SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 15 September 2021

doi: 10.2903/j.efsa.2021.6863

Maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 11: *Sulfonamides*

EFSA Panel on Biological Hazards (BIOHAZ),
Konstantinos Koutsoumanis, Ana Allende, Avelino Alvarez-Ordóñez, Declan Bolton, Sara Bover-Cid, Marianne Chemaly, Robert Davies, Alessandra De Cesare, Lieve Herman, Friederike Hilbert, Roland Lindqvist, Maarten Nauta, Giuseppe Ru, Marion Simmons, Panagiotis Skandamis, Elisabetta Suffredini, Dan I. Andersson, Vasileios Bampidis, Johan Bengtsson-Palme, Damien Bouchard, Aude Ferran, Maryline Kouba, Secundino López Puente, Marta López-Alonso, Søren Saxmose Nielsen, Alena Pechová, Mariana Petkova, Sebastien Girault, Alessandro Broglia, Beatriz Guerra, Matteo Lorenzo Innocenti, Ernesto Liébana, Gloria López-Gálvez, Paola Manini, Pietro Stella and Luisa Peixe

Abstract

The specific concentrations of sulfonamides 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. Levels in feed that showed to have an effect on growth promotion/increased yield were identified for three sulfonamides: sulfamethazine, sulfathiazole and sulfamerazine. 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: Sulfonamides, antimicrobial resistance, sub-inhibitory concentration, growth promotion, yield increase, food-producing animals

Requestor: European Commission

Question number: EFSA-Q-2021-00511

Correspondence: biohaz@efsa.europa.eu

Panel members: Ana Allende, Avelino Alvarez-Ordóñez, Declan Bolton, Sara Bover-Cid, Marianne Chemaly, Robert Davies, Alessandra De Cesare, Lieve Herman, Friederike Hilbert, Konstantinos Koutsoumanis, Roland Lindqvist, Maarten Nauta, Luisa Peixe, Giuseppe Ru, Marion Simmons, Panagiotis Skandamis and Elisabetta SuffrediniAna Allende, Avelino Alvarez-Ordóñez, Declan Bolton, Sara Bover-Cid, Marianne Chemaly, Robert Davies, Alessandra De Cesare, Lieve Herman, Friederike Hilbert, Konstantinos Koutsoumanis, Roland Lindqvist, Maarten Nauta, Luisa Peixe, Giuseppe Ru, Marion Simmons, Panagiotis Skandamis and Elisabetta Suffredini.

Declarations of interest: The declarations of interest of all scientific experts active in EFSA's work are available at https://ess.efsa.europa.eu/doi/doiweb/doisearch.

Acknowledgments: The BIOHAZ Panel, leading Panel in charge of the adoption of the scientific opinion and assessment of Term of Reference 1 (ToR1, antimicrobial resistance) wishes to thank the following for the support provided to this scientific output: EFSA Panel on Animal Health and Welfare (AHAW Panel), who supported ToR1 assessments development and endorsement of those sections under their remit (animal production, main use of antimicrobials); EFSA Panel for Additives and Products or Substances used in Animal Feed (FEEDAP), in charge of the assessment and endorsement of ToR2, and providing advice and data needed for ToR1 assessments; European Medicines Agency (EMA), who was represented by an external expert and EMA secretariat as members of the Working Group (WG); Valeria Bortolaia, who was member of the WG until 17 April 2020; EFSA staff members: Angelica Amaduzzi, Gina Cioacata, Pilar García-Vello, Michaela Hempen, Rita Navarrete, Daniel Plaza and Anita Radovnikovic; EMA staff members: Barbara Freischem, Zoltan Kunsagi, Nicholas Jarrett, Jordi Torren, and Julia Fábrega (currently EFSA staff). The BIOHAZ Panel wishes also to acknowledge the EMA Committee for Medicinal Products for Veterinary Use (CVMP) and their experts.

Suggested citation: EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021. Scientific Opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 11: *Sulfonamides*. EFSA Journal 2021;19(10):6863, 26 pp. https://doi.org/10.2903/j.efsa.2021.6863

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.

Table of contents

1. Introduction

The European Commission requested 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 sulfonamides.

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

The sulfonamides are folate pathway antagonists used since the 1930s. Sulfonamides are structural analogues of *para*-aminobenzoic acid (PABA) substrate and a potent inhibitor of dihydropteroate synthase (DHPS), which catalyses the formation of dihydropteroate. This blocks the synthesis of folate which is an essential co-factor in the biosynthesis of thymidine and thus in DNA synthesis (Anderson et al., 2011). As bacteria cannot take up folate from the environment, disruption of this metabolic pathway results in inhibition of bacterial growth (Boothe, 2015; Fernández-Villa et al., 2019).

More than 150 sulfonamides, differing in their heterocyclic ring structure, have been used in human and veterinary medicine. The effect is bacteriostatic but can be bactericidal at high concentrations. Although all of the sulfonamides have the same mechanism of action, differences in terms of antimicrobial spectrum, antimicrobial activity and in terms of pharmacokinetics (PK) exist. The differences are due to the variation of physiochemical characteristics seen among the sulfonamides.

Sulfonamides are mainly used in combination with trimethoprim. Combinations of a sulfonamide and a diaminopyrimidine result in synergistic, bactericidal actions on susceptible organisms. The optimal ratio *in vitro* for the combination of trimethoprim and a sulfonamide is 1:20 but could vary in function of the microorganism considered. However, the licensed products, supported by PK considerations, use generally a ratio of 1:5 resulting in an optimal ratio at the site of infection.

[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.

1.3.2. Main use[2]

Sulfonamides are the oldest manufactured therapeutic antibacterial agents and were initially used in veterinary medicine for bovine mastitis therapy in 1937 (EMA/CVMP/CHMP, 2020). Several substances belonging to sulfonamides class are approved for use in food-producing and companion animals (e.g. sulfadiazine, sulfadimidine, sulfamethoxazole, sulfadimethoxine). Sulfonamides are extremely important in veterinary medicine in view of the variety of their uses and the nature of the diseases treated. These classes when administered alone or in combination are of critical importance in the treatment of a wide variety of diseases (bacterial infections, coccidial infections and protozoan infections) in many animal species. Products containing sulfonamides exist in formulations for use in groups (oral formulation) and individual animals (injectable formulation), for systemic and local treatments (Lees et al., 2021). For examples, sulfathiazole, sulfamethazine and sulfadiazine are mainly used in prophylaxis/metaphylaxis and therapy in animal husbandry and veterinary medicine (Baptiste and Kyvsgaard, 2017; Charuaud et al., 2019). For veterinary, different combinations of sulfonamides with trimethoprim are used and they are registered to be used in most of the domestic animals (livestock and pets) for the treatment and metaphylaxis of diseases caused by a broad spectrum of gram-positive and many gram-negative bacteria. For example, a sulfadiazine/trimethoprim combination is used in calves, lambs, swine, rabbits and poultry for the treatment and metaphylaxis of respiratory and digestive diseases. Veterinary products containing this combination are also authorised in horses for the treatment of respiratory tract infections associated with *Streptococcus* spp. and *Staphylococcus aureus*; gastrointestinal infections associated with *E. coli*; urogenital infections associated with beta-haemolytic streptococci; infections of open or drained wounds and abscesses associated with *Streptococcus* spp. and *Staphylococcus aureus* (EMA/CVMP/CHMP, 2020).

Other combinations, e.g. sulfamethoxazole/trimethoprim, are approved for oral administration in fattening pigs for the treatment and metaphylaxis of post-weaning diarrhoea caused by *Escherichia coli* K88, K99 or 987P-positive ß-hemolytic strains and secondary bacterial infections caused by *Pasteurella multocida*, *Actinobacillus pleuropneumoniae*, *Streptococcus* spp. and *Haemophilus parasuis*. In broilers, the approved indications are treatment and metaphylaxis of colibacillosis caused by *Escherichia* and coryza caused by *Avibacterium paragallinarum*.

1.3.3. Main pharmacokinetic data

Sulfonamides share similar PK features in terms of absorption and elimination. Most sulfonamides are rapidly absorbed from the gastrointestinal tract. Sulfonamides are then mainly metabolised by the liver both by phase 1 (oxidation) and phase 2 (acetylation, glucuronidation or sulfation) reactions, depending on the animal species (Prescott, 2013). Phase 2 reactions increase the hydrophilic character of metabolites, thereby favouring renal excretion. The elimination of sulfonamides, as the parent drug or metabolites, occurs mainly through renal excretion. For the parent compounds, glomerular filtration is predominant, while the metabolites are mainly eliminated via tubular secretion. Despite these common features, the percentages of the drug absorbed after oral administrations vary among sulfonamides and thus, each drug will be assessed separately.

Sulfadiazine

The bioavailability of sulfadiazine is around 69 ± 10% in sheep (Batzias et al., 2005), 83.9 ± 17.0% in goats (Elbadawy et al., 2016), 74.44 ± 12.03% in fed horses receiving sulfadiazine in a paste (Winther et al., 2011) and ranged from 67% to 92% in non-fasted broilers (Löscher et al., 1990; Baert et al., 2003). The bioavailability in non-fasted pigs is complete (Baert et al., 2001).

In pigs, sulfadiazine was shown to accumulate towards the distal segments of the intestines following absorption through the gut wall (De Smet et al., 2017). Mean concentrations of sulfadiazine in caecum, proximal colon ascendens, distal colon ascendens and colon descendens of pigs fed with cross-contaminated feed with 12.5 mg sulfadiazine/kg feed were 0.47, 0.45, 0.67 and 0.54 μg/g respectively (Peeters et al., 2016). With standard doses of sulfadiazine, maximal concentrations found in the faeces were 26.93 ± 8.36 μg/g and 19.36 ± 1.86 μg/g after oral gavage and consumption of

[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.

medicated feed in pigs respectively (De Smet et al., 2017). These studies suggest that sulfadiazine, after absorption, is partly subjected to a mechanism of excretion in the intestines.

Sulfadimethoxine

The bioavailability is around 100% in sheep (Ferran et al., 2020) and higher than 90% in pigs (Shimoda et al., 1990). The main metabolite of sulfadimethoxine in cattle is known to be pharmacologically inactive and mostly excreted in urine without being extensively reabsorbed by the kidney (Chiesa et al., 2012).

Sulfamethazine = sulfadimidine

The bioavailability of sulfamethazine is around 58% in sheep (Bulgin et al., 1991) and 44.9 ± 16.4% in goats (Elbadawy et al., 2016).

The bioavailability of sulfamethazine in pigs was 48.0 ± 11.5% when administered mixed with pelleted feed for 3 consecutive days (Nouws et al., 1986). However, another study showed that after oral administration of labelled sulfamethazine to pigs, only 16% of the dose was eliminated via the faeces suggesting an absorption higher than 48% (Giera et al., 1982).

In calves and cows, sulfamethazine is metabolised predominantly to the N4- acetyl metabolite; hydroxylation is absent or is a minor metabolic pathway (Nouws et al., 1991).

Sulfamethoxazole

The oral bioavailability of sulfamethoxazole is 99.4 ± 7.6% in fasted calves (Nishida et al., 1997) and around 46% in fasted hens (Queralt and Castells, 1985).

Binding of sulfachlorpyridazine

In vitro binding of sulfachlorpyridazine in caecal contents of horses after incubation for 3 h at 37°C ranged from 58% to 69% (Van Duijkeren et al., 1996).

1.3.4. Main resistance mechanisms

Resistance to sulfonamides can be both chromosomally and plasmid mediated. Altered proteins conferring reduced affinity with the compound is the most common mechanism of resistance. For example, in staphylococci, resistance is chromosomally mediated by mutations in genes encoding for dihydropteroate synthetase. Concerning horizontal spread of resistance, two plasmids were originally characterised harbouring genes expressing drug-insensitive variants of the target enzymes dihydropteroate synthase (*sul1* and *sul2*). The *sul1* genes are often linked to the Tn*21* type integron, while *sul2* is usually located on small plasmids such as IncQ family. Also, *sul1* gene is part of class 1 integrons and thus often associated with other resistance genes. Another variant of *sul* gene, *sul*3 has also been identified from various animals and foods. Resistance to sulfonamides has spread extensively and rapidly. Nowadays, high prevalence rates of sulfonamide resistance genes *sul1*, *sul2*, and *sul3* have been observed in Gram-negative bacteria isolated from humans, animals, and aquaculture. Overproduction of PABA can create a competition with sulfonamides preventing the inhibition of dihydropteroate synthetase. Low-level resistance may also be mediated by alternate folic acid synthesis pathways (Antunes et al., 2005; Jiang et al., 2019; Sköld, 2000, Sköld, 2001; Boothe, 2015).

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' (EFSA BIOHAZ Panel, 2021a, 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 sulfonamides: examples

Few studies were identified on effects of sub-MIC of sulfonamides on selection of resistance, HGT or virulence. Generally, the MICs against specific susceptible bacteria for sulfonamides are lowered when administered in combination with trimethoprim. The resistance development potential due to the antimicrobial combination is lower than that to each individual agent. This aspect is of importance in view of the common resistance to sulfonamides and the rapid development of resistance to diaminopyrimidines when used alone.

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

- It has been demonstrated in *in vitro* mutagenic experiments that sub-MIC levels of sulfonamides produced statistically significant increases in mutant frequency with a maximal increase of 17.1-, 6,3-, 8.7-fold, respectively for trimethoprim, sulfamethoxazole and trimethoprim/sulfamethoxazole. A wild-type strain and a *rec*A mutant strain were tested. The corresponding MIC values for sulfamethoxasole, trimethoprim and sulfamethoxasole/ trimethoprim were, for the wild-type strain, 256, 0.5 and 0.5/9.5 mg/L and for the *rec*A mutant strain 256, 0.25 and 0.25/4.75 mg/L. The mutagenic effect was tested for five different concentrations, including two lower and two higher than the MIC (i.e. 1/4 of MIC, 1/2 of MIC, MIC, 2 × MIC and 4 × MIC). The concentration of each antimicrobial producing the highest effect was re-tested using five independent replicates to confirm the results. It was concluded that while most antimicrobials produced mild increases in mutagenesis, trimethoprim, sulfamethoxazole and trimethoprim/sulfamethoxazole produced the highest increases in mutant frequency in both tests (rifampicin and fosfomycin resistance) (Thi et al., 2011).
- Another *in vitro* experiment conducted on a multiresistant strain of *P. aeruginosa* showed that sub-inhibitory concentrations of sulfonamides upregulated expression of specific resistant genes (*sul1*) and efflux pumps (*mex*D) (Bruchmann et al., 2013).

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

Regarding effects of sub-MIC levels of sulfonamides on HGT and virulence, some published *in vitro* studies have showed effects. Even if scarce, this information provides some insight on the ability of low concentrations to influence bacterial behaviour.

- In the study of Jutkina et al. (2018), an *in vitro* method was developed to assess the transfer of resistance gene to a recipient bacterial strain when exposed to low concentrations. In a study published in 2018, the same authors using this methodology had identified for example that exposure to sulfamethoxazole at 1 mg/L (i.e. 1/16 of the MIC) significantly increases the *in vitro* HGT of in conjugation assay from a bacterial community to a *E. coli* recipient strain (MIC for sulfamethoxazole 16 mg/L).
- Bruchmann et al. (2013) also showed that for the *in vitro* experiments mentioned above on multiresistant strain of *P. aeruginosa*, the application of sulfamethoxazole, erythromycin and roxithromycin induced changes in biofilm dynamics regarding biomass formation, spatial structure and specific gene expression.
- In the study of Zhanel and Nicolle (1992), sub-inhibitory concentrations were reported to alter the adherence of *E. coli* to uroepithelial cells. It has been shown for example that both trimethoprim and sulfonamides consistently decrease bacterial adherence at concentrations ranging from 1/32 to 1/2 of MIC and 1/4 to 1/2 of MIC, respectively (Zhanel and Nicolle, 1992).

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', the estimation of this value for sulfonamides 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 sulfonamides as indicated in Table 1. However, no MSC data required to do the calculations are available.

Table 1: Calculation of sulfonamides 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 ($MIC_{lowest}/MIC_{test}/MSC_{test}$)
Sulfonamides	NA	NA	NA	1	NA

MIC: minimum inhibitory concentration. MSC: minimal selective concentration. MSC_{test}: MSC experimentally determined. MIC_{lowest}: lowest MIC data for sulfamethoxazole calculated based on data from the EUCAST database as described in Bengtsson-Palme and Larsson (2016), and Methodology Section 2.2.1.3.1.1 in the Scientific Opinion Part 1 (see also the Virtual Issue). (EUCAST database last accessed 15 May 2021[3]). NA: not available.

Due to the lack of PMSC, no FARSC could be calculated. If PMSC was available, the FARSC ($FARSC_{intestine}$ and $FARSC_{rumen}$) 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 the different molecules of sulfonamides:

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

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

With daily faeces being the daily fresh faecal output in kg, I the inactive fraction, F the fraction available, GE 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.

Sulfadiazine

Sulfadiazine is well absorbed in all species and one study suggests an intestinal elimination in pigs. There is no information on the potential binding of sulfadiazine to intestinal contents.

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 sulfadiazine for the different animal species

Sulfadiazine data	Scenario #1	Scenario #2	Scenario #3
Inactive fraction (I)	NA	NA	NA
Bioavailability (F) sheep	0.7	0.6	0.8
Bioavailability (F) goat	0.8	0.6	0.9
Bioavailability (F) pig	1	0.85	1
Bioavailability (F) horse	0.75	0.5	0.85
Bioavailability (F) broilers	0.75	0.65	0.95
Gastrointestinal elimination (GE) pig	0.1	0.2	0

NA: not available. 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 ($1 - F + F \times GE$).

[3] https://mic.eucast.org/search/

Sulfadimethoxine

Sulfadimethoxine is well absorbed in pigs and sheep. There are no data for other species. There is no information on the intestinal elimination nor on the binding of sulfadiazine to intestinal contents.

The values of *F*, *GE* and *I* extracted from literature for the calculations of FARSC are summarised in Table 3.

Table 3: Pharmacokinetic (PK) values used for the calculation of Feed Antimicrobial Resistance Selection Concentration (FARSC) of sulfadimethoxine for the different animal species

Sulfadimethoxine data	Scenario #1
Inactive fraction (*I*)	NA
Bioavailability (*F*) sheep	1
Bioavailability (*F*) pig	0.90
Gastrointestinal elimination (*GE*) pig	NA

NA: not available. 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 $(1 - F + F \times GE)$.

Sulfamethazine = sulfadimidine

Sulfadimethazine appears to be less absorbed in pigs, goats and sheep than other sulfonamides. There are no data for other species. There is no information on the intestinal elimination nor on the binding of sulfadiazine to intestinal contents.

The values of *F*, *GE* and *I* extracted from literature for the calculations of FARSC are summarised in Table 4. 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 4: Pharmacokinetic (PK) values used for the calculation of Feed Antimicrobial Resistance Selection Concentration (FARSC) of sulfadimethazine for the different animal species

Sulfadimethazine data	Scenario #1	Scenario #2	Scenario #3
Inactive fraction (*I*)	NA	NA	NA
Bioavailability (*F*) sheep	0.6	–	–
Bioavailability (*F*) goat	0.45	0.30	0.6
Bioavailability (*F*) pig	0.5	0.35	0.85
Gastrointestinal elimination (*GE*)	NA	NA	NA

NA: not available. 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 $(1 - F + F \times GE)$.

Sulfamethoxazole

The bioavailability of sulfamethoxazole was only found for fasted calves and hens. There are no data for fed animals and for other species. There is no information on the intestinal elimination nor on the binding of sulfadiazine to intestinal contents.

The values of *F*, *GE* and *I* extracted from literature for the calculations of FARSC are summarised in Table 5. 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 5: Pharmacokinetic (PK) values used for the calculation of Feed Antimicrobial Resistance Selection Concentration (FARSC) of sulfamethoxazole for the different animal species

Sulfamethoxazole data	Scenario #1	Scenario #2	Scenario #3
Inactive fraction (*I*)	NA	NA	NA
Bioavailability (*F*) calf	1	0.9	1
Bioavailability (*F*) hen	0.45	–	–
Gastrointestinal elimination (*GE*)	NA	NA	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. NA: not available. The fraction remaining in the digestive tract and that could be available for the bacteria is equal to $(1 - F + F \times GE)$.

Due to the absence of MSC and other PK data the estimation of the FARSC for the different sulfonamides 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 sulfonamides under assessment:

i) MSC data: no data is available.
ii) MIC data: only data for sulfamethoxazole are available in the EUCAST database.
iii) Bioavailability: quantitative data are not available for each species and each sulfonamide.
iv) Inactive fraction: the data only come from one study conducted in horses with sulfachlorpyridazine.
v) Intestinal secretion: the results of one study suggest that there is an intestinal secretion of sulfadiazine in pigs, but no quantitative data are available for the value of *GE*.
vi) Ruminants: no data are available for sulfonamides administered to adult 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 antimicrobials concentrations in feed which have an effect in terms of growth promotion/increased yield

3.3.1. Sulfonamides

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 2,336 papers mentioning sulfonamides and any of the food-producing animal species considered[4] and any of the performance parameters identified as relevant for the assessment of the possible growth-promoting effects of sulfonamides.[5] After removing the reports not matching the eligibility criteria, 75 publications were identified.

[4] 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); crustaceans; other animal species.

[5] (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.

3.3.1.2. Evaluation of the studies

The 75 publications identified in the literature search were appraised for suitability for the assessment of the effects of sulfonamides 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).[6] A total of 70 publications were not considered suitable for the assessment, because of several shortcomings identified in the design of the studies or in the reporting of the results. The list of excluded publications and their shortcomings are presented in Appendix A (Table A.1).

The publications considered suitable for the assessment are described and assessed in Section 3.3.1.3.

3.3.1.3. Assessment of the effects of sulfonamides on growth performance and yield

Five publications were considered suitable for the assessment of the effects of sulfonamides on growth and yield performance in food-producing animals. The effects of the administration of the antimicrobial on the endpoints described in Section 2.2.2.2.2 of the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (see also the Virtual Issue) were evaluated. The selected publications and the effects on the relevant endpoints are described below. The summary of the studies includes the description of the source of sulfonamide used —either as the base or as any specific form/commercial preparation—, and the concentration(s) applied as reported in each study.

3.3.1.3.1. Study in ruminants

Only one study in lambs for fattening was identified. The study by Calhoun and Shelton (1973), involved a total of 120 females and castrated males Crossbred lambs (white-faced and black-faced), which were used in three identical successive experiments. In each experiment, the lambs were distributed in eight pens in groups of five animals per pen and allocated to four dietary treatments. In each experiment, three basal diets (for phases days 0–7, 8–14, 15–56) containing 40% roughage were either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisted of sulfamethazine (unspecified chemical form; Sulmet® 7.7 % sulfamethazine American Cyanamid Company, Princeton, New Jersey, USA) at a concentration of 55 mg/kg feed. All three experiments lasted 56 days. The animals were weighed on days 0, 7, 14, 18 and 56. Feed consumption was determined daily for the first week and thereafter at weekly intervals. Rectal temperature was measured twice a week for the first 28 days. Data from the three experiments were pooled and analysed, and at the end of the experiments the lambs' fed rations supplemented with sulfamethazine showed, compared to the control group, a higher feed consumption (1.34 vs. 1.28 kg/day) and a higher daily weight gain (210 vs 184 g). The study showed that sulfamethazine at 55 mg/kg feed had a growth-promoting effect in lambs.

3.3.1.3.2. Study in poultry

In the study of Stutz and Lawton (1984), Experiment 4, a total of 168 two-day-old male chickens for fattening (Hubbard) were allocated to six dietary treatments and distributed in six (control) or three (experimental) pens per treatment, in groups of eight birds per pen. The basal diet based on maize and soybean meal was either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of sulfathiazole (unspecified form) supplementation at a concentration of 110 mg/kg feed. The experiment lasted eight days (from day 3 to day 11 of age). Body weight (BW) and cumulative feed intake (FI) were recorded and feed to gain ratio (F:G) calculated at the end of the experiment. At the end of the experiment, 32 chickens (control) or 16 chickens (sulfathiazole treatment) were slaughtered for relative ileal weight determination, whereas ileal digesta from 12 chickens (control) or 6 chickens (sulfathiazole treatment) were used for enumeration of *C. perfringens*. At the end of the experiment, the birds treated with sulfathiazole at 110 mg/kg feed, compared to the control group, showed no differences in daily weight gain, F:G or *C. perfringens* count, but had increased relative ileum weight (1.57% vs. 1.46% BW). Dietary sulfathiazole supplementation at 110 mg/kg feed did not have a growth-promoting effect in chickens for fattening.

[6] 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)'.

3.3.1.3.3. Studies in fish

In the study of Boujard and Le Gouvello (1997), in Experiment 1, a total of 360 rainbow trouts (*Oncorhynchus mykiss*) were allocated to four dietary treatments and distributed in three tanks (replicates) per treatment, in groups of 30 fish per tank. One basal diet based on fish meal and maize starch was either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of sulfamerazine sodium supplementation (Sigma Chem.) at a concentration of 10,000 mg/kg feed (corresponding to 9,200 mg sulfamerazine/kg feed). The study lasted 20 days, and fish in the sulfamerazine sodium group received the basal diet without sulfamerazine sodium supplementation between days 1 and 10 of the experiment and with sulfamerazine sodium supplementation between days 11 and 20 of the experiment. Mortality and health status were checked every day. Fish weight was recorded on days 1, 10, and 20 of the study. Feed intake was recorded and specific growth rate and F:G calculated at the end of the experiment. Overall, mortality was low (1.4%). Feed intake was decreased in the sulfamerazine sodium group by more than 50% during the last 10 days compared to the control group. Consequently, specific growth rate decreased in the sulfamerazine sodium group during the last ten days (1.1 vs. 2.0%), when compared to the control group. Feed to gain ratio remained unaffected during the whole period. Overall, negative effects were observed on performance parameters at 10,000 mg sulfamerazine sodium/kg feed (corresponding to 9,200 mg sulfamerazine/kg feed) of for rainbow trout.

In the study by Glencross et al. (2006) a total of 960 juvenile rainbow trout (*Oncorhynchus mykiss*) of 35.6 g BW were allocated to 48 tanks (four tanks/treatment, 20 fish/tank). One basal diet was either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and treatments consisting of sulfamerazine sodium supplemented at 5,000 mg/kg feed and 10,000 mg/kg feed (corresponding to 4,600 and 9,200 mg sulfamerazine/kg feed). The experiment lasted 42 days. The following parameters were measured: survival of fish, growth parameters (BW, FI) and nutrient utilisation (nitrogen, phosphorous and energy). Further calculated parameters were weight gain and feed conversion ratio. Final BW of the fish in the groups supplemented with sulfamerazine was lower (117.4 and 97.8 g for the groups fed sulfamerazine sodium at 5,000 or 10,000 mg/kg feed, respectively) compared with the control fish (126.8 g); the weight gain of the fish in the supplemented groups was decreased (81.9 and 62 g for the groups fed sulfamerazine sodium at 5,000 or 10,000 mg/kg feed, respectively) compared with the control fish (91.2 g/fish). Feed intake was lower (1.64 and 1.28 g/fish per day for the groups fed sulfamerazine sodium at 5,000 or 10,000 mg/kg feed, respectively) compared to control fish (1.89 g/fish per day). The supplementation of sulfamerazine sodium at the concentrations of 5,000 and 10,000 mg/kg feed (corresponding to 4,600 and 9,200 mg sulfamerazine/kg feed) negatively affected the performance of rainbow trout.

3.3.1.4. Discussion

In the four studies assessed, only three sulfonamides were tested (sulfamethazine, sulfathiazole and sulfamerazine), being this fact a limitation when considering the large variety of substances that the functional group 'sulfonamides' comprises.

From the studies examined, the test item has been described as (i) 'sulfamerazine sodium' (two studies), (ii) 'sulfamerazine' (unspecified form; one study) or (iii) 'sulfathiazole' (unspecified form; one study). Therefore, for the cases (ii) and (iii), an uncertainty on the exact product/concentration applied has been identified.

A detailed analysis of the uncertainties for sulfonamides is included in Appendix B (Table B.1) of this document, and the Section 3.3 of the Scientific Opinion Part 1 (see also the Virtual Issue).

3.3.1.4.1. Ruminants

In one study in lambs for fattening, dietary sulfamethazine supplementation at 55 mg/kg feed improved the growth performance of lambs (Calhoun and Shelton, 1973).

3.3.1.4.2. Poultry

From one study in poultry, sulfathiazole supplementation at 110 mg/kg feed had no growth-promoting effect in chickens for fattening (Stutz and Lawton, 1984).

3.3.1.4.3. Fish

From two studies in rainbow trout, dietary sulfamerazine sodium supplementation adversely affected the performance of rainbow trout fingerlings at 10,000 mg/kg feed (Boujard and Le Gouvello, 1997) and that of juvenile rainbow trout at 5,000 mg/kg feed (Glencross et al., 2006).

3.3.1.5. Concluding remarks

It is judged 33–66% certain ('about as likely as not') that sulfonamides have growth-promoting/ increase yield effects in lambs for fattening at the concentration of 55 mg sulfamethazine/kg complete feed (one study).

It is judged 33–66% certain ('about as likely as not') that sulfonamides have negative effects on performance of rainbow trout at concentrations ranging from 4,600 to 9,200 mg sulfamerazine/kg complete feed (two studies).

No data are available in the scientific literature showing effects of substances from the functional group 'sulfonamides' on growth promotion/increased yield when added (i) to lambs for fattening feed at concentrations below 55 mg sulfamethazine/kg, or (ii) to feed of any other food-producing animal species or categories for all sulfonamides.

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 sulfonamides 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 sulfonamides 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 is available.

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

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

- It is judged 33–66% certain ('about as likely as not') that sulfonamides have growth-promoting/increase yield effects in lambs for fattening at the concentration of 55 mg/kg complete feed (one study).
- It is judged 33–66% certain ('about as likely as not') that sulfonamides have negative effects on performance of rainbow trout at concentrations ranging from 4,600 to 9,200 mg sulfamerazine/kg complete feed (two studies).
- No data are available in the scientific literature showing effects of substances from the functional group 'sulfonamides' on growth promotion/increased yield when added (i) to lambs for fattening feed at concentrations below 55 mg sulfamethazine/kg, or (ii) to feed of any other food-producing animal species or categories for all sulfonamides.

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 the sulfonamides (e.g. sulfadiazine, sulfadimethoxine, sulfamethazine = sulfadimidine and sulfamethoxazole).

References

Alzieu JP, Mage C, Maes L and Mûelenaere C, 1999. Economic benefits of prophylaxis with diclazuril against subclinical coccidiosis in lambs reared indoors. Veterinary Record, 144, 442–444. https://doi.org/10.1136/vr.144.16.442

Anderson DD, Quintero CM and Stover PJ, 2011. Identification of a de novo thymidylate biosynthesis pathway in mammalian mitochondria. Proc Natl Acad Sci USA. 108, 15163–15168. https://doi.org/10.1073/pnas.1103623108

Anderson NG, Alpaugh WC and Baughn CO, 1974. Effect of sulfachloropyrazine in the drinking water of chickens infected experimentally with fowl cholera. Avian Diseases, 18, 410–415. https://doi.org/10.2307/1589108

al-Ankari AS and Homeida AM, 1996. Effect of antibacterial growth promoters on the immune system of broiler chicks. Veterinary Immunology and Immunopathology, 53, 277–283. https://doi.org/10.1016/S0165-2427(96)05609-7

Antunes P, Machado J, Sousa JC and Peixe L, 2005. Dissemination of sulfonamide resistance genes (*sul1*, *sul2*, and *sul3*) in Portuguese *Salmonella enterica* strains and relation with integrons. Antimicrobial Agents and Chemotherapy, 49, 836–839. https://doi.org/10.1128/AAC.49.2.836-839.2005

Atabaigi Elmi V, Moradi S, Harsini SG and Rahimi M, 2020. Effects of *Lactobacillus acidophilus* and natural antibacterials on growth performance and *Salmonella* colonization in broiler chickens challenged with *Salmonella* Enteritidis. Livestock Science, 233. https://doi.org/10.1016/j.livsci.2020.103948

Backstrom L, McDonald J, Collins MT, Chung WB, Shryock TR and Ose EE, 1994. Efficacy of tilmicosin, and a combination of tylosin and sulfamethazine, for control of swine atrophic rhinitis involving infection with toxigenic *Pasteurella multocida* type D. Journal of Swine Health and Production, 2, 11–14.

Baert K, De Baere S, Croubels S, Gasthuys F and De Backer P, 2001. Pharmacokinetics and bioavailability of sulfadiazine and trimethoprim (trimazin 30%) after oral administration in non-fasted young pigs. Journal of Veterinary Pharmacology and Therapeutics, 24, 295–298. https://doi.org/10.1046/j.1365-2885.2001.00337.x

Baert K, De Baere S, Croubels S and De Backer P, 2003. Pharmacokinetics and oral bioavailability of sulfadiazine and trimethoprim in broiler chickens. Veterinary Research Communications, 27, 301–309. https://doi.org/10.1023/A:1024084108803

Baptiste KE and Kyvsgaard NC, 2017. Do antimicrobial mass medications work? A systematic review and meta-analysis of randomised clinical trials investigating antimicrobial prophylaxis or metaphylaxis against naturally occurring bovine respiratory disease. Pathogens and Disease, 75. https://doi.org/10.1093/femspd/ftx083

Batzias GC, Delis GA and Koutsoviti-Papadopoulou M, 2005. Bioavailability and pharmacokinetics of sulphadiazine, N4-acetylsulphadiazine and trimethoprim following intravenous and intramuscular administration of a sulphadiazine/trimethoprim combination in sheep. Veterinary Research Communications, 29, 699–712. https://doi.org/10.1007/s11259-005-3868-6

Bengtsson-Palme J and Larsson DGJ, 2016. Concentrations of antibiotics predicted to select for resistant bacteria: proposed limits for environmental regulation. Environment International, 86, 140–149. https://doi.org/10.1016/j.envint.2015.10.015

Bhandari SK, Xu B, Nyachoti CM, Giesting DW and Krause DO, 2008. Evaluation of alternatives to antibiotics using an *Escherichia coli* K88+ model of piglet diarrhea: effects on gut microbial ecology. Journal of Animal Science, 86, 836–847. https://doi.org/10.2527/jas.2006-822

Boothe DM, 2015. Sulfonamides and sulfonamide combinations. MSD Veterinary Manual. Available online: https://www.msdvetmanual.com/pharmacology/antibacterial-agents/sulfonamides-and-sulfonamide-combinations

Boujard T and Le Gouvello R, 1997. Voluntary feed intake and discrimination of diets containing a novel fluoroquinolone in self-fed rainbow trout. Aquatic Living Resources, 10, 343–350. https://doi.org/10.1051/alr:1997037

Bridge R, Owen BD and Beames RM, 1982. The effectiveness of adding penicillin to a mixture of chlortetracycline and sulfamethazine to improve growth of weanling pigs. Canadian Journal of Animal Science, 62, 967–969. https://doi.org/10.4141/cjas82-119

Bruchmann J, Kirchen S and Schwartz T, 2013. Sub-inhibitory concentrations of antibiotics and wastewater influencing biofilm formation and gene expression of multi-resistant *Pseudomonas aeruginosa* wastewater isolates. Environmental Science and Pollution Research, 20, 3539–3549. https://doi.org/10.1007/s11356-013-1521-4

Bulgin MS, Lane VM, Archer TE, Baggot JD and Craigmill AL, 1991. Pharmacokinetics, safety and tissue residues of sustained-release sulfamethazine in sheep. Journal of Veterinary Pharmacology and Therapeutics, 14, 36–45. https://doi.org/10.1111/j.1365-2885.1991.tb00802.x

Burnell TW, Cromwell GL and Stahly TS, 1988. Effects of dried whey and copper sulfate on the growth responses to organic acid in diets for weanling pigs. Journal of Animal Science, 66, 1100–1108. https://doi.org/10.2527/jas1988.6651100x

Cabel MC and Waldroup PW, 1991. Effects of different coccidiostats on performance of large white turkeys. Poultry Science, 70, 241–249. https://doi.org/10.3382/ps.0700241

Cabel MC, Norton RA, Yazwinski TA and Waldroup PW, 1991. Efficacy of different anticoccidials against experimental coccidiosis in large white turkeys. Poultry Science, 70, 289–292. https://doi.org/10.3382/ps.0700289

Calhoun MC and Shelton M, 1973. Effect of chlortetracycline and sulfamethazine supplementation on the performance of lambs fed a high concentrate diet. Journal of Animal Science, 37, 1433–1437. https://doi.org/10.2527/jas1973.3761433x

Charuaud L, Jarde E, Jaffrezic A, Thomas MF and Le Bot B, 2019. Veterinary pharmaceutical residues from natural water to tap water: sales, occurrence and fate. Journal of Hazardous Materials, 361, 169–186. https://doi.org/10.1016/j.jhazmat.2018.08.075

Chiesa OA, Li H, Kijak PJ, Li JX, Lancaster V, Smith ML, Heller DN, Thomas MH and Von Bredow J, 2012. Tissue/fluid correlation study for the depletion of sulfadimethoxine in bovine kidney, liver, plasma, urine, and oral fluid. Journal of Veterinary Pharmacology and Therapeutics, 35, 249–258. https://doi.org/10.1111/j.1365-2885.2011.01327.x

Cho JH, Chen YJ, Min BJ, Kim HJ, Kwon OS, Shon KS, Kim IH, Kim SJ and Asamer A, 2006. Effects of essential oils supplementation on growth performance, IgG concentration and fecal noxious gas concentration of weaned pigs. Asian-Australasian Journal of Animal Sciences, 19, 80–85. https://doi.org/10.5713/ajas.2006.80

Daft BM, Bickford AA and Hammarlund MA, 1989. Experimental and field sulfaquinoxaline toxicosis in Leghorn chickens. Avian Diseases, 33, 30–34. https://doi.org/10.2307/1591064

Davami A, Peterson RA, Jones WT and Ilardi RL, 1987. Compatibility of sulfadimethoxine and ormetoprim with lasalocid and monensin on performance of male broiler chickens. Poultry Science, 66, 373–375. https://doi.org/10.3382/ps.0660373

De Smet J, Croubels S, De Backer P and Devreese M, 2017. Effect of administration route and dose alteration on sulfadiazine-trimethoprim plasma and intestinal concentrations in pigs. International Journal of Antimicrobial Agents, 50, 707–714. https://doi.org/10.1016/j.ijantimicag.2017.06.012

Dean WF, Price JI and Leibovitz L, 1973. Effect of feed medicaments on bacterial infections in ducklings. Poultry Science, 52, 549–558. https://doi.org/10.3382/ps.0520549

Dritz SS, Tokach MD, Goodband RD, Nelssen JL and Owen KQ, 1993. Comparison of feed-grade antibiotics in starter diets containing spray-dried blood products. In: Kansas State University Swine Day 1993. Report of Progress 695, Kansas State University, Kansas, pp. 75–77.

Edmonds MS, Izquierdo OA and Baker DH, 1985. Feed additive studies with newly weaned pigs: efficacy of supplemental copper, antibiotics and organic acids. Journal of Animal Science, 60, 462–469. https://doi.org/10.2527/jas1985.602462x

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021a. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 1: Methodology, general data gaps and uncertainties. EFSA Journal 2021;19(10):6852, 57 pp. https://doi.org/10.2903/j.efsa.2021.6852

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021b. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 2: Aminoglycosides/aminocyclitols: apramycin, paromomycin, neomycin and spectinomycin. EFSA Journal 2021;19(10):6853, 40 pp. https://doi.org/10.2903/j.efsa.2021.6853

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021c. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 3: Amprolium. EFSA Journal 2021;19(10):6854, 20 pp. https://doi.org/10.2903/j.efsa.2021.6854

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021d. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 4: β-Lactams: amoxicillin and penicillin V. EFSA Journal 2021;19(10):6855, 26 pp. https://doi.org/10.2903/j.efsa.2021.6855

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021e. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 5: Lincosamides: lincomycin. EFSA Journal 2021;19(10):6856, 21 pp. https://doi.org/10.2903/j.efsa.2021.6856

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021f. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 6: Macrolides: tilmicosin, tylosin and tylvalosin. EFSA Journal 2021;19(10):6858, 52 pp. https://doi.org/10.2903/j.efsa.2021.6858

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021g. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 7: Amphenicols: florfenicol and thiamphenicol. EFSA Journal 2021;19(10):6859, 27 pp. https://doi.org/10.2903/j.efsa.2021.6859

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021h. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 8: Pleuromutilins: tiamulin and valnemulin. EFSA Journal 2021;19(10):6860, 27 pp. https://doi.org/10.2903/j.efsa.2021.6860

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021i. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 9: Polymyxins: colistin. EFSA Journal 2021;19(10):6861, 26 pp. https://doi.org/10.2903/j.efsa.2021.6861

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021j. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 10: Quinolones: flumequine and oxolinic acid. EFSA Journal 2021;19(10):6862, 18 pp. https://doi.org/10.2903/j.efsa.2021.6862

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021k. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 12: Tetracyclines: tetracycline, chlortetracycline, oxytetracycline, and doxycycline. EFSA Journal 2021;19(10):6864, 115 pp. https://doi.org/10.2903/j.efsa.2021.6864

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021l. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 13: Diaminopyrimidines: trimethoprim. EFSA Journal 2021;19(10):6865, 21 pp. https://doi.org/10.2903/j.efsa.2021.6865

Elbadawy M, Ishihara Y, Aboubakr M, Sasaki K and Shimoda M, 2016. Oral absorption profiles of sulfonamides in Shiba goats: a comparison among sulfadimidine, sulfadiazine and sulfanilamide. Journal of Veterinary Medical Science, 78, 1025–1029. https://doi.org/10.1292/jvms.15-0601

EMA/CVMP/CHMP (European Medicines Agency/Committee for Medicinal Products for Veterinary Use/Committee for Medicinal Products for Human Use), 2020. Categorisation of antibiotics in the European Union. Answer to the request from the European Commission for updating the scientific advice on the impact on public health and animal health of the use of antibiotics in animals. EMA/CVMP/CHMP/682198/2017. 12 December 2019. Available online: https://www.ema.europa.eu/en/documents/report/categorisation-antibiotics-european-union-answer-request-european-commission-updating-scientific_en.pdf

Fayer R, 1992. Activity of sulfadimethoxine against cryptosporidiosis in dairy calves. Journal of Parasitology, 78, 534–537. https://doi.org/10.2307/3283662

Fernández-Villa D, Aguilar MR and Rojo L, 2019. Folic acid antagonists: antimicrobial and immunomodulating mechanisms and applications. International Journal of Molecular Sciences, 20, 4996. https://doi.org/10.3390/ijms20204996

Ferran AA, Lacroix MZ, Bousquet-Mélou A, Duhil I and Roques BB, 2020. Levers to improve antibiotic treatment of lambs via drinking water in sheep fattening houses: the example of the sulfadimethoxine/trimethoprim combination. Antibiotics, 9, 561. https://doi.org/10.3390/antibiotics9090561

Furusawa N, 2001. Transference of dietary veterinary drugs into eggs. Veterinary Research Communications, 25, 651–662. https://doi.org/10.1023/A:1012743230180

Gallo GF and Berg JL, 1995. Efficacy of a feed-additive antibacterial combination for improving feedlot cattle performance and health. Canadian Veterinary Journal, 36, 223–229.

Gardiner JL, 1958. A comparison of the effect of aureomycin in combination with three levels of sulfamethazine in feed for the control of cecal coccidiosis of chickens. Poultry Science, 37, 549–554. https://doi.org/10.3382/ps.0370549

Gerhold RW, Fuller AL, Lollis L, Parr C and McDougald LR, 2011. The efficacy of anticoccidial products against *Eimeria* spp. in northern bobwhites. Avian Diseases, 55, 59–64. https://doi.org/10.1637/9572-101310-Reg.1

Gibb DJ, Schwartzkopf-Genswein KS, McAllister TA, Genswein BMA, Streeter M and Genswein BMA, 2006. Effect of sub-therapeutic antibiotics and auction exposure on health, performance, and feeding behavior of weaned calves. Canadian Journal of Animal Science, 86, 457–460. https://doi.org/10.4141/A06-024

Giera DD, Sieck RF, Graper LK, Mertz JL and Herberg RJ, 1982. Metabolism of [^{14}C]sulfamethazine in swine. Journal of Agriculture and Food Chemistry, 30, 263–266. https://doi.org/10.1021/jf00110a012

Glencross B, Hawkins W, Evans D, Rutherford N, Dods K, Maas R, McCafferty P and Sipsas S, 2006. Evaluation of the nutritional value of prototype lupin protein concentrates when fed to rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 251, 66–77. https://doi.org/10.1016/j.aquaculture.2005.05.023

Glencross B, Rutherford N and Jones B, 2011. Evaluating options for fishmeal replacement in diets for juvenile barramundi (*Lates calcarifer*). Aquaculture Nutrition, 17, e722–e732. https://doi.org/10.1111/j.1365-2095.2010.00834.x

Glisson JR, Hofacre CL and Mathis GF, 2004. Comparative efficacy of enrofloxacin, oxytetracycline, and sulfadimethoxine for the control of morbidity and mortality caused by *Escherichia coli* in broiler chickens. Avian Diseases, 48, 658–662. https://doi.org/10.1637/7166

Goren E, Jong WA and Doornenbal P, 1984. Some pharmacokinetic aspects of four sulphonamides and trimethoprim, and their therapeutic efficacy in experimental *Escherichia coli* infection in poultry. The Veterinary Quarterly, 6, 134–140. https://doi.org/10.1080/01652176.1984.9693927

Goren E, Jong WA and Doornenball P, 1987. Additional studies on the therapeutic efficacy of sulphadimidine sodium in experimental *Escherichia coli* infection of broilers. The Veterinary Quarterly, 9, 86–87. https://doi.org/10.1080/01652176.1987.9694081

Harper AF, Kornegay ET, Bryant KL and Thomas HR, 1983. Efficacy of virginiamycin and a commercially-available lactobacillus probiotic in swine diets. Animal Feed Science and Technology, 8, 69–76. https://doi.org/10.1016/0377-8401(83)90044-5

Hathaway MR, Dayton WR, White ME, Henderson TL and Henningson TB, 1996. Serum insulin-like growth factor I (IGF-I) concentrations are increased in pigs fed antimicrobials. Journal of Animal Science, 74, 1541–1547. https://doi.org/10.2527/1996.7471541x

Hathaway MR, Dayton WR, White ME, Henderson TL, Young DA and Doan TN, 1999. Effect of feed intake on antimicrobially induced increases in porcine serum insulin-like growth factor I. Journal of Animal Science, 77, 3208–3214. https://doi.org/10.2527/1999.77123208x

Hathaway MR, Dayton WR, White ME and Pampusch MS, 2003. Effects of antimicrobials and weaning on porcine serum insulin-like growth factor binding protein levels. Journal of Animal Science, 81, 1456–1463. https://doi.org/10.2527/2003.8161456x

Holderread D, Nakaue HS and Arscott GH, 1983. Anticoccidial drugs and duckling performance to four weeks of age. Poultry Science, 62, 1125–1127. https://doi.org/10.3382/ps.0621125

Hong JW, Kwon OS, Min BJ, Lee WB, Shon KS, Kim IH and Kim JW, 2004. Evaluation effects of spray-dried egg protein containing specific egg yolk antibodies as a substitute for spray-dried plasma protein or antibiotics in weaned pigs. Asian-Australasian Journal of Animal Sciences, 17, 1139–1144. https://doi.org/10.5713/ajas.2004.1139

Howarth B and Marks HL, 1973. Thyroidal 131 I uptake of Japanese quail in response to three different dietary goitrogens. Poultry Science, 52, 326–331. https://doi.org/10.3382/ps.0520326

Huang CW, Lee TT, Shih YC and Yu B, 2012. Effects of dietary supplementation of Chinese medicinal herbs on polymorphonuclear neutrophil immune activity and small intestinal morphology in weanling pigs. Journal of Animal Physiology and Animal Nutrition, 96, 285–294. https://doi.org/10.1111/j.1439-0396.2011.01151.x

Jiang H, Cheng H, Liang Y, Yu S, Yu T, Fang J and Zhu C, 2019. Diverse mobile genetic elements and conjugal transferability of sulfonamide resistance genes (*sul1*, *sul2*, and *sul3*) in *Escherichia coli* Isolates from *Penaeus vannamei* and pork from large markets in Zhejiang, China. Frontiers in Microbiology, 10. https://doi.org/10.3389/fmicb.2019.01787

Joachim A and Mundt H-C, 2011. Efficacy of sulfonamides and Baycox® against *Isospora suis* in experimental infections of suckling piglets. Parasitology Research, 109, 1653–1659. https://doi.org/10.1007/s00436-011-2438-9

Johnson MR and Smith KL, 1994. Effect of pellet size and drug concentration on the efficacy of Romet-medicated feed for controlling *Edwardsiella ictaluri* infections in channel catfish fingerlings. Journal of Aquatic Animal Health, 6, 53–58. https://doi.org/10.1577/1548-8667(1994)006<0053:EOPSAD>2.3.CO;2

Johnson MR, Smith KL and Boyle CR, 1992. Field efficacy trials of the antibacterial sarafloxacin-hydrochloride (A-56620) for treatment of *Edwardsiella ictaluri* infections in channel catfish. Journal of Aquatic Animal Health, 4, 244–251. https://doi.org/10.1577/1548-8667(1992)004<0244:FETOTA>2.3.CO;2

Johnson MR, Smith KL and Rode RA, 1993. Field trials of Sarafin- and Romet-medicated feeds for treatment of *Edwardsiella ictaluri* infections in channel catfish. Journal of Aquatic Animal Health, 5, 51–58. https://doi.org/10.1577/1548-8667(1993)005<0051:FTOSAR>2.3.CO;2

Jutkina J, Marathe NP, Flach CF and Larsson DGJ, 2018. Antibiotics and common antibacterial biocides stimulate horizontal transfer of resistance at low concentrations. Science of the Total Environment, 616–617, 172–178. https://doi.org/10.1016/j.scitotenv.2017.10.312

Lees P, Pelligand L, Giraud E and Toutain P-L, 2021. A history of antimicrobial drugs in animals: evolution and revolution. Journal of Veterinary Pharmacology and Therapeutics, 44, 137–171. https://doi.org/10.1111/jvp.12895

Limbu SM, Zhou L, Sun S-X, Zhang M-L and Du Z-Y, 2018. Chronic exposure to low environmental concentrations and legal aquaculture doses of antibiotics cause systemic adverse effects in Nile tilapia and provoke differential human health risk. Environment International, 115, 205–219. https://doi.org/10.1016/j.envint.2018.03.034

Löscher W, Fabbender CP, Weissing M and Kietzmann M, 1990. Drug plasma levels following administration of trimethoprim and sulphonamide combinations to broilers. Journal of Veterinary Pharmacology and Therapeutics, 13, 309–319. https://doi.org/10.1111/j.1365-2885.1990.tb00782.x

Mengelers MJB, Kuiper HA, Pijpers A and Verheijden JHM, 2000. Prevention of pleuropneumonia in pigs by in-feed medication with sulphadimethoxine and sulphamethoxazole in combination with trimethoprim. The Veterinary Quarterly, 22, 157–162. https://doi.org/10.1080/01652176.2000.9695047

Mitrovic M, Fusiek G and Schildknecht EG, 1969. Antibacterial activity of sulfadimethoxine potentiated mixture (Ro 5-0013) in chickens. Poultry Science, 48, 1151–1155. https://doi.org/10.3382/ps.0481151

Mitrovic M, Schildknecht EG, Maestrone G and Luther HG, 1980. Rofenaid in the control of *Pasteurella anatipestifer* and *Escherichia coli* infections in ducklings. Avian Diseases, 24, 302–308. https://doi.org/10.2307/1589697

Morand-Fehr P, Richard A, Tessier J and Hervieu J, 2002. Effects of decoquinate on the growth and milk performance of young female goats. Small Ruminant Research, 45, 109–114. https://doi.org/10.1016/S0921-4488(02)00089-5

Mosleh N, Shomali T, Namazi F, Marzban M, Mohammadi M and Boroojeni AM, 2016. Comparative evaluation of therapeutic efficacy of sulfadiazine-trimethoprim, oxytetracycline, enrofloxacin and florfenicol on *Staphylococcus aureus*–induced arthritis in broilers. British Poultry Science, 57, 179–184. https://doi.org/10.1080/00071668.2016.1148263

NCR-89 Committee on Confinement Management of Swine, 1984. Effect of space allowance and antibiotic feeding on performance of nursery pigs. NCR-89 Committee on Confinement Management of Swine. Journal of Animal Science, 58, 801–804. https://doi.org/10.2527/jas1984.584801x

Neveling DP, van Emmenes L, Ahire JJ, Pieterse E, Smith C and Dicks LMT, 2017. Safety assessment of antibiotic and probiotic feed additives for *Gallus gallus domesticus*. Scientific Reports, 7. https://doi.org/10.1038/s41598-017-12866-7

Nishida Y, Takahashi Y, Oda K and Hayama T, 1997. Bioavailability of ruminally protected sulfamethoxazole after oral administration in ruminating calves. Journal of Veterinary Medical Science, 59, 289–291. https://doi.org/10.1292/jvms.59.289

Nouws JFM, Vree TB, Baakman M, Driessens F, Vellenga L and Mevius DJ, 1986. Pharmacokinetics, renal clearance, tissue distribution, and residue aspects of sulphadimidine and its N4-acetyl metabolite in pigs. Veterinary Quarterly, 8, 123–135. https://doi.org/10.1080/01652176.1986.9694031

Nouws JF, Vree TB, Degen M and Mevius D, 1991. Pharmacokinetics of sulphamethoxazole in calves and cows. Veterinary Quarterly, 13, 10–15. https://doi.org/10.1080/01652176.1991.9694279

Nyachoti CM, Kiarie E, Bhandari SK, Zhang G and Krause DO, 2012. Weaned pig responses to *Escherichia coli* K88 oral challenge when receiving a lysozyme supplement. Journal of Animal Science, 90, 252–260. https://doi.org/10.2527/jas.2010-3596

Olson LD, 1977a. Comparison of low-level rofenaid, low-level chlortetracycline, and vaccination with commercial bacterin for preventing pulmonary form of fowl cholera in turkeys. Avian Diseases, 21, 160–166. https://doi.org/10.2307/1589336

Olson LD, 1977b. Evaluation of Rofenaid and a commercial bacterin for prevention of cranial form of fowl cholera in turkeys. Poultry Science, 56, 1098–1101. https://doi.org/10.3382/ps.0561098
Patterson DC, 1984. Effects of the dietary inclusion of copper sulphate, avoparcin and carbadox/sulphadimidine supplements on the performance of pigs weaned at 25 days. Animal Science, 38, 487–493. https://doi.org/10.1017/S0003356100041696
Peeters LEJ, Daeseleire E, Devreese M, Rasschaert G, Smet A, Dewulf J, Heyndrickx M, Imberechts H, Haesebrouck F, Butaye P and Croubels S, 2016. Residues of chlortetracycline, doxycycline and sulfadiazine-trimethoprim in intestinal content and feces of pigs due to cross-contamination of feed. BMC Veterinary Research, 12, 209. https://doi.org/10.1186/s12917-016-0803-8
Piva A, Grilli E, Fabbri L, Pizzamiglio V and Campani I, 2007. Free versus microencapsulated organic acids in medicated or not medicated diet for piglets. Livestock Science, 108, 214–217. https://doi.org/10.1016/j.livsci.2007.01.065
Prescott JF, 2013. Sulfonamides, diaminopyrimidines, and their combinations. In: Giguère S, Prescott JF and Dowling PM (eds.). Antimicrobial Therapy in Veterinary Medicine. pp. 279–294.
Queralt J and Castells I, 1985. Pharmacokinetics of sulfamethoxazole and trimethoprim association in hens. Poultry Science, 64, 2362–2367. https://doi.org/10.3382/ps.0642362
Radecki SV, Juhl MR and Miller ER, 1988. Fumaric and citric acids as feed additives in starter pig diets: effect on performance and nutrient balance. Journal of Animal Science, 66, 2598–2605. https://doi.org/10.2527/jas1988.66102598x
Radwan AA, Elhabbak MME, Elaggoury SA and Badawy NA, 1991. Influence of sulfanilamide on intestinal calcium-absorption, uterine contractile activity and egg-shell thickness in the domestic-fowl. Archiv Fur Geflugelkunde, 55, 4–9.
Rawles SD, Kocabas A, Gatlin DM III, Du WX and Wei CI, 1997. Dietary supplementation of Terramycin and Romet-30 does not enhance growth of channel catfish but does influence tissue residues. Journal of the World Aquaculture Society, 28, 392–401. https://doi.org/10.1111/j.1749-7345.1997.tb00286.x
Robinson EH, Brent JR, Crabtree JT and Tucker CS, 1990. Improved palatability of channel catfish feeds containing Romet-30®. Journal of Aquatic Animal Health, 2, 43–48. https://doi.org/10.1577/1548-8667(1990)002<0043:IPOCCF>2.3.CO;2
Rollins LD, Gaines SA, Pocurull DW, Mercer HD and Frobish LT, 1976. Persistence of transferable drug resistance in the lactose fermenting enteric flora of swine following antimicrobial feeding. Canadian Journal of Comparative Medicine: Revue Canadienne de Médecine Comparée, 40, 175–183.
Rozeboom DW, Shaw DT, Tempelman RJ, Miguel JC, Pettigrew JE and Connolly A, 2005. Effects of mannan oligosaccharide and an antimicrobial product in nursery diets on performance of pigs reared on three different farms. Journal of Animal Science, 83, 2637–2644. https://doi.org/10.2527/2005.83112637x
Ruff MD and Wilkins GC, 1990. Prevention of coccidiosis in the chukar partridge (*Alectoris chukar*) by medication with sulfadimethoxine and ormetoprim (Rofenaid). Poultry Science, 69, 1675–1680. https://doi.org/10.3382/ps.0691675
Sedqyar M, Kandiel MMM, Weng Q, Nagaoka K, Watanabe G and Taya K, 2012. Effects of sulfamethazine on induction of precocious puberty in japanese quails (*Coturnix japonica*) assessed through monitoring the hormonal changes and gonadal development. Journal of Reproduction and Development, 58, 563–568. https://doi.org/10.1262/jrd.2012-036
Shimoda M, Kokue E, Suzuki R, Hayama T and Vree TB, 1990. Oral dosage regimen in the nonlinear pharmacokinetics of sulphadimethoxine in pigs. Veterinary Quarterly, 12, 7–13. https://doi.org/10.1080/01652176.1990.9694235
Sköld O, 2000. Sulfonamide resistance: mechanisms and trends. Drug Resistance Updates, 3, 155–160. https://doi.org/10.1054/drup.2000.0146
Sköld O, 2001. Resistance to trimethoprim and sulfonamides. Veterinary Research, 32, 261–273. https://doi.org/10.1051/vetres:2001123
Stanford K, Gibb DJ, Schwartzkopf-Genswein KS, van Herk F and McAllister TA, 2015. Feeding subtherapeutic antimicrobials to low-risk cattle does not confer consistent performance benefits. Canadian Journal of Animal Science, 95, 589–597. https://doi.org/10.4141/CJAS-2015-008
Stutz MW and Lawton GC, 1984. Effects of diet and antimicrobials on growth, feed efficiency, intestinal *Clostridium perfringens*, and ileal weight of broiler chicks. Poultry Science, 63, 2036–2042. https://doi.org/10.3382/ps.0632036
Svensson C, 1998. Prevention of *Eimeria alabamensis* coccidiosis by a long-acting baquiloprim/sulphadimidine bolus. Veterinary Parasitology, 74, 143–152. https://doi.org/10.1016/s0304-4017(97)00154-4
Ternus GS, Vetter RL and Danley MM, 1971. Feeder lamb response to chlortetracycline-sulfamethazine supplementation. Journal of Animal Science, 33, 878–880. https://doi.org/10.2527/jas1971.334878x
Thaler RC, Nelssen JL, Anderson GA, Blecha F, Chitko CG, Chapes SK and Clough ER, 1989. Evaluation of a biological response modifier: effects on starter pig performance. Journal of Animal Science, 67, 2341–2346. https://doi.org/10.2527/jas1989.6792341x
Thi TD, López E, Rodríguez-Rojas A, Rodríguez-Beltrán J, Couce A, Guelfo JR, Castañeda-García A and Blázquez J, 2011. Effect of *recA* inactivation on mutagenesis of *Escherichia coli* exposed to sublethal concentrations of antimicrobials. Journal of Antimicrobial Chemotherapy, 66, 531–538. https://doi.org/10.1093/jac/dkq496

Unno T, Kim J, Guevarra RB and Nguyen SG, 2015. Effects of antibiotic growth promoter and characterization of ecological succession in swine gut microbiota. Journal of Microbiology and Biotechnology, 25, 431–438. https://doi.org/10.4014/jmb.1408.08063

Van Duijkeren E, Kessels BGF, Van Oldruiten-Borgh-Oosterbaan MMS, Breukink HJ, Vulto AG and Van Miert ASJPAM, 1996. *In vitro* and *in vivo* binding of trimethoprim and sulphachlorpyridazine to equine food and digesta and their stability in caecal contents. Journal of Veterinary Pharmacology and Therapeutics, 19, 281–287. https://doi.org/10.1111/j.1365-2885.1996.tb00050.x

Veum TL, Lauxen R and Yen J-T, 1980. Efficacy of feed additives in enhancing performance of growing pigs. Animal Production, 30, 95–103. https://doi.org/10.1017/S0003356100023849

Weber TE, Schinckel AP, Houseknecht KL and Richert BT, 2001. Evaluation of conjugated linoleic acid and dietary antibiotics as growth promotants in weanling pigs. Journal of Animal Science, 79, 2542–2549. https://doi.org/10.2527/2001.79102542x

Winther L, Guardabassi L, Baptiste KE and Friis C, 2011. Antimicrobial disposition in pulmonary epithelial lining fluid of horses. Part I. Sulfadiazine and trimethoprim. Journal of Veterinary Pharmacology and Therapeutics, 34, 277–284. https://doi.org/10.1111/j.1365-2885.2010.01228.x

Woods GT, Jensen AH, Gossling J, Rhoades HE and Nickelson WF, 1972. The effect of medicated feed on the nasal microflora and weight gain of pigs. Canadian Journal of Comparative Medicine: Revue Canadienne de Médecine Comparéee, 36, 49–54.

Yilmaz S, Ergun S and Yigit M, 2018. Effects of dietary FARMARIN (R) XP supplement on immunological responses and disease resistance of rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 496, 211–220. https://doi.org/10.1016/j.aquaculture.2018.07.024

Young LG, Forshaw RP and Smith GC, 1973. Simplified diets based on barley for reproducing swine. Journal of Animal Science, 37, 898–905. https://doi.org/10.2527/jas1973.374898x

Zhanel GG and Nicolle LE, 1992. Effect of subinhibitory antimicrobial concentrations (sub-MICs) on *in-vitro* bacterial adherence to uroepithelial cells. Journal of Antimicrobial Chemotherapy, 29, 617–627. https://doi.org/10.1093/jac/29.6.617

Zinn RA, 1986. Short-term effect of antibiotic feeding on site and extent of digestion of growing and finishing diets in feedlot cattle. Journal of Animal Science, 63, 2013–2017. https://doi.org/10.2527/jas1986.6362013x

Zinn RA, 1993. Influence of oral antibiotics on digestive function in Holstein steers fed a 71% concentrate diet. Journal of Animal Science, 71, 213–217. https://doi.org/10.2527/1993.711213x

Abbreviations

AQ	assessment question
BW	body weight
DHPS	Dihydropteroate synthase
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
F:G	feed conversion ratio or feed to gain ratio
FI	feed intake
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
PABA	para-aminobenzoic acid
PK	pharmacokinetic(s)
PMSC	predicted MSC
rRNA	ribosomal ribonucleic acid
ToRs	Terms of Reference

Appendix A – List of excluded publications and their shortcomings

The publications excluded from the assessment of the effects of sulfonamides 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 sulfonamides 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)
Al-Ankari and Homeida (1996)	Poultry								X	
Alzieu et al. (1999)	Ruminants				X		X			
Anderson et al. (1974)	Poultry				X	X			X	
Atabaigi Elmi et al. (2020)	Poultry	X			X	X				X[(1)]
Backstrom et al. (1994)	Pigs	X			X	X	X			
Bhandari et al. (2008)	Pigs	X			X	X				
Bridge et al. (1982)	Pigs	X								
Burnell et al. (1988)	Pigs	X								X[(2)]
Cabel et al. (1991)	Pigs	X			X	X				
Cabel and Waldroup (1991)	Poultry	X			X		X			X[(2)]
Cho et al. (2006)	Pigs	X							X	
Daft et al. (1989)	Poultry				X		X	X		X[(3)]

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)
Davami et al. (1987)	Poultry	X							X	X(4)
Dean et al. (1973)	Poultry				X	X	X		X	
Dritz et al. (1993)	Pigs	X							X	X(2)
Edmonds et al. (1985)	Pigs	X							X	
Fayer (1992)	Ruminants				X	X			X	X(5)
Furusawa (2001)	Poultry								X	X(6)
Gallo and Berg (1995)	Ruminants	X								
Gardiner (1958)	Poultry				X	X			X	
Gerhold et al. (2011)	Poultry	X			X	X				
Gibb et al. (2006)	Ruminants	X			X					X(2)
Glencross et al. (2011)	Fish								X	
Glisson et al. (2004)	Poultry				X	X	X			
Goren et al. (1984)	Poultry				X	X			X	X(7)
Goren et al. (1987)	Poultry				X	X			X	
Harper et al. (1983)	Pigs	X								
Hathaway et al. (1996)	Pigs	X								
Hathaway et al. (1999)	Pigs	X							X	

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)
Hathaway et al. (2003)	Pigs	X								
Holderread et al. (1983)	Poultry								X	X[8][9]
Hong et al. (2004)	Pigs	X							X	X[2][10]
Howarth and Marks (1973)	Poultry								X	X[11]
Huang et al., 2012;	Pigs	X								
Joachim and Mundt (2011)	Pigs				X	X				
Johnson and Smith (1994)	Fish	X			X		X			
Johnson et al. (1992)	Fish	X			X		X			
Johnson et al. (1993)	Fish	X			X		X			
Limbu et al. (2018)	Fish				X				X	X[12]
Mengelers et al. (2000)	Pigs				X		X		X	
Mitrovic et al. (1969)	Poultry	X			X	X			X	
Mitrovic et al. (1980)	Poultry	X			X	X				
Morand-Fehr et al. (2002)	Ruminants								X	X[2]
Mosleh et al. (2016)	Poultry				X	X			X	X[13]

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)
NCR-89 (1984)	Pigs	X			X					
Neveling et al. (2017)	Poultry	X								
Nyachoti et al. (2012)	Pigs	X			X	X			X	
Olson (1977a)	Poultry	X			X	X				
Olson (1977b)	Poultry	X			X	X				
Patterson (1984)	Pigs	X			X					
Piva et al. (2007)	Pigs	X							X	X(2)
Radecki et al. (1988)	Pigs	X								
Radwan et al. (1991)	Poultry				X				X	X(9)
Rawles et al. (1997)	Fish	X								
Robinson et al. (1990)	Fish	X							X	
Rollins et al. (1976)	Pigs	X							X	
Rozeboom et al. (2005)	Pigs	X								
Ruff and Wilkins (1990)	Poultry	X			X	X				
Sedqyar et al. (2012)	Poultry								X	
Stanford et al. (2015)	Ruminants	X			X					
Svensson (1998)	Ruminants	X		X	X	X				X(14)

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)
Ternus et al. (1971)	Ruminants	X			X					
Thaler et al. (1989)	Pigs	X								X(15)
Unno et al. (2015)	Pigs	X								X(15)
Veum et al. (1980)	Pigs	X			X					
Weber et al. (2001)	Pigs	X			X					
Woods et al. (1972)	Pigs	X			X		X		X	
Yilmaz et al. (2018)	Fish	X			X	X				X(2)
Young et al. (1973)	Pigs	X								
Zinn (1986)	Ruminants	X			X					X(7)
Zinn (1993)	Ruminants	X			X					

(1): No specific information on sulfadiazine administration in water could be found.
(2): No control group used in the experiment.
(3): The study aimed to experimentally reproduce sulfaquinoxaline toxicosis. Mortalities of 33% and 44% were observed in sulfaquinoxaline-treated groups due to sulfaquinoxaline toxicosis.
(4): Animals were subsequently treated with lasalocid or monensin.
(5): The antimicrobial was administered orally, but in the form of oral bolus.
(6): Designed to study the transfer of antibiotics to eggs.
(7): No replicates.
(8): The study investigated the adverse effects of anticoccidial drugs administered to ducklings.
(9): Low number of animals.
(10): Antibiotic diet vs. diets with SDEP (spray-dried egg protein). Low number of animals (4 pens of 3 pigs/treatment).
(11): Sulfaguanidine was used as a goitrogenic compound.
(12): Small number of animals per group.
(13): The study investigated the effects of sulfanilamide on calcium absorption and egg shell thickness.
(14): Oral administration, but not administered with feed (slow release bolus).
(15): The study focused on sequencing the microbiota.

Appendix B – Table of uncertainties

Table B.1: Potential sources of uncertainty identified in the levels of sulfonamides in feed which have growth promotion/increase yield effect and assessment of the impact that these uncertainties could have on the conclusion

Source of the uncertainty	Nature or cause of uncertainty	Impact of the uncertainty on the conclusion on the level(s) which have growth promotion/increase yield effect
Form(s) of antimicrobial used	The specific form of the antimicrobial used in the study (as the '(free) base' substance, its salts or specific products/formulations containing the base substance) has not been clearly described in several publications. In summarising the results, the concentrations have been reported as for 'base' substance when the form of the antimicrobial is not specified (conservative assumption).	Underestimation of the concentration which may have shown growth-promoting effect.
Evidence synthesis and integration	As described in Section 2.2.3 of the Scientific Opinion Part 1 (see also the Virtual Issue), the low number of studies retrieved prevented evidence synthesis.	Underestimation/Overestimation