine and porcine respiratory tract pathogens. *Antimicrob. Agents Chemother.* **2003**, *47*, 2703–2705.