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Maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 10: *Quinolones: flumequine and oxolinic acid*

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Abstract

The specific concentrations of flumequine and oxolinic acid in non-target feed for food-producing animals, below which there would not be an effect on the emergence of, and/or selection for, resistance in bacteria relevant for human and animal health, as well as the specific antimicrobial concentrations in feed which have an effect in terms of growth promotion/increased yield were assessed by EFSA in collaboration with EMA. Details of the methodology used for this assessment, associated data gaps and uncertainties, are presented in a separate document. To address antimicrobial resistance, the Feed Antimicrobial Resistance Selection Concentration (FARSC) model developed specifically for the assessment was applied. However, due to the lack of data on the parameters required to calculate the FARSC, it was not possible to conclude the assessment until further experimental data are available. To address growth promotion, data from scientific publications obtained from an extensive literature review were used. No suitable data for the assessment were available. It was recommended to carry out studies to generate the data that are required to fill the gaps which prevented the calculation of the FARSC for these antimicrobials.

Keywords: flumequine, oxolinic acid, antimicrobial resistance, sub-inhibitory concentration, growth promotion, yield increase, food-producing animals

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1. Introduction

The European Commission requested the European Food Safety Authority (EFSA) to assess, in collaboration with the European Medicines Agency (EMA), (i) the specific concentrations of antimicrobials resulting from cross-contamination in non-target feed for food-producing animals, below which there would not be an effect on the emergence of, and/or selection for, resistance in microbial agents relevant for human and animal health (term of reference 1, ToR1), and (ii) the levels of the antimicrobials which have a growth promotion/increase yield effect (ToR2). The assessment was requested to be conducted for 24 antimicrobial active substances specified in the mandate.[1]

For the different substances (grouped by class if applicable)[1], separate scientific opinions included within the 'Maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed' series (Scientific Opinions Part 2 - Part 13, EFSA BIOHAZ Panel, 2021b–l – see also the Virtual Issue; for practical reasons, they will be referred as 'scientific opinion Part X' throughout the current document) were drafted. They present the results of the assessments performed to answer the following questions: *Assessment Question 1 (AQ1),* which are the specific antimicrobial concentrations in non-target feed below which there would not be emergence of, and/or selection for, resistance in the large intestines/rumen, and *AQ2*: which are the specific antimicrobial concentrations in feed of food-producing animals that have an effect in terms of growth promotion/increased yield. The assessments were performed following the methodology described in Section 2 of the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (EFSA BIOHAZ Panel, 2021a, see also the Virtual Issue). The present document reports the results of the assessment for the quinolones: flumequine and oxolinic acid.

1.1. Background and Terms of Reference as provided by the requestor

The background and ToRs provided by the European Commission for the present document are reported in Section 1.1 of the Scientific Opinion "Part 1: Methodology, general data gaps and uncertainties" (See also the Virtual Issue).

1.2. Interpretation of the Terms of Reference

The interpretation of the ToRs, to be followed for the assessment is in Section 1.2 of the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (see also the Virtual Issue).

1.3. Additional information

1.3.1. Short description of the class/substance

Quinolones are broad-spectrum bactericidal antimicrobials that share a bicyclic core structure related to the substance 4-quinolone. The first quinolone introduced was nalidixic acid (1962). Like nalidixic acid, oxolinic acid is a non-fluorinated quinolone. Later, more potent fluorinated quinolones were introduced, with flumequine being the first fluoroquinolone (Willems, 1986). Both oxolinic acid and flumequine, belong to the first-generation quinolones and are today used exclusively for animals. Quinolones interfere with bacterial DNA replication by targeting two essential bacterial type II topoisomerase enzymes, DNA gyrase and DNA topoisomerase IV, enzymes that are heterotetramers with two subunits. Quinolones can differ in their relative potency for the two enzymes depending on the bacterial species and the specific quinolone. These enzymes catalyse a DNA double-strand break, passing another DNA strand through the break, and resealing the break. Quinolones bind reversibly to the complexes of DNA, with gyrase and topoisomerase IV, blocking the resealing of the DNA double-strand break, thereby serving as a barrier to DNA replication and transcription, ultimately resulting in lethal double-strand DNA breaks (Drlica and Zhao, 1997; Hawkey, 2003; Fàbrega et al., 2009; Aldred et al., 2014; Bush et al., 2020). As a result of the DNA strand breaks quinolones also trigger the bacterial SOS responses to repair DNA damage (Drlica and Zhao, 1997; Qin et al., 2015).

Oxolinic acid has activity against several Enterobacterales, including *Escherichia coli* and indole-positive *Proteus* species, but demonstrates only little activity against *P. aeruginosa*. It also possesses *in vitro* activity for *Staphylococcus aureus* but has no activity for other Gram-positive aerobic cocci

[1] Aminoglycosides: apramycin, paromomycin, neomycin, spectinomycin; Amprolium; Beta-lactams: amoxicillin, penicillin V; Amphenicols: florfenicol, thiamphenicol; Lincosamides: lincomycin; Macrolides: tilmicosin, tylosin, tylvalosin; Pleuromutilins: tiamulin, valnemulin; Sulfonamides; Polymyxins: colistin; Quinolones: flumequine, oxolinic acid; Tetracyclines: tetracycline, chlortetracycline, oxytetracycline, doxycycline; Diaminopyrimidines: trimethoprim.

(Neussel and Linzenmeier, 1973; Gleckman et al., 1979). Oxolinic acid is about 10-fold more active than the structurally related nalidixic acid (Staudenbauer, 1976). Like nalidixic and oxolinic acid, flumequine is generally more active against enterobacteria than other bacteria. *In vitro* activity of flumequine is considerably higher than nalidixic acid and slightly less than for oxolinic acid against both nalidixic acid-susceptible and -resistant *E. coli* strains: its activity was four times greater than that of nalidixic acid and one-half that of oxolinic acid (Greenwood, 1978).

1.3.2. Main use[2]

Flumequine and oxolonic acid are effective against Gram-negative bacteria including many fish pathogens, and they are among the most commonly used quinolones in aquaculture (Husevåg et al., 1991; Lunestad and Samuelsen, 2008).

The main target infections including those caused by atypical *Aeromonas salmonicida*, *Pasteurella* spp., *Pseudomonas anguilliseptica*, *Vibrio anguillarum*, *Vibrio ordalii*, *Tenacibaculum maritimum* (causing flexibacteriosis) and *Edwardsiella* spp. (Guardabassi et al., 2008; Alarcón et al., 2016; Rimstad et al., 2017; Scholz et al., 2018; Walde et al., 2019). Oxolinic acid is primarily used for eels, carp, goldfish, salmonids and turbot, and flumequine is used in salmonids and other marine species (Guardabassi et al., 2008).

In others food-producing animals, these antimicrobial substances are approved in products to be administered orally in calves, ruminants, pigs and poultry. The approved indications for use are treatment and metaphylaxis of digestives infections due to *E. coli* and *Salmonella enterica*, respiratory infections due to *Pasteurella multocida* and *Mannheimia haemolytica*. In pigs, urinary tract infections due to *E. coli* are also part of the indications.

1.3.3. Main pharmacokinetic data

The oral bioavailability (i.e. the fraction of the antimicrobials absorbed from the digestive tract to the plasma) of quinolones is relatively high in species with available data.

Flumequine

Oral bioavailability of flumequine is 57 ± 6% in chickens for fattening (Anadón et al., 2008), and in pre-ruminant calves, the values ranged between 55.7% and 100% depending on the dose (Ziv et al., 1986; Mevius et al., 1989). In Atlantic salmon (*Salmo salar*) held in seawater at 10°C, the bioavailability was 44.7% (Martinsen and Horsberg, 1995).

In cattle, about 55% of the administered radioactive-labelled flumequine was excreted in urine and 35% in faeces. Both in urine and faeces, the flumequine represented about 80% of the radioactivity measured, its hydroxylated metabolite 20% after oral route (EMEA/CVMP, 1999). However, the main urinary metabolite of flumequine is the glucuronide conjugate (approximately 40% recovery within 48 h of intravenous injection). Only 3.2–6.5% of flumequine is excreted in the urine unchanged. After oral administration, a 'first-pass' effect was observed, with a significant increase in the excretion of conjugated drug (Mevius et al., 1990).

Oxolinic acid

Oral bioavailability of oxolinic acid is of 82% in healthy chickens, about 100% in diseased chickens (EMEA/CVMP, 2002) and 30.1% in Atlantic salmon held in seawater at 10°C (Martinsen and Horsberg, 1995). Bioavailability was described to be high in pigs and calves but no value was provided (EMEA/CVMP, 2002).

There are no available data on oxolinic acid metabolism and elimination in food-producing animal species.

1.3.4. Main resistance mechanisms

Resistance to quinolone can be conferred by a number of different mechanisms, including (i) target alterations (mutations in DNA gyrase and/or topoisomerase IV), (ii) reduced uptake due to porin mutations (most common in Gram-negative bacteria), (iii) increased efflux due to activation of various

[2] Antimicrobials are currently used in food-producing animal production for treatment, prevention and/or metaphylaxis of a large number of infections, and also for growth promotion in non-EU countries. In the EU, in future, use of antimicrobials for prophylaxis or for metaphylaxis is to be restricted as addressed by Regulation (EU) 2019/6 and use in medicated feed for prophylaxis is to be prohibited under Regulation (EU) 2019/4.

pumps, (iv) protection of the target molecules DNA by Qnr proteins and (v) modification of the quinolone by a variant of the common aminoglycoside-modifying acetyl-transferase AAC(6′)-Ib. Of these mechanisms, (i) and (ii) are due to chromosomal mutations, whereas (iii) can be due to either mutations in chromosomal regulatory genes or plasmid-encoded quinolone efflux pumps: OqxAB and QepA; (iv) and (v) are caused by plasmid-carried *qnr* or *aac* genes (Redgrave et al., 2014; Hooper and Jacoby, 2016; Bush et al., 2020). By themselves, these mechanisms confer relatively moderate increases in resistance and high-level resistance in clinical isolates is often the result of a step-wise accumulation of several mutations of small effect (e.g. in *E. coli* combinations of mutations in *gyrAB*-encoding DNA gyrase, *parCE*-encoding topoisomerase IV and *marR/acrR*-encoding repressors of the efflux pump AcrAB are generally present in isolates with high-level quinolone resistance) (Marcusson et al., 2009).

2. Data and methodologies

The data sources and methodology used for this opinion are described in a dedicated document, the Scientific Opinion "Part 1: Methodology, general data gaps and uncertainties" (see also the Virtual Issue).

3. Assessment

3.1. Introduction

As indicated in the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (see also the Virtual Issue), exposure to low concentrations of antimicrobials (including sub-minimum inhibitory concentrations (sub-MIC)) may have different effects on bacterial antimicrobial resistance evolution, properties of bacteria and in animal growth promotion. Some examples including emergence of, and selection for, antimicrobial resistance, mutagenesis, virulence and/or horizontal gene transfer (HGT), etc. for the antimicrobials under assessment are shown below.

3.1.1. Resistance development/spread due to sub-MIC concentrations of quinolones including flumequine and oxolinic acid: examples

Several studies have shown that sub-MIC levels of quinolones (in particular the ones used in human medicine) can cause resistance selection of both pre-existing resistant mutants as well as by *de novo* selection. In addition, because of their SOS-inducing effects quinolones can increase the mutation rate and the emergence of new resistance mutations. However, for the target antimicrobials, flumequine and oxolinic acid, there are only a few studies available, whereas there are many studies (examples summarised below) for other quinolones (mainly ciprofloxacin).

3.1.1.1. Effects of sub-MIC concentrations on selection for resistance and mutagenesis

- Exposure of six isolates of *Enterobacter aerogenes* (one isolate), *E. cloacae* (2), *Pseudomonas aeruginosa* (2) and *Klebsiella pneumoniae* (1) to sub-inhibitory concentrations of seven quinolones derivatives or related organic acids, including oxolinic acid, caused a stepwise decrease in susceptibility with all seven drugs, and the resistant cultures demonstrated a concomitant cross-resistance to the other antimicrobials. For oxolinic acid, depending on the bacteria (wild-type MIC varied from 0.4 for *E. aerogenes* to 5 mg/L for *K. pneumoniae*), the MIC increase varied between 2 and 4 step increase (Barry and Jones, 1984).
- Exposure to sub-inhibitory concentrations of oxolinic acid induced genetic instability (deletions and/or amplifications of DNA sequences) in *Streptomyces ambofaciens*. The concentrations tested were 0–10 mg/L, and with 7.5 mg/L, the frequency of the studied mutants increased almost to 100%, even at a high survival rate (55%). The instability could be related to the induction of the SOS response or directly related to the *gyrA* (Volff et al., 1993).
- Exposure to low-levels (0.1 mg/L) of different antimicrobials including flumequine selected for low-levels resistance mutants of virulent wild-type *Aeromonas salmonicida* (wild-type isolates tested had MIC to flumequine ranging from 0.05 to 2 mg/L, with all isolates mutants were observed). Those mutants also lacked exoprotease activity. The effects observed seemed to be related to point mutations and outer membrane protein changes (Wood et al., 1986).

For other quinolones:

- Exposure to ciprofloxacin in *E. coli* at 1/230 of MIC caused enrichment of a pre-existing *gyrA* mutant. The calculated MSC for ciprofloxacin was 0.1 ug/L (Gullberg et al., 2011).
- Exposure to ciprofloxacin in *E. coli* at 1/5 of MIC selected *de novo* for stable, low-level resistance due to mutations in *gyrA, marR* and *acrR* (Ching and Zaman, 2020).
- Exposure to ciprofloxacin in *P. aeruginosa* growing planktonically or in biofilms at 1/2 to 1/5 of MIC selected for resistant mutants (Ahmed et al., 2018).
- Exposure to ciprofloxacin (at 0.01 mg/L) in *Aeromonas* spp. at 1/100 of the clinical resistance breakpoint resulted in *de novo* selection for resistance (Beka et al., 2018).
- Exposure to ciprofloxacin/levofloxacin at sub-inhibitory levels (concentration not exactly stated) in *S. aureus* selected for increased resistance (Tattevin et al., 2009).
- Exposure to ciprofloxacin/levofloxacin *P. aeruginosa* at 1/3 to 1/7 of MIC caused a 2- to 7-fold increase in mutation frequency (Tanimoto et al., 2008).
- Exposure to norfloxacin in *E. coli* at sub-inhibitory levels (1/4 of MIC) resulted in a genome-wide increase in mutation rate. This response was associated with an increased expression of error-prone DNA polymerases (Long et al., 2016).
- Exposure to ciprofloxacin in *Mycobacterium fortuitum* at 1/8 to 1/2 of MIC caused a 2- to 120-fold increases in mutation frequencies (Gillespie et al., 2005).
- Exposure to norfloxacin in *S. pneumoniae* at sub-inhibitory levels (1/2 of MIC) caused an up to 5-fold increase in mutation frequency (Henderson-Begg et al., 2006).

3.1.1.2. Effects of sub-MIC concentrations on horizontal gene transfer and virulence

Several studies have shown that sub-inhibitory levels of quinolones can stimulate HGT and affect expression (reducing in most studies but sometimes also increasing) of virulence-associated functions.

- Exposure to low concentrations of oxolinic acid that were just inhibitory to *Azotobacter vinelandii* growth (3 mg/L; 84% growth inhibition) promoted the production of the catechol siderophores, azotochelin and aminochelin, in the presence of normally repressive concentrations of Fe^{3+}. These effects were subtle, affecting the iron regulation of catechol siderophore synthesis without severe inhibition of growth (Page and Patrick, 1988).
- Exposure to sub-MIC concentrations of oxolinic acid (how far below MIC not reported) to different *E. coli* strains, induced filaments in most of the bacterial cultures, and inhibition of haemagglutination and/or adhesion, with the effect being strain-specific (Hammami et al., 1987).

For other quinolones:

- Exposure to ciprofloxacin or levofloxacin at 1/8 to 1/32 of MIC caused an upregulation of expression of conjugation related genes on the PR4 plasmid associated with an approximately 10-fold increase in conjugation frequency of the plasmid from *E. coli* to *P. aeruginosa* (Shun-Mei et al., 2018).
- Sub-MIC levels (1/2 to 1/4 of MIC) of enoxacin, lomefloxacin and ciprofloxacin in *S. aureus, E. coli*, *P. aeruginosa*, *S.* Typhimurium and *Y. pseudotuberculosis* may reduce the virulence by inhibition of adherence, hydrophobicity, enhanced susceptibility to phagocytosis by PMNs, or elimination of the ability to produce toxins (Sonstein and Burnham, 1993).
- Sub-MIC levels (1/2 of MIC) exposure of ofloxacin and moxifloxacin in fluoroquinolone resistant *C. difficile* resulted in upregulation of colonisation factors (Denève et al., 2009).
- Exposure to sub-MIC levels (1/4 of MIC) of ciprofloxacin in resistant *S. aureus* led to increased expression of fibronectin-binding proteins and adhesion (Bisognano et al., 1997).
- Exposure to sub-MIC levels (1/2 to 1/4 of MIC) of ciprofloxacin in enterotoxigenic *E. coli* decreased adhesiveness and toxin production (Oviedo et al., 2000).
- Exposure to sub-MIC levels (1/4 of MIC) of ciprofloxacin in *S. saprophyticus* resulted in increased adherence to abiotic and biotic surfaces (Erdeljan et al., 2012).
- Exposure to sub-MIC levels (1/8 to 1/64 of MIC) of gatifloxacin in *E. coli* and *S. aureus* reduced adhesiveness, haemagglutination, hydrophobicity and swarming whereas phagocytosis was increased (Dal Sasso et al., 2002)
- Sub-MIC levels (1/4 of MIC) of ciprofloxacin in *P. aeruginosa* inhibited quorum sensing mechanisms thereby causing a reduction of biofilm formation and virulence (Gupta et al., 2016).

In summary, these studies show that sub-MIC concentrations (from 1/230 to 1/2 of MIC depending on the specific compound and bacterial species) of quinolones can have a number of effects, including selection for *de novo* resistance, enrichment of pre-existing resistance, increased mutagenesis, stimulation of HGT as well as increasing/decreasing virulence. With regard to the concentrations of quinolones where the biological effects are observed, the concentration can be as low as 0.1 μg/L even though the majority of the effects reported are seen at higher concentrations.

3.2. ToR1. Estimation of the antimicrobial levels in non-target feed that would not result in the selection of resistance: Feed Antimicrobial Resistance Selection Concentration (FARSC)

As explained in the Methodology Section (2.2.1.3) of the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (see also the Virtual Issue), the estimation of this value for these two quinolones for different animal species, if suitable data were available, would follow a two-step approach as described below:

The first step would be the calculation of the predicted minimal selective concentration (PMSC) for flumequine and oxolinic acid as indicated in Table 1. However, no MSC data required to do the calculations is available those substances.

Table 1: Calculation of the flumequine and oxolinic acid predicted minimal selective concentration (PMSC)

Antimicrobial (all values in mg/L)	MIC_{test}	MSC_{test}	MIC_{test}/MSC_{test} ratio	MIC_{lowest}	Predicted MSC (PMSC) for most susceptible species ($MIC_{lowest}/MIC_{test}/MSC_{test}$)
Flumequine	NA	NA	NA	0.064	NA
Oxolinic acid	NA	NA	NA	NA	NA

MIC: minimum inhibitory concentration; MSC: minimal selective concentration; MSC_{test}: MSC experimentally determined; MIC_{lowest}: lowest MIC data for flumequine calculated based on data from the EUCAST database as described in Bengtsson-Palme and Larsson (2016), see Methodology Section 2.2.1.3.1.1 in the Scientific Opinion Part 1. No MIC data for oxolinic acid in the EUCAST database (EUCAST database (https://mic.eucast.org/search/) last accessed 15 May 2021); NA: not available.

Due to the lack of PMSC, no FARSC ($FARSC_{intestine}$ and $FARSC_{rumen}$) could be calculated. If PMSC was available, the FARSC corresponding to the maximal concentrations in feed would be calculated for each species from the equations below (for details, see Section 2.2.1.3.2 of the Scientific Opinion Part 1; see also the Virtual Issue) by including specific values for flumequine and oxolinic acid:

$$FARSC_{intestine}\ (\text{mg/kg feed}) = \frac{\text{PMSC} \times \text{daily faeces}}{(1-I) \times (1-F+F \times GE) \times \text{daily feed intake}}$$

$$FARSC_{rumen}\ (\text{mg/kg feed}) = \frac{\text{PMSC} \times \text{volume of rumen}}{(1-I) \times \text{daily feed intake}}$$

With daily faeces being the daily fresh fael output in kg, I the inactive fraction, F the fraction available, GE is the fraction of the antimicrobial that is secreted back into the intestinal tract for elimination, after initially being absorbed into the bloodstream, and daily feed intake being the daily dry-matter feed intake expressed in kg.

Flumequine

Flumequine is well absorbed in chickens and in pigs. However, a fraction of the absorbed antimicrobial is afterwards eliminated in the intestines. If we consider that 35% is eliminated in faeces and that flumequine represented about 80% (measured by radioactive-labelled flumequine), then 28% (35 × 0.8) of the absorbed antimicrobial would be eliminated in the intestines. Among these drugs eliminated in the intestines, a fraction might be conjugated and inactivated as in urine but there are no data available. The value of 28% corresponds to a scenario leading to lowest FARSC in which the drug secreted in the intestine is fully active. There is no information on the potential binding of flumequine to the intestinal content.

The values of *F*, *GE* and *I* extracted from literature for the calculations of FARSC are summarised in Table 2. The first set of values (scenario 1) corresponds to the average of published values while

scenario 2 corresponds to scenario that would lead to lower FARSC and scenario 3 to scenario that would lead to higher FARSC.

Table 2: Pharmacokinetic (PK) values used for the calculation of Feed Antimicrobial Resistance Selection Concentration (FARSC) of flumequine for the different animal species

Flumequine data	Scenario #1	Scenario #2	Scenario #3
Inactive fraction (*I*)	NA	–	–
Bioavailability (*F*) calves	0.9	0.55	1
Bioavailability (*F*) chickens	0.6	0.5	0.65
Gastrointestinal elimination (*GE*) calves	NA	0.3	–
Gastrointestinal elimination (*GE*) chickens	NA	–	–

Inactive fraction (*I*) is the fraction of antimicrobial that would not have any activity on bacteria. Bioavailability (*F*) is the fraction of antimicrobial that is absorbed from the digestive tract to the blood. Gastrointestinal elimination (*GE*) is the fraction of the antimicrobial that is secreted back into the intestinal tract for elimination, after initially being absorbed into the bloodstream. The fraction remaining in the digestive tract and that could be available for the bacteria is equal to $(1 - F + F \times GE)$. NA: not available.

Due to the absence of MSC and other PK data, the estimation of the FARSC for flumequine was not possible.

Oxolinic acid

The oral bioavailability of oxolinic acid is only available for chickens and there is no published value for other species. There are no data on the fate of oxolinic acid after absorption and especially on the metabolism before gut elimination in the food-producing animal species either.

In consequence, no PK parameter value, that could be used to calculate FARSC for oxolinic acid was proposed.

Due to the absence of MSC and other PK data, the estimation of the FARSC for oxolinic acid was not possible.

3.2.1. Associated data gaps and uncertainties

With regard to the uncertainties and data gaps described in the Scientific Opinion Part 1 (Sections 3.1 and 3.3; see also the Virtual Issue), we identified the following for the quinolones under assessment:

i) MSC data: no data for MSCs for oxolinic acid or flumequine are available. Data for ciprofloxacin are available, but due to the difference in resistance mechanisms, potency and PK, no extrapolations are possible.
ii) MIC data: MIC data only exist for few bacterial species for flumequine and are not available for oxolinic acid in EUCAST database (accessed on 15 May 2021).
iii) Bioavailability: data for oxolinic acid were only available for chickens. Quantitative data for flumequine are not available for each species.
iv) Fraction eliminated in gut: there is an intestinal secretion of flumequine but there is no information on the activity of the secreted drug. There are no quantitative data to consider this process for oxolinic acid.
v) Inactive fraction: no data on the possible binding of flumequine or oxolinic acid in digestive tract are available.
vi) Ruminants: no data are available for flumequine or oxolinic acid administered to ruminants by oral route.

3.2.2. Concluding remarks

Due to the lack of data on the parameters required to calculate the FARSC, it is not possible to conclude the ToR1 assessment until further experimental data are available.

3.3. ToR2. Specific antimicrobial concentrations in feed which have an effect in terms of growth promotion/increased yield

3.3.1. Flumequine

3.3.1.1. Literature search results

The literature search, conducted according to the methodology described in Section 2.2.2.1 of the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (see also the Virtual Issue), resulted in 366 papers mentioning flumequine and any of the food-producing animal species considered[3] and any of the performance parameters identified as relevant for the assessment of the possible growth-promoting effects of flumequine.[4] After removing the reports not matching the eligibility criteria, seven publications were identified.

3.3.1.2. Evaluation of the studies

The seven publications identified in the literature search were appraised for suitability for the assessment of the effects of flumequine on growth or yield of food-producing animals; this appraisal was performed by checking each study against a series of pre-defined exclusion criteria (see Section 2.2.2.2.1 of the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (see also the Virtual Issue).[5] None of the publications was considered suitable for the assessment because of several shortcomings identified in their design or in the reporting of the results. The list of excluded publications and their shortcomings are presented in Appendix A.1 (Table A.1).

3.3.1.3. Concluding remark

Owing to the lack of suitable data, levels of flumequine in feed which may have a growth promotion/production yield effect in any food-producing animal species could not be identified.

3.3.2. Oxolinic acid

3.3.2.1. Literature search results

The literature search, conducted according to the methodology described in Section 2.2.2.1 of the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (see also the Virtual Issue), resulted in 336 papers mentioning oxolinic acid and any of the food-producing animal species considered[3] and any of the performance parameters identified as relevant for the assessment of the possible growth-promoting effects of oxolinic acid.[4] After removing the reports not matching the eligibility criteria, three publications were identified.

3.3.2.2. Evaluation of the studies

The three publications identified in the literature search were appraised for suitability for the assessment of the effects of oxolinic acid on growth or yield of food-producing animals; this appraisal was performed by checking each study against a series of pre-defined exclusion criteria (see Section 2.2.2.2.1 of the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (see also the Virtual Issue).[5] None of the publications was considered suitable for the assessment because of several shortcomings identified in their designs or in the reporting of the results. The list of excluded publications and their shortcomings are presented in Appendix A.2 (Table A.2).

[3] Ruminants: growing and dairy (cattle, sheep, goats, buffaloes); pigs: weaned, growing and reproductive; equines; rabbits; poultry: chickens and turkeys for fattening, laying hens, turkeys for breeding, minor avian species (ducks, guinea fowl, geese, quails, pheasants, ostrich); fish: salmon, trout, other farmed fish (seabass, seabream, carp, other); crustaceans; other animal species.

[4] (i) Intake-related parameters: feed intake, feed/gain ratio, feed efficiency, feed intake/milk yield, feed intake/egg mass; (ii) Weight-related parameters: body weight, body weight gain; (iii) Carcass-related parameters: carcass weight, carcass yield, carcass chemical composition, relative weight of the (different sections of) intestine; (iv) Milk or egg production/quality: milk yield, fat/protein yield, egg production/laying rate, egg weight, egg mass; (v) Digestibility/utilisation of nutrients: utilisation of some nutrients (e.g. DM, Ca, P), digestibility; (vi) Health-related parameters: reduction of morbidity and/or mortality; (vii) Herd/flock related parameters; (viii) Other endpoints: e.g. intestinal morphological characteristics (*villi* height/width), changes in microbiota.

[5] The following exclusion criteria were applied: 'Combination of substances administered to the animals', 'Antimicrobial used different from the one under assessment', 'Administration via route different from oral', 'Use of the antimicrobial with a therapeutic scope', 'Animals subjected to challenges with pathogens', 'Animals in the study sick or not in good health, Zootechnical parameters not reported', 'Insufficient reporting/statistics', 'Other (indicate)'.

3.3.2.3. Concluding remark

Owing to the lack of suitable data, levels of oxolinic acid in feed which may have a growth promotion/production yield effect in any food-producing animal species could not be identified.

4. Conclusions

ToR1: to assess the specific concentrations of antimicrobials resulting from cross-contamination in non-target feed for food-producing animals, below which there would not be an effect on the emergence of, and/or selection for, resistance in microbial agents relevant for human and animal health.

AQ1. Which are the specific concentrations of flumequine and oxolinic acid in non-target feed below which there would not be emergence of, and/or selection for, resistance in the large intestines/ rumen?

- Due to the lack of data on the parameters required to calculate the Feed Antimicrobial Resistance Selection Concentration (FARSC) corresponding to the concentrations of those antimicrobials in non-target feed below which there would not be expected to be an effect on the emergence of, and/or selection for, resistance in microbial agents relevant for human and animal health, it is not possible to conclude until further experimental data are available.

ToR2: to assess which levels of the antimicrobials have a growth promotion/increase yield effect.

AQ2. Which are the specific concentrations of flumequine and oxolinic acid in feed of food-producing animals that have an effect in terms of growth promotion/increased yield?

With regard to flumequine:

- Owing to the lack of suitable data, levels of flumequine in feed which may have a growth promotion/production yield effect in any food-producing animal species could not be identified.

With regard to oxolinic acid:

- Owing to the lack of suitable data, levels of oxolinic acid in feed which may have a growth promotion/production yield effect in any food-producing animal species could not be identified.

The results from these assessments for the different animal species are summarised in Annex F (Tables F.1 and F.2) of EFSA BIOHAZ Panel, 2021a - Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (see also the Virtual Issue).

5. Recommendation

To carry out studies to generate the data that are required to fill the gaps which have prevented calculation of the FARSC for flumequine and oxolinic acid.

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Abbreviations

AQ	assessment question
EUCAST	European Committee on Antimicrobial Susceptibility testing
F	fraction of the antimicrobial that is absorbed from the digestive tract to the blood
FARSC	Feed Antimicrobial Resistance Selection Concentration
GE	fraction of the antimicrobial that is secreted back into the intestinal tract for elimination, after initially being absorbed into the bloodstream
I	fraction of the antimicrobial present in the digestive tracts that would be inactive on the microbiota
MIC	minimum inhibitory concentration
MIC_{lowest}	minimum inhibitory concentration of the most susceptible species/strain included in the EUCAST database for a certain antimicrobial used to calculate the PMSC (see below)
MIC_{res}	minimum inhibitory concentration of the resistant strain

MIC_{susc}	minimum inhibitory concentration of the susceptible strain
MIC_{test}	minimum inhibitory concentration of the susceptible isolate used in the competition experiments to calculate the MSC
MSC	minimal selective concentration
PK	pharmacokinetic
PMNs	PMNs Polymorphonuclear leukocytes
PMSC	predicted MSC
ToRs	Terms of Reference

Appendix A – List of excluded publications and their shortcomings

A.1. Flumequine

The publications excluded from the assessment of the effects of flumequine on growth promotion/increased yield following the criteria defined in Section 2.2.2.2.1 of the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (see also the Virtual Issue) are summarised in Table A.1.

Table A.1: Publications not relevant for the assessment of the effects of flumequine on growth promotion/increased yield and excluding criteria

Author, year	SPECIES	Excluding criteria								
		Combination of substances administered to the animals	Antimicrobial used different from the one under assessment	Administration via route different from oral	Use of the antimicrobial with a therapeutic scope	Animals subjected to challenges with pathogens	Animals in the study sick or not in good health	Zootechnical parameters not reported	Insufficient reporting/ statistics	Other (indicate)
Ahmad et al. (2007)	Poultry					X			X	
Choubert et al. (1991a)	Fish	X						X	X	
Choubert et al. (1991b)	Fish	X							X	
Elema et al. (1994)	Fish							X		
Goren et al. (1982)	Poultry								X	X(1)
Goren et al. (1988)	Poultry	X			X	X			X(2)	
Lehel et al. (1995)	Poultry	X								

(1): Absence of replicates.
(2): No description of statistical analysis.

A.2. Oxolinic acid

The publications excluded from the assessment of the effects of oxolinic acid on growth promotion/increased yield following the criteria defined in Section 2.2.2.2.1 of the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (see also the Virtual Issue) are summarised in Table A.2.

Table A.2: Publications not relevant for the assessment of the effects of oxolinic acid on growth promotion/increased yield and excluding criteria

Author, year	SPECIES	Excluding criteria								
		Combination of substances administered to the animals	Antimicrobial used different from the one under assessment	Administration via route different from oral	Use of the antimicrobial with a therapeutic scope	Animals subjected to challenges with pathogens	Animals in the study sick or not in good health	Zootechnical parameters not reported	Insufficient reporting/ statistics	Other (indicate)
Hustvedt et al. (1991)	Fish									X(1)
Rigos et al. (1999)	Fish							X		
Yasunaga and Yasumoto (1988)	Fish				X	X			X(2)	

(1): Short-term (6-h) study on the tolerance of therapeutic use in trouts. No untreated control-group.
(2): No description of statistical analysis.