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Veterinary Medicines Division

Scientific advice under Article 107(6) of Regulation (EU) 2019/6 for the establishment of a list of antimicrobials which shall not be used in accordance with Articles 112, 113 and 114 of the same Regulation or which shall only be used in accordance with these articles subject to certain conditions

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Introduction

On the 17 February 2020, the European Medicines Agency (EMA, the Agency) received a request from the European Commission to provide scientific advice for the establishment of a list of antimicrobials that, as per Article 107(6) of Regulation (EU) 2019/6 (the Regulation) [1], shall not be used in accordance with Articles 112, 113 and 114 of the Regulation or may only be used in accordance with these articles subject to certain conditions. The list will be established by means of implementing acts adopted by the European Commission.

The purpose of this list, together with the list of antimicrobials reserved for human use established under Article 37(5) and enacted under Commission Implementing Regulation (EU) 2022/1255 [2], is to help preserve the efficacy of certain antimicrobials for humans and/or animals by promoting prudent antimicrobial use and thereby reducing the risk from antimicrobial resistance (AMR).

According to the request from the Commission, the scientific advice should also take into account the fact that sufficient availability of antimicrobials should be ensured to secure animal health, including for limited markets and exceptional circumstances.

The Committee for Veterinary Medicinal Products of EMA (CVMP) formed an expert group to prepare the scientific advice. In line with the Commission's request to ensure coherence and complementarity between the two advices, several of the experts had also been members of the working group for the CVMP's advice for the Article 37(5) Human Reserved List [3]. These included two experts on human infectious diseases, nominated each through the European Centre for Disease Prevention and Control (ECDC) and CHMP's Infectious Diseases Working Party (IDWP), and one expert nominated from European Food Safety Authority (EFSA). The group was also composed of ten members selected from the European network of experts on the basis of recommendations from the national competent authorities and two Agency staff members, all with expertise in the area of antimicrobial resistance.

The expert group submitted their report to the CVMP on 2 May 2023.

The CVMP adopted the scientific advice on 15 June 2023.

Summary

Legal context

Promoting the responsible use of antimicrobials in animals with the aim to reduce the risk of antimicrobial resistance to human, animal and public health is a cornerstone of the Regulation. Article 107(6) is one of several measures included in the Regulation in this respect.

Article 107(6) provides that the Commission may, by means of implementing acts, and taking into consideration scientific advice of the Agency, establish a list of antimicrobials which:

> (a) shall not be used in accordance with Articles 112, 113 and 114; or
>
> (b) shall only be used in accordance with Articles 112, 113 and 114 subject to certain conditions.

When establishing the list above, the same provision states that the Commission shall take account the following criteria:

> (a) risks to animal or public health if the antimicrobial is used in accordance with Articles 112, 113 and 114;

(b) risk for animal or public health in case of development of antimicrobial resistance;

(c) availability of other treatments for animals;

(d) availability of other antimicrobial treatments for humans;

(e) impact on aquaculture and farming if the animal affected by the condition receives no treatment.

As explained in following sections, these same criteria were taken into account by EMA for the present scientific advice.

In turn, Articles 112, 113 and 114[1] of the Regulation provide, amongst others, that, by way of derogation from Article 106(1) of the Regulation, when no veterinary medicinal product is authorised for an indication in a particular animal species, the veterinarian may exceptionally use a veterinary or human medicinal product outside the terms of a marketing authorisation [1]. The purpose of these derogations is to facilitate treatment of diseases and in animal species for which authorised veterinary medicinal products are not available, in order to avoid causing unacceptable animal suffering.

Article 107(6) is complementary to Article 37(5) of the Regulation, which tasks the Commission with the responsibility to designate antimicrobials or groups of antimicrobials that are reserved for the treatment of certain infections in humans (the Human Reserved List). Accordingly, antimicrobials or groups of antimicrobials in the Human Reserved List cannot be authorised in veterinary medicines or, as provided by Article 107(5) of the Regulation, used in accordance with Articles 112, 113 and 114.

Whilst Article 107(6) aims further to preserve the efficacy of certain antimicrobials for human and animal health, this should be balanced against the aim of Articles 112, 113 and 114 stated above.

It bears noting that Article 107(7) allows a Member State to further restrict or prohibit the use of certain antimicrobials in animals on its own territory if the administration is contrary to national policy on prudent use.

Considerations behind the development of the advice

The **criteria** under Article 107(6), listed above, are discussed in detail in the context of the development of the Agency's advice in Section 3.1.1. of this document. In brief:

Criterion (a) is understood primarily to address the risks to the safety of the target (treated) animal and to the consumer of food-animal produce that may result from the use of an antimicrobial in accordance with Articles 112, 113 and 114.

Criterion (b) is dependent on the importance of the antimicrobial for treating diseases in humans and animals, the likelihood of selection and transmission of resistance and the extent of use of the antimicrobial in the EU.

In relation to criteria (c) and (d), the availability of other antimicrobials for human diseases, and of alternative treatments for animal diseases, is also important in determining the consequences and hence risk to animal or human health in case of development of resistance to a particular antimicrobial class. In this perspective, (c) and (d) are linked to criterion (b). In addition, if conditions are proposed to limit the use of certain antimicrobials animals in accordance with Articles 112, 113 and 114, then it is necessary to consider the availability of alternative treatments, particularly for limited markets and exceptional circumstances.

[1] Articles 112, 113 and 114 relate, respectively, to non-food-producing animal species, food-producing terrestrial animal species and food-producing aquatic species.

Criterion (e) is understood to relate to the situation whereby a proposed prohibition or conditions on antimicrobial use in accordance with Articles 112, 113 and 114 leave no treatment options for animal(s) affected by certain diseases. Impacts on aquaculture and farming could include production losses and effects on animal health and welfare, amongst others.

Considerations on the potential conditions to be placed on use of medicinal products in accordance with Articles 112, 113 and 114

Article 107(6)(b) is silent on the nature of the conditions that may qualify as "certain conditions" applicable to the use in accordance with Articles 112, 113 and 114. In developing the present scientific advice, potential conditions were considered based around the types of use allowed stepwise under Articles 112, 113 and 114, such as: use to treat indications or animal species not included in the Summary of Product Characteristics (SPC); use of different (including human-authorised) formulations and routes of administration; use of veterinary medicines authorised in third countries.

The potential usefulness for AMR risk management and impacts of the conditions that were considered are discussed in Section 3.1.2. The proposed conditions include, for example, restrictions on use for certain indications, limitation to use in individual animals only and restrictions on the route of administration. A condition requiring target pathogen identification and antimicrobial susceptibility testing was also considered to be an important risk management measure and is discussed further in the Annex 1. The conditions are summarised in Summary Table **1**.

Considerations relating to Articles 112, 113 and 114 that are of particular relevance for the present advice

The following additional considerations were particularly relevant for the evaluation of different antimicrobial classes:

- The use in accordance with Articles 112, 113 and 114 should be 'exceptional' and 'in particular to avoid causing unacceptable suffering', as stated in the first paragraph of said provisions;
- Articles 113(4) and 114(6) require that substances used to treat food-producing species in accordance with Articles 113 and 114 shall be allowed in accordance with Table 1 of the Annex to Regulation (EC) No 470/2009 (relating to establishment of residue limits) [4].
- Article 115(5) provides a derogation from Articles 113(1) and (4) of the Regulation for substances listed as essential for the treatment of equine species or bringing added clinical benefit compared with other treatment options available for equine species; although at time of preparation of this advice, Commission Regulation (EC) 1950/2006 remains in force. The antimicrobials/indications included in Regulation (EC) 1950/2006, as clarified by the European Commission, have not been assessed under Article 107(6) of Regulation (EU) 2019/6.

Matters outside scope of this advice

The present scientific advice only addresses antimicrobial use in accordance with Articles 112, 113 and 114 of the Regulation, i.e. illegal use under EU legislation is not addressed in this advice.

Reference in this scientific advice to the use of antimicrobials outside the terms of their marketing authorisations shall not be construed as a scientific opinion of the CVMP in favour of such uses.

Methodology

Methodology for Antibiotics

Antibiotics were primarily addressed in pharmacological classes. Background information was first compiled relating to each antibiotic class with potential veterinary use in the EU. This information related to, for example, the availability of different formulations authorised in veterinary medicinal products (VMPs) for use in different animal species, the authorised indications and the maximum residue limit (MRL) status of individual substances in the class. Information on use of the class outside the terms of the marketing authorisation was also gathered from published sources and from an 'open call for data' published by the Agency. The evaluation of the class was then conducted in four steps. In the first step, the Article 107(6) criteria (b), (c) and (d) were reviewed in relation to the risk to public and animal health due to AMR associated with the use of the antimicrobial in animals and availability of alternative treatments. It was considered that for some classes of antibiotics, based on this evaluation, a decision could be made to recommend that no restrictions should be placed on use under Articles 112, 113 and 114 and the evaluation stopped at the end of Step 1. For the remaining classes, Step 2 considered the conditions that could be placed on use of the class outside the terms of the marketing authorisation(s) to mitigate any additional AMR risk associated with such use. In Step 3, criteria (a) and (e) were then evaluated considering use of the antibiotic outside the terms of the marketing authorisation and in the context of the proposed conditions. Finally, in Step 4, taking into account the previous steps, the conditions were concluded. As part of this exercise, it was considered if conditions alone would be sufficient to fulfil the aim of Article 107(6), or if it should be recommended that the antibiotic should not be used in accordance with Articles 112, 113 and 114. The evaluations are presented in Section 4. of the advice, with separate monographs for each antibiotic class.

Methodology for Antivirals

There are currently no direct-acting antivirals authorised in veterinary medicinal products in the EU, and none are compliant with the requirement of Articles 113(4) and 114(6) (i.e. 'allowed' in accordance with Table 1 of the Annex to the MRL Regulation (EC) No 470/2009 [4]); therefore, they can only be used in non-food-producing animals, including non-food-producing equines, under Article 112 of the Regulation (an exception is made for substances/indications for equine species as referred to under Article 115(5), see above). Consequently, uses in accordance with Articles 113 and 114 were not considered.

Firstly, a review of the literature was undertaken to identify potential therapeutic uses of antiviral substances in non-food-producing animals in the EU. The findings are presented in Section 5.1. of the advice. The following antivirals were identified as having widespread use for treatment of specific diseases in animals under Article 112: cidofovir, famciclovir, idoxuridine, remdesivir and valacyclovir/acyclovir. These substances were then evaluated against the criteria of Article 107(6) using the same step-wise process as outlined above for the antibiotics. In respect of criterion (b), it is important to note that, except for remdesivir, those antivirals for which it was previously assessed under Article 37(6) that there is a risk of transmission of antiviral resistant organisms from animals to humans are included in the Annex to Regulation (EU) 2022/1255 and are reserved for use in humans [2]. Therefore, for the remaining antivirals that have been reviewed in this exercise, it had already been concluded by CVMP that there is no significant risk for human health due to antiviral-resistance developing from their use in animals in the EU.

Methodology for Antiprotozoals and Antifungals

Antiprotozoals and antifungals were grouped in pharmacological classes and all those found to have potential veterinary use in the EU were evaluated. As for the antibiotics, background information was compiled, but with particular reference to publications identified for the advice relating to the Article 37(5) Human Reserved List [3]. Each class was then evaluated against each of the Article 107(6) criteria (a) to (e), where found applicable. The results of the evaluations are presented in Sections 6. (Antifungals) and 7. (Antiprotozoals) of this advice. In particular, regarding criterion (b), for many antiprotozoal and antifungal drugs, there is a paucity of evidence which in some cases makes it difficult to perform an assessment of the potential risk to animal health and public health due to drug-resistance. However, for other classes/substances, more certain conclusions can be drawn if the class is not related to drugs used in human medicine or where it is used to treat diseases in humans or animals that are not zoonotic/contagious and hence there is no obvious transmission pathway for drug resistance. Based on criteria (b), (c) and (d), it was considered if conditions should be placed on use under Articles 112, 113 and 114.

Conditions were proposed only for use of echinocandins and amphotericin B in accordance with Article 112. As these substances cannot be used in food-producing animals in the absence of MRL status and there is no evidence for their need in other farmed animals, criterion (e) did not need to be evaluated. In the light of the conclusions for the criteria (a) to (d), it was then considered if conditions alone would be sufficient to fulfil the aim of Article 107(6). As this was the case for both echinocandins and amphotericin B, no antiprotozoals or antifungals have been recommended to be prohibited from use under Articles 112, 113 and 114.

Background information and sources

In considering this advice, the working group has paid attention to publications from international bodies mentioned in the Commission's request, including the *OIE*[2] *List of Antimicrobial agents of Veterinary Importance* and the *WHO's CIA List* and *AWaRe classification of antibiotics,* and to previous publications from the Agency including the AMEG's *Categorisation of antibiotics in the European Union* and the *Reflection paper on off-label use*[3] *of antimicrobials in veterinary medicine in the European Union [5-9]*. Recommendations in these publications have been considered, insofar as they were relevant to the present scientific advice; however, the context and criteria differ to greater or lesser extent from those in the legislation underlying this advice.

This advice also refers in many places to the Agency's advice provided in relation to the Article 37(5) Human Reserved List [3], noted above.

Other sources of information used include official reports and opinions from EMA, ECDC and EFSA, Summaries of Product Characteristics for EU-authorised medicines, textbooks and studies and reviews published in scientific journals. For the latter, relevance to the EU-situation has been considered for international publications. In addition, evidence was gathered from an 'open call for data' in which interested parties were invited to submit information on the uses and availability of antimicrobials in the EU to treat serious infections in animals, including uses outside the terms of a marketing authorisation. See Sections 2.2.4. , 2.2.5. and Annex 3.

[2] The acronym for the World Organisation for Animal Health has recently changed from OIE to WOAH to reflect the full name of the organisation.
[3] The CVMP's reflection paper makes a distinction between 'off-label use' – *the use of a veterinary medicinal product that is not in accordance with the summary of product characteristics, including the misuse and serious abuse of the product* – and cascade use, that falls within the narrower definition of the legal derogations in force at the time.

The data sources mentioned provide a significant body of evidence relating to the importance of different antimicrobial classes/substance in human and veterinary medicine, the occurrence of AMR in animals and its transmission between animals and from animals to humans.

Uncertainties and data gaps

Uncertainties and data gaps were identified in the process of developing the advice, key of which is the lack of research or systematic collection of data indicating the types or extent of use of antimicrobials outside the terms of a VMP marketing authorisation. See Section 3.2.

However, pursuant to Article 107(6)(b) of the Regulation, when conditions are recommended for the use of antimicrobial VMPs in accordance with Articles 112, 113 and 114, the conditions that are proposed are considered by the CVMP to be justified based on the referenced available evidence and expert judgement.

Recommendations

Based on the evaluations presented in Sections 4. , 5. , 6. and 7. of this advice report, the following recommendations are made relating to the use of antimicrobial medicinal products and the provisions of Article 107(6) of the Regulation:

Table (a) Antimicrobials that **shall not be used** in accordance with Articles 112, 113 and 114

Antimicrobial class/substance
None

Table (b) Antimicrobials that shall only be used in accordance with Articles 112, 113 and 114 **subject to certain conditions**

Antimicrobial class/substance	**Conditions for use outside the terms of the marketing authorisation**
Aminopenicillin-beta lactamase inhibitor (BLI) combinations (See Section 4.4.)	• For those indications not included in the SPC of the concerned product, use must be based on target pathogen identification and antimicrobial susceptibility testing that demonstrates that aminopenicillin-BLI are likely to be effective and that antimicrobials from a lower AMEG category would not be effective, unless it can be justified that this is not possible. • Not to be used in poultry • Not to be used in food-producing aquaculture
3rd- and 4th-generation cephalosporins (See Section 4.7.)	• For those indications not included in the SPC of the concerned product, use must be based on target pathogen identification and antimicrobial susceptibility testing that demonstrates that 3rd- and 4th-generation cephalosporins are likely to be effective and that antimicrobials from a lower AMEG category would not be effective, unless it can be justified that this is not possible. • Use of 3rd- and 4th-generation cephalosporins under Article 113 to treat salmonellosis should be restricted to use of injectable products in individual animals with potentially life-threatening infections.

Antimicrobial class/substance	Conditions for use outside the terms of the marketing authorisation
	• Not to be used in poultry. • Not to be used in food-producing aquaculture • To be used in individual animals only. Exemption: Ornamental or conservation aquatic animals kept in closed water tanks.
Polymyxins (See Section 4.8.)	Conditions do not apply to use of polymyxin B for systemic treatment for endotoxaemia associated with severe colic and other gastrointestinal diseases in equines.[4] • For those indications not included in the SPC of the concerned product, use must be based on target pathogen identification and antimicrobial susceptibility testing that demonstrates that polymyxins are likely to be effective and that antimicrobials from a lower AMEG category would not be effective, unless it can be justified that this is not possible. • Formulations intended for oral group administration must not be used for treatment or metaphylaxis of *Salmonella* spp. • Must not be used for the treatment or metaphylaxis of *Salmonella* spp. in poultry. • Not for use in food-producing aquaculture. • When the intended route of administration is outside that included in the SPC of the concerned VMP, or when using an extemporaneous formulation, the product should be administered to individual animals, only. • Human medicinal products should be administered to individual animals only.
Amphenicols (See Section 4.18.)	• For those indications not included in the SPC of the concerned product, use must be based on target pathogen identification and antimicrobial susceptibility testing that demonstrates that amphenicols are likely to be effective and that antimicrobials from a lower AMEG category would not be effective, unless it can be justified that this is not possible.
Quinolones and Fluoroquinolones (See Section 4.20.)	• For those indications not included in the SPC of the concerned product, use must be based on target pathogen identification and antimicrobial susceptibility testing that demonstrates that (fluoro)quinolones are likely to be effective and that antimicrobials from a lower AMEG category would not be effective, unless it can be justified that this is not possible. • Use of (fluoro)quinolones under Article 113 to treat salmonellosis should be restricted to use of injectable products in individual animals with potentially life-threatening infection. • Must not be used for the treatment or metaphylaxis of *Salmonella* spp. in poultry.

[4] Substance / indication included in Commission Regulation (EC) 1950/2006.

Antimicrobial class/substance	Conditions for use outside the terms of the marketing authorisation
	• When the proposed route of administration is outside the terms of the SPC, or when using an extemporaneous formulation, the product should be administered to individual animals, only. • Human medicinal products should be administered to individual animals, only.
Rifamycins (See Section 4.23.)	Conditions apply to use of human medicinal products, extemporaneous preparations and VMPs authorised in third countries, only. They do not apply to EU-authorised VMPs containing rifaximin. In addition, they do not apply to the use of rifampicin for the treatment of *Rhodococcus equi* infections in equines.[4] • Use must be based on target pathogen identification and antimicrobial susceptibility testing that demonstrates that rifamycins are likely to be effective and that antimicrobials from a lower AMEG category would not be effective, unless it can be justified that this is not possible. See 'Special note regarding the diagnosis of mycobacterial infections in companion animals and antimicrobial susceptibility testing' in Annex 1. • For treatment of mycobacteria and MDR staphylococci, only. • Not to be used for prophylaxis of *Rhodococcus equi* infection. • To be used in individual animals only.
Substances used solely to treat tuberculosis or other mycobacterial diseases ('TB drugs') (See Section 4.24.)	• Use must be based on target pathogen identification and antimicrobial susceptibility testing that demonstrates that TB drugs are likely to be effective. See 'Special note regarding the diagnosis of mycobacterial infections in companion animals and antimicrobial susceptibility testing' in Annex 1. • To be used in individual animals only
Riminofenazines (See Section 4.25.)	• Use must be based on target pathogen identification and antimicrobial susceptibility testing that demonstrates that riminofenazines are likely to be effective. See 'Special note regarding the diagnosis of mycobacterial infections in companion animals and antimicrobial susceptibility testing' in Annex 1. • To be used in individual animals only.
Pseudomonic acids (See Section 4.27.)	• Use must be based on target pathogen identification and antimicrobial susceptibility testing that demonstrates that Pseudomonic acids are likely to be effective and that antimicrobials from a lower AMEG category would not be effective, unless it can be justified that this is not possible. See 'Special note on the use of AST for pathogens treated topically or locally' in Annex 1. • To be used only for treatment of MRSA and MRSP infections. Veterinary-authorised topical treatments for staphylococcal infections should not have been effective.

Antimicrobial class/substance	Conditions for use outside the terms of the marketing authorisation
	• Not to be used for routine decolonisation of MRSA/P. • To be used in individual animals only. • For topical administration only.
Remdesivir (See Section 5.3.)	• For treatment of feline infectious peritonitis only.
Echinocandins (See Section 6.)	• For use only as a last resort treatment for individual animals, where alternative treatments have been shown not to be, or unlikely to be, effective and preferably after target pathogen identification and susceptibility testing.
Amphotericin B (See Section 7.)	• In cases where used for treatment of leishmaniasis, or for treatment of other diseases in animals in regions where leishmaniasis is endemic, amphotericin B is to be used only as last resort when other treatments have failed, or can be expected to fail.

Further considerations

- For certain antimicrobial classes, it has been recommended that a condition should be applied for their use in accordance with Articles 112, 113 and 114 to be based on the results of target pathogen identification and antimicrobial susceptibility testing. This condition is elaborated in more detail in Annex 1. Specific circumstances have been taken into account, e.g. the availability of reliable testing methods for certain pathogens or antimicrobials, or to allow exemptions for particular animal species.
- This advice has been established based on current scientific knowledge. It is suggested that the recommendations should be reviewed, as and when appropriate, in the light of new scientific evidence or emerging information. This new information could include, in both human and veterinary contexts, emergence of new diseases or changes in the epidemiology of existing diseases, changes in antimicrobial drug resistance and changes in availability and patterns of antimicrobial use.

Scientific advice under Article 107(6) of Regulation (EU) 2019/6 for the establishment of a list of antimicrobials which shall not be used in accordance with Articles 112, 113 and 114 of the same Regulation or which shall only be used in accordance with these articles subject to certain conditions

Table of Contents

1. Terms of reference and scope

1.1. Request from the European Commission for scientific advice regarding implementing measures under Article 107(6) of the Regulation

On the 17 February 2020, the Agency received a request from the European Commission to provide scientific advice for the establishment of the list of antimicrobials that, as per Article 107(6) of Regulation (EU) 2019/6, shall not be used in accordance with Articles 112, 113 and 114 of the Regulation or may only be used in accordance with these articles subject to certain conditions. The list will be established by means of implementing acts adopted by the European Commission.

The purpose of this list, together with the list of antimicrobials reserved for human use (Article 37(5)) [3], is to help preserve the efficacy of certain antimicrobials by promoting prudent antimicrobial use and thereby reducing the risk to public and animal health due to antimicrobial resistance.

According to Article 107(6), whilst establishing the list, five criteria (a) to (e) should be taken into account. These criteria are set out in Section 1.2. below, 'Legislative background'.

The Commission's request additionally notes the need to ensure sufficient availability of antimicrobials to secure animal health, including for limited markets[5] and exceptional circumstances.

Attention is drawn to several relevant background documents:

- The *OIE List of Antimicrobial agents of Veterinary Importance* and the recommendations of the OIE AMR working group, particularly in relation to fluoroquinolones, 3rd- and 4th-generation cephalosporins and colistin [5].
- The *WHO CIA List* and the *AWaRe classification of antibiotics*, noting that recommendations are made as to use of certain antibiotics in order to preserve their efficacy [6, 7].
- The considerations in the Agency's *Reflection paper on off-label use of antimicrobials in veterinary medicine in the European Union [9]*.
- The AMEG's *Categorisation of antibiotics in the European Union*, although considering that there are divergences with the WHO CIA List and that it was developed for a different purpose [8].

The Commission's request also advises that bans or conditions on the use of antimicrobials in accordance with Articles 112, 113 and 114, shall not apply to animals or products of animal origin imported into the EU from third countries.

It should be noted that at the time of the submission to the Commission of this advice for Article 107(6), the list of substances which are essential for the treatment of equine species under Article 115(5), and the list of substances which may be used in aquatic species in accordance with Article 114(1), were still under development.

1.2. Legislative background

Promoting the responsible use of antimicrobials in animals in order to reduce the risk of antimicrobial resistance to human, animal and public health is a cornerstone of Regulation (EU) 2019/6 on veterinary medicinal products ('the Regulation'). In this respect, recital (41) of the Regulation notes that '*use that is not covered by the marketing authorisation of certain new or critically important antimicrobials for humans should be restricted in the veterinary sector*.' In addition, recital (42)

[5] 'limited market' refers to (a) VMPs for diseases that occur infrequently or limited geographical areas; (b) VMPs for species other than cattle, sheep for meat production, pigs, chickens, dogs and cats.

indicates that, in the context of applications for antimicrobial VMPs and considering potential risks to humans or animals from development of antimicrobial resistance, if necessary, conditions may be needed restricting use of the product that is not in accordance with the terms of the marketing authorisation. In this respect, Article 107 of the Regulation, relating to the use of antimicrobial medicinal products, includes several relevant provisions of which Article 107(6) specifically relates to use outside the terms of the marketing authorisation.

Article 107(6): Provisions on use of antimicrobial medicinal products in accordance with Articles 112, 113 and 114

Article 107(6) of the Regulation states that the Commission may establish, by means of implementing acts, a list of antimicrobials which:

> (a) shall not be used in accordance with Articles 112, 113 and 114; or
>
> (b) shall only be used in accordance with Articles 112, 113 and 114 subject to certain conditions

Whilst establishing the list, the following criteria should be taken into account:

> a) risks to animal or public health if the antimicrobial is used in accordance with Articles 112, 113 and 114;
>
> b) risk for animal or public health in case of the development of antimicrobial resistance;
>
> c) availability of other treatments for animals;
>
> d) availability of other antimicrobial treatments for humans;
>
> e) impact on aquaculture and farming if the animal affected by the condition receives no treatment.

Articles 112, 113 and 114 - Use of medicinal products outside the terms of the marketing authorisation

In turn, Articles 112, 113 and 114 of the Regulation provide, amongst others, that, by way of derogation from Article 106(1)[6] of the Regulation, when no veterinary medicinal product is authorised for an indication in a particular animal species, the veterinarian may exceptionally use a veterinary or human medicinal product outside the terms of a marketing authorisation. Articles 112, 113 and 114 refer to such use in non-food-producing animal species, food-producing terrestrial animal species and food-producing aquatic species, respectively.

Prescribing outside the terms of the marketing authorisation is expected to be exceptional and 'in particular to avoid causing unacceptable suffering'. The provisions allow use of veterinary medicines authorised in another member state, for different species or for different indications. If no such suitable veterinary medicinal products are available, use of authorised human medicinal products, or otherwise, extemporaneously prepared products, is allowed. In the absence of any of these options, a veterinary medicinal product authorised in a third country for the same animal species and indication may be used.

Use of medicines according to Articles 112, 113 and 114 is under the direct personal responsibility of a prescribing veterinarian, who may delegate the administration to another person.

[6] Article 106(1) states: 'Veterinary medicinal products shall be used in accordance with the terms of the marketing authorisation.'

In line with Articles 113(4) for terrestrial and 114(6) for aquatic food-producing animal species, any active substance prescribed under these Articles shall be 'allowed' in accordance with Table 1 of the Annex to Regulation (EC) No 470/2009 for the establishment of residue limits [4]. If no withdrawal period is stated in the SPC for the species under treatment, then it should be set by the veterinarian in accordance with the provisions of Article 115. In addition, for aquatic food-producing animals, it is proposed that in future a list of substances for use in accordance with Article 114(1)(b) and (c) will be established, paying specific attention to risks to the environment alongside other considerations.

Antimicrobials reserved for treatment of certain infections in humans

In addition to the provisions of Article 107(6), Article 37(5) of the Regulation states that the Commission shall, by means of implementing acts, designate antimicrobials or groups of antimicrobials reserved for the treatment of certain infections in humans (the Human Reserved List). According to Article 107(5), these antimicrobials shall **not** be used in accordance with Articles 112, 113 and 114. The Annex to Commission Implementing Regulation (EU) 2022/1255 lists these designated substances, which are hence out of scope of this advice [2].

National restrictions in individual Member States

It bears noting that Article 107(7) allows a Member State to further restrict or prohibit the use of certain antimicrobials in animals on its territory if the administration is contrary to national policy on prudent use.

Particular considerations relating to equine species

Article 115(5) provides a derogation from Articles 113(1) and (4) of the Regulation for substances listed as essential for the treatment of equine species or bringing added clinical benefit compared with other treatment options available for equine species; although at time of preparation of this advice, Commission Regulation (EC) 1950/2006 remains in force. The antimicrobials/indications included in Regulation (EC) 1950/2006,[7] as clarified by the Commission, should not be assessed against the criteria laid down in Article 107(6) of Regulation (EU) 2019/6 in the context of this advice.

Definition of 'antimicrobial'

According to Article 4(12) of the Regulation, 'antimicrobial' means any substance with a direct action on micro-organisms used for treatment or prevention of infections or infectious disease, including antibiotics, antivirals, antifungals and antiprotozoals.

2. Background information

2.1. Documents referenced in the request for scientific advice

2.1.1. The WOAH List of Antimicrobials of Veterinary Importance

The World Organisation for Animal Health /OIE List of Antimicrobials of Veterinary Importance addresses antimicrobials (antibiotics and certain anticoccidials) authorised for use in food-producing animals and does not include substances used only in human medicine [5]. It is based on two criteria:

- Criterion 1. Identification of the veterinary importance of the antimicrobial by more than 50% of OIE member countries responding to a questionnaire.

[7] To be replaced by implementing acts to be adopted by the Commission pursuant to Article 115(5) of Regulation (EU) 2019/6.

- Criterion 2. The class is identified as essential against a specific infection where there is a lack of sufficient therapeutic alternatives.

According to these criteria, antimicrobial agents are classified in three categories, Veterinary Critically Important Antimicrobial Agents (VCIA), Veterinary Highly Important Antimicrobial Agents (VHIA) and Veterinary Important Antimicrobial Agents (VIA).

Recognising that fluoroquinolones, 3rd- and 4th-generation cephalosporins and colistin are also considered critically important for human health, amongst others the following recommendations are made:

'Not to be used as a first line treatment unless justified, when used as a second line treatment, it should ideally be based on the results of bacteriological tests; and

Extra-label/off-label use should be limited and reserved for instances where no alternatives are available. Such use should be in agreement with the national legislation in force'

In relation to antimicrobial (sub) classes that are used only in human medicine and not included in the WOAH/OIE List, a recommendation is made: "*Recognising the need to preserve the effectiveness of the antimicrobial agents in human medicine, careful consideration should be given regarding their potential use (including extra-label/off-label use) / authorisation in animals."*

2.1.2. The WHO's List of Critically Important Antimicrobials for Human Medicine (6th revision)

The WHO's List of Critically Important Antimicrobials for Human Medicine is a ranking of medically important antimicrobials[8] for risk management of antimicrobial resistance arising due to non-human use [6]. It is built on two criteria:

- Criterion 1 (C1): The antimicrobial class is the sole, or one of limited available therapies, to treat serious bacterial infections in people.
- Criterion 2 (C2): The antimicrobial class is used to treat infections in people caused by either: (1) bacteria that may be transmitted to humans from non-human sources, or (2) bacteria that may acquire resistance genes from non-human sources.

On this basis, antimicrobial classes are classified as critically important (CIA), highly important (HIA) or important (IA) for human medicine.

CIAs are further prioritized in terms of the resources to be allocated to risk management strategies based on three additional prioritisation factors. These relate to: the number of people that might need treatment (P1), the frequency and intensity of use in humans (P2) and the evidence available to show transmission of resistance (P3). Considering these additional criteria, the 3rd-, 4th- and 5th-generation cephalosporins, glycopeptides, macrolides and ketolides, polymyxins and quinolones have been identified as highest priority CIAs (HPCIAs).

It should be noted that at the time of preparation of this advice, the WHO's List was under revision.

[8] The scope of the WHO List is limited to antibacterial antimicrobials that are used in human medicine.

2.1.3. The WHO's AWaRe Classification of Antibiotics

The AWaRe classification places antibiotic substances into three stewardship groups: Access, Watch and Reserve, to highlight the importance of their optimal uses and potential for antimicrobial resistance [7].

ACCESS – first and second choice antibiotics for the empiric treatment of most common infectious syndromes, e.g. amoxicillin, doxycycline, gentamicin

WATCH – antibiotics with higher resistance potential whose use as first and second choice treatment should be limited to a small number of syndromes or patient groups, e.g. macrolides, fluoroquinolones, and certain 3rd-generation cephalosporins; and

RESERVE – antibiotics to be used mainly as 'last resort' treatment options for confirmed or suspected infections due to multidrug resistant organisms. These antibiotics should be prioritized as key targets for stewardship, e.g. polymyxins, novel tetracyclines and 5th-generation cephalosporins.

2.1.4. The AMEG Categorisation of antibiotics

The AMEG categorisation of antibiotics differs from the above lists in that it takes account of the importance of classes/substances in both human and veterinary medicine and that it was developed from the EU perspective [8]. The AMEG's categorisation is built on four criteria:

1. If the (sub)class or group is authorised for use as a veterinary medicine in the EU

2. The importance of the (sub)class or group to human medicine according to the WHO ranking (6th revision) and taking into account the EU situation

3. The knowledge of factors influencing the likelihood and possible consequences of AMR transfer from animals to humans, in particular considering mechanisms where a single gene confers multiresistance (or resistance to several classes)

4. The availability of alternative antibiotic (sub)classes in veterinary medicine with lower AMR risk to animal and public health

According to these criteria, antibiotics are placed into 4 categories: A 'Avoid', B 'Restrict', C 'Caution' and D 'Prudence'.

Category A includes antibiotic classes/substances not authorised in veterinary medicines but authorised in human medicines in the EU. Other than virginiamycin (streptogramins), none of these substances is included in the Annex to Regulation (EU) 37/2010 on maximum residue limits (MRLs) and therefore they cannot be used in food-producing species in the EU.

The AMEG advice notes: *'These antibiotic classes may only be used exceptionally in individual companion animals in compliance with the prescribing "cascade"…. The extent of use of these classes, and hence overall selection pressure for AMR, would be low provided the restrictions detailed in the prescribing "cascade" are complied with.'*

Category B includes the 3rd- and 4th-generation cephalosporins, quinolones and polymyxins. These are the WHO's HPCIAs (at the time of writing), but with the macrolides and those classes in category A being excluded. The AMEG considered that for Category B substances, the risk to public health resulting from veterinary use needs to be mitigated by specific restrictions. Especially for this category, use should be based on the results of susceptibility testing, whenever possible.

Category C includes substances for which there are in general alternatives in human medicine in the EU but there are few alternatives in veterinary medicine for certain indications. Substances in this category may also select for resistance to a substance in category A through specific multiresistance genes.

Category D includes substances where the AMR risk to public health due to veterinary use is considered low and for which there are no specific recommendations to avoid use beyond general principles for the prudent use of antimicrobials.

2.1.5. EMA/CVMP Reflection paper on off-label use of antimicrobials in veterinary medicine in the EU

The CVMP's reflection paper on off-label use of antimicrobials [9] addresses the common reasons for use of antibiotics outside the terms of a marketing authorisation, which include:

- Unmet medical needs e.g. limited availability of products for limited markets
- Use of alternative routes of administration to improve distribution to the site of infection or for practical reasons of administration
- To address individual patient characteristics e.g. underlying disease or physiology
- Use of alternative dosing regimens to accommodate changes in pathogen susceptibility or chronic disease

The CVMP's reflection paper makes a distinction between 'off-label use' – *the use of a veterinary medicinal product that is not in accordance with the summary of product characteristics, including the misuse and serious abuse of the product*[9] – and 'cascade' use that falls within the narrower definition relating to the provisions of Articles 10 and 11 of Directive 2001/82/EC, as amended and in force at the time of publication of the paper.

It is of interest to note certain conclusions from the CVMP in relation to off-label use of antimicrobials –

"As there is no organized collection of data on the volume of off-label antimicrobial use in the EU, and a limited number of mainly descriptive published studies devoted to the topic, it is only possible to speculate about the risks to animal and public health and acceptability of these practices based on general principles…"

"… Where an antimicrobial product is used in the **intended target species** for an **unauthorised indication** at the dose regimen detailed in the SPC, and if this use is supported by bacterial culture and susceptibility testing with appropriate clinical monitoring, then there is unlikely to be any additional risk to animal or public health due to AMR compared to authorised use."

"Where an antimicrobial product is used under the cascade in an **unauthorised species**, by a **different route** of administration and/or there is an adjustment to the dosing regimen, then consideration should be given to potential risks for lack of effectiveness and increased selection pressure for AMR due to (i) a change in bacterial exposure to the antimicrobial in the animal, and (ii) possible antimicrobial residues in food produce. Measures to mitigate the potential risks include limiting such use to the treatment of individual animals, use of culture and susceptibility testing, attention to differences in pharmacokinetics and application of statutory minimum withdrawal periods.

[9] Article 1(16) of Directive 2001/82/EC

Cascade use for **groups** of animals as compared to individuals requires particularly careful consideration because of the higher antimicrobial exposure…"

"…the cascade use of **human-only authorised antimicrobials** in individual companion animals should be kept to an absolute minimum following a careful benefit-risk assessment as these are often last-resort antimicrobials and close contact between humans and pets is a prime opportunity for exchange of multidrug resistant organisms."

"Some types of off-label antimicrobial use cannot be considered as cascade use and the associated risks cannot be justified. These include use of antimicrobials for practical or economic reasons alone, systematic preventive use in groups of animals, intentional under-dosing and concomitant use of two or more antimicrobials without proper diagnosis. Such practices are of high concern when they also involve group treatments and/or use of CIAs."

Recommendations include, amongst others:

"Prescribing under the cascade should be limited to individual animals, if feasible, although it is recognised that this may not be applicable to all husbandry systems e.g. fish, poultry or for minor species e.g. food rabbits. Off-label use, in particular that of antimicrobial substances/classes categorised as critically important with regard to their use in human and animal health (WHO, AMEG), should be supported by a full diagnostic investigation including bacterial culture and antimicrobial susceptibility testing (AST), where possible."

"When prescribing under the cascade, veterinarians should take into account the importance of the antimicrobial to human medicine and the risk for transmission of AMR from treated animals to humans. In particular, veterinarians should take these factors into account in the benefit-risk assessment before prescribing antimicrobials that are presently only authorised for use in human medicine (AMEG Category 3) [*now AMEG Category A*] [8, 10], which are CIAs for use in human medicine as one of few alternatives to treat serious disease, and for which the AMEG considered the risk for spread of resistance to be high. This could be facilitated by use of treatment guidelines that have already considered these aspects (see below). Use of Category 3 [*Category A*] antimicrobials should be kept to an absolute minimum."

2.2. Other relevant publications relating to use of antimicrobials outside the terms of the marketing authorisation

2.2.1. European guidelines for the prudent use of antimicrobials in veterinary medicine

In 2015, the European Commission published a Notice on Guidelines for the prudent use of antimicrobials in veterinary medicine [11] that sets out measures to be considered by Member States when developing and implementing national strategies to combat antimicrobial resistance.

Recommendations are available for critically important antimicrobials that are only authorised for human use. In particular, it is stated that the off-label use of products containing such antimicrobials in non-food-producing animals should be avoided and strictly limited to very exceptional cases and where antimicrobial susceptibility tests have confirmed that no other antimicrobial would be effective. Off-label use of such products may be necessary to avoid the suffering of diseased animals and should take into consideration ethical and public health concerns.

2.2.2. EMA and EFSA joint scientific opinion on measures to reduce the need to use antimicrobial agents in animal husbandry in the EU (RONAFA report)

The RONAFA report [12] makes the following recommendations in relation to 'cascade' use:

- Further research should be done into the nature and extent of off-label use of antimicrobials in food-producing animals in the EU, and the associated potential for impacts on AMR.
- When prescribing under the cascade, the risk to public health due to AMR should be taken into account alongside the need to protect animal welfare.
- Evidence-based treatment guidelines can support responsible off-label use of antimicrobials by taking into account the local AMR situation and product availability in the member state in addition to the general clinical evidence base for such use. The potential impact on public health should be included in the risk assessment underlying this guidance.

2.2.3. CVMP Recommendations and Opinions

In previous years, the CVMP has published reflection papers making recommendations in relation to specific antimicrobial classes and has conducted referral procedures that addressed issues concerning certain antimicrobial products or classes. The recommendations and conclusions from these reviews have been considered in the evaluations conducted under this scientific advice. This advice refers in many places to the complementary scientific advice provided in relation to the Article 37(5) Human Reserved List [3].

2.2.4. Published literature sources

In addition to the documents mentioned above, the expert working group has made use of publications from various sources. Examples include:

- Studies and reviews published in peer-reviewed scientific journals
- Official reports from EU Agencies e.g. EFSA, ECDC and EMA surveillance reports, scientific opinions, EPARs
- Summaries of Product Characteristics for authorised human and veterinary medicines obtained from the Union Product Database [13] or databases maintained by National Competent Authorities
- Treatment guidelines published by professional bodies
- Textbooks

The references used have been included in the related parts of the report. Annex 3. includes the reports, textbooks etc. that were most frequently used.

A systematic literature review was not undertaken. Owing to the nature of the topic, there are few high-quality studies available investigating the efficacy or safety, or even providing evidence for the use antimicrobials outside the terms of a marketing authorisation in animals. In addition, these studies can be difficult to identify through search terms. Due to the very reduced evidence available, very limited comment can be made upon, and this advice does not endorse, the efficacy or safety of the reported uses *(Disclaimer: this advice report is not intended to be used as treatment guidance.)* To gain more information on the wider use of antimicrobials in animals in the EU, the Agency launched an open call for data in 2019 (see 2.2.5.). Information from this call has been cited in the evaluations.

2.2.5. Open call for data on use of antimicrobials in in animals in the EU

In order to support the Agency in the preparation of this scientific advice and that for the Article 37(5) Human Reserved List [3], interested parties were invited to submit information via a questionnaire on the use and availability of antimicrobials in the EU to treat serious infections in animals, including use outside the terms of a marketing authorisation (referred to as 'cascade' use), and to provide any scientific evidence of the impact on public and animal health that the CVMP should consider (throughout this advice this is referred to as the 'open call for data' or 'open call').

The open call for data was posted on 9 December 2019. Responses were accepted until 6 March 2020 and were received from 133 interested parties representing 17 European countries.

Background information and a partial summary report on the findings of the questionnaire are presented in Section 4 of the Annex to the Agency's advice relating to Article 37(5) [3]. The limitations of the questionnaire are noted in the report. Some of the information from the questionnaire has been included in the antimicrobial monographs in this advice.

3. Methodology

3.1. Considerations behind the development of the advice

3.1.1. Considerations on criteria (a) to (e) of Article 107(6)

(a) risks to animal or public health if the antimicrobial is used in accordance with Articles 112, 113 and 114

Criterion (a) is primarily understood to address the risks to the target (treated) animal and to the consumer of food-animal produce that may result from use of an antimicrobial outside the marketing authorisation. It should also be noted that there could be a risk to public health if zoonotic diseases could not be treated.

Risks to users or to the environment, as clarified by the Commission, were not considered to be relevant to public health within the context of Article 107(6) and hence this advice.

General points and caveats:

- Noting the flexibility of the provisions in Articles 112, 113 and 114, which allow administration of substances for unauthorised indications, species and formulations/routes of administration, it is not possible to be aware of all potential scenarios or to consider them individually.
- Use of any medicine outside the marketing authorisation (not antimicrobials alone) may result in increased exposure of target animals to the active substance compared with that through authorised use e.g. due to use at a higher dose, over a longer duration or through administration to a group rather than individual animals. It may also result in exposure through unconventional routes of administration that may affect the bioavailability of the active substance.
- However, as a generality, risks associated with use of a medicine outside the marketing authorisation are accepted as being of low significance compared with risks associated with authorised use, owing to the 'exceptional' nature of such use, as laid out in the legislation.
- It may not be possible to extrapolate the target animal or consumer safety profile across any given 'class' of antimicrobials due to different properties of individual substances.
- It is the responsibility of the veterinarian to apply proportionate and effective risk management measures to address risks to the target animal and consumer when prescribing any veterinary medicine outside the marketing authorisation.

In relation to safety for target animals, information in the authorised SPC and European Public Assessment Report (EPAR) is likely to be applicable and may be extrapolated to the altered conditions of use outside the marketing authorisation (e.g. effects at overdose may be relevant if the dose is increased). Certain additional information from standard textbooks has been considered in the evaluations.

Regarding consumer safety, the risk relating to use outside the marketing authorisation is considered as mitigated through the application of a withdrawal period set in accordance with Article 115.

(b) risk for animal or public health in case of development of resistance

In relation to Article 107(6), the risk for animal and public health in case of development of AMR, as indicated in recital (41), is fundamental to the need to place conditions on the use of a particular antimicrobial substance/class outside the terms of the marketing authorisation.

The AMR risk associated with any antimicrobial use is dependent on many factors. Significantly these include:

- the importance of the antimicrobial for treating diseases in human and animals;
- the likelihood of development, selection and transmission of resistance from animals to humans and other animals;
- the extent of use of the antimicrobial.

Information in the Agency's advice for the Article 37(5) Human Reserved List has been used [3], where relevant considering the different objective of this scientific advice, to support the evaluation of criterion (b) of Article 107(6).

It is notable that there is very little published information on the extent of use of antimicrobials outside the marketing authorisation, although this can be influenced by, for example, restrictions on use in food-producing animals due to residues legislation and the availability of authorised formulations for group versus individual animal administration only.

(c) availability of other treatments for animals

The availability of other treatments (including alternative antimicrobials) for the animal diseases treated with any specific antimicrobial (class) is also important in determining the consequences and hence risk to animal health in case of development of resistance to that substance/class. Therefore, criterion (c) is linked to criterion (b).

In addition, noting that the derogations provided in Articles 112, 113 and 114 are intended to address the lack of availability of veterinary medicines to treat certain indications or target species, and that the Commission's request highlights the need to ensure availability of antimicrobials to secure animal health including for limited markets and exceptional circumstances, then if conditions are proposed to limit use of certain antimicrobials outside the marketing authorisation, it is necessary to consider the availability of alternative treatments. As previously noted, there is limited published information in this respect. For antivirals, antiprotozoals and antifungals, due to no or limited availability of veterinary-authorised medicines for many of the indications, in most cases the use of alternatives will also be outside a marketing authorisation.

(d) availability of alternative antimicrobial treatments for humans

As for criterion (c), the availability of alternative antimicrobials treatments for the human diseases treated with any specific antimicrobial (class) is also important in determining the consequences and hence risk to human health in case of development of resistance to that substance/class. Therefore, criterion (d) is also linked to criterion (b).

When considering alternatives for both animal and human diseases (criteria (c) and (d)), substances have been proposed in the evaluations but (even if authorised) may not be appropriate substitutes according to the specific circumstances of the disease, underlying medical conditions in the patient, product availability etc.

(e) impact on aquaculture and farming if the animal affected by the condition receives no treatment

Criterion (e) is understood to relate to the situation whereby a proposed ban or conditions on antimicrobial use outside the marketing authorisation leave no treatment options for animal(s) affected by certain diseases. Impacts might include diminished productivity and quality of yield, reduction in animal health and welfare, economic losses and societal costs [14]. Information on the prevalence of these diseases and their outcomes has been considered where available to assess this impact; however, there is little published information on the burden of animal diseases in Europe, particularly for those diseases associated with the minor species and indications.

3.1.2. Consideration of potential 'conditions' to reduce the AMR risk relating to the types/nature of use of medicinal products in accordance with Articles 112, 113 and 114

The legal provisions relating to use of medicinal products outside the terms of the marketing authorisation are laid out in Articles 112, 113 and 114 of the Regulation and summarised in Section 1.2. of this advice report. Various potential conditions have been considered based around the types of use allowed in the stepwise approach provided under Articles 112, 113 and 114, such as: use to treat different indications or animal species not included in the SPC; use of different (including human-authorised) formulations and routes of administration; use of veterinary medicines authorised in third countries.

Although there is published evidence of specific uses of some antimicrobials outside the marketing authorisation and further information has been collected through the open call for data, it is not possible to have data on all current uses outside the marketing authorisation. Therefore, in addition to considering evidenced uses, some consideration has also been given to reasonably anticipated uses of antimicrobials that could result in a significant increase in AMR selection pressure compared with use in accordance with authorised SPCs, for example, the administration of particular antimicrobials such as 3rd- and 4th-generation cephalosporins in group formulations, if, to date, they have only been authorised for administration to individual animals.

Note that discrepancies between Member States in, for example, indications in the SPCs for certain related VMPs, are expected to be resolved as part of the SPC harmonisation exercise foreseen under Article 69 of the Regulation.

(i) Indications not included in the SPC

One intention of the derogations from Article 106(1) is to enable treatment of less common or minor indications that may not be included in the SPCs for authorised antimicrobial VMPs. In a questionnaire conducted to respond to a previous mandate [10], stakeholders were asked to provide examples of indications for which there is a lack of antimicrobial VMPs and for which new antimicrobials are needed. The responses referred to coliform infections in food-producing and companion animals (neonatal diarrhoea, sepsis, mastitis), *Brachyspira hyodysenteriae* in pigs, enterococcal and mycoplasma respiratory infections in poultry, bovine respiratory disease (*Pasteurellaceae* and *Mycoplasma* spp.) and bovine interdigital dermatitis. Although many of these indications occur commonly and authorised products are available, it might be inferred that use of alternative medicines outside the marketing authorisation is also sometimes necessary to treat them effectively e.g. in case of development of resistance to authorised antibiotics. According to the 'open call for data' conducted for this advice, cascade use was reported for a wide variety of indications across different species. Frequently cited were sepsis, bacteraemia, *E. coli* infections and eye infections in various species, and *Rhodococcus*

equi in horses. At present, there are no truly reliable data on the frequency of use of antimicrobial medicines for indications not included in an SPC.

The CVMP's reflection paper on off-label use [9] concluded that where an antimicrobial product is used in the authorised target species for an unauthorised indication at the dose regimen detailed in the SPC, and if this use is **supported by bacterial culture [*target pathogen identification*] and susceptibility testing** with appropriate clinical monitoring, then there is unlikely to be any additional risk to animal or public health due to AMR compared with authorised use.

Certain zoonotic target pathogens could be associated with a specific public health risk e.g. *Salmonella* Enteritidis. In this case, it could be considered on a case-by-case basis if a condition should be placed on use outside the SPC for these indications e.g. if the same antibiotic is important to treat the infection in humans and animals.

The possibility to include conditions on other indications will be dependent on specific knowledge of **lack of efficacy for certain target pathogens**. In cases where a target pathogen is intrinsically resistant to the antibiotic, then this is usually mentioned in the SPC at the time of VMP authorisation if considered relevant. In cases where acquired resistance has developed in previously/approved target pathogens since authorisation, then this is often mentioned in warnings included in post-authorisation revisions to the SPC, and indications may have been deleted if resistance is particularly common (e.g. resistance in *Brachyspira hyodysenteriae* to tylosin). It is considered that these circumstances are better and more flexibly addressed through SPC revisions than through legislative provisions.

Use for prophylaxis – The Regulation includes stringent provisions in relation to the prophylactic use of antimicrobials under Article 107(3). Additionally, administration of medicated feed containing antimicrobial VMPs as prophylaxis is prohibited according to the Medicated Feed Regulation (EU) 2019/4 (Article 17(3)). These measures equally apply to the use of antimicrobials under Articles 112, 113 and 114. There is limited requirement to apply further restrictions on prophylaxis unless there is knowledge of a specific risk identified for particular antimicrobials/classes and/or circumstances.

(ii) Use to treat an animal species not included in the SPC

A further purpose of the derogations from Article 106(1) is to ensure the availability of treatments for **minor species** for which there are few authorised medicines, such as rabbits, ducks, bees, fish and exotic animals. Information collected in the open call for data showed that use of antimicrobial medicines outside of the SPC was particularly important to treat horses, goats and mink. According to ESVAC (2021 data), pigs, cattle, poultry and sheep/goats account for 36%, 30%, 14% and 10%, respectively, of the overall PCU for the 29 EU and EEA reporting countries [15]. Although the overall extent of use of antimicrobials in the EU for individual species, especially minor species, is unknown, it should be considered that more than 90% of the food-producing animal biomass relates to major species.

AMR hazards could be related to specific animal species due to certain pathogenic or commensal organisms that they harbour e.g. *Salmonella* spp. in poultry. If a specific AMR risk is identified, it may be considered if there is a need to **prohibit use of a certain antimicrobial in a major animal species if it is not already authorised in VMPs for use in that species.**

Where a certain antimicrobial is already authorised for use in a major species, the relative extent of additional exposure due to use in a limited market species is likely to be relatively small. Therefore, considering the need to maintain availability of treatments for minor species and not to disadvantage smaller livestock sectors where there is greater reliance on use of medicines outside the SPC, it is

proposed **to avoid conditions on use of specific antimicrobials in minor species not included in the SPC**, without specific justification**.**

It is also noted that although sheep reared for meat do not qualify under 'limited market provisions' in the Regulation, there are fewer antibiotic VMPs authorised for use in this species, with some important classes not authorised (e.g. 3rd- and 4th-generation cephalosporins, aminopenicillin-BLI combinations), and use of medicines outside the SPC is important to maintain welfare.

(iii) Use of a different formulation (including extemporaneous formulations) or route of administration

The CVMP's reflection paper on off-label use noted the use of alternative routes of administration to those authorised, particularly for treatment of infection sites where good antimicrobial penetration is difficult to achieve (e.g. for joint or bone infections) [9]. It was concluded that where sporadic treatment of individual animals is concerned, the AMR public health impact is consequently limited. Despite this, certain deviations might be considered as 'bad practice' e.g. administration of a topical product orally where there is no knowledge of the bioavailability of the formulation by this route. This presents both a potential animal health risk (lack of efficacy, safety) and an unnecessary AMR public health risk if the treatment is ineffective.

Greater concern was expressed in relation to practices where antimicrobials are regularly administered by an unauthorised route for practical reasons to groups of animals, or when the effectiveness and risks of the practice are poorly evidenced e.g. anecdotal administration of antimicrobials by nebulisation in poultry sheds.

According to AMEG [8] a ranking has been provided listing **routes of administration** and associated **formulations** according to the effect on the selection of AMR. Oral medications administered to groups of animals via feed, drinking water or milk replacer are postulated in general as higher risk considering the number of animals treated, potential for inaccuracy of achieving the correct dose uptake and the impact on the exposed gut microbiota compared with formulations intended for individual animal administration by parenteral or local routes of administration. Parenterally administered antibiotics that are actively excreted into the intestine as parent substance or active metabolites and/or antibiotics that persist in the body due to a long half-life may also exert a more detrimental AMR selection pressure. Further research is needed into the AMR impact of these products, although an advantage is that they are intended for use in individual or small numbers of animals [8].

Of the veterinary-authorised antibiotics, all classes are available in formulations for group oral treatment except cephalosporins (all generations), rifaximin, anti-staphylococcal penicillins, nitrofurans, nitroimidazoles and steroid antibacterials. Particularly for the 3rd- and 4th-generation cephalosporins (AMEG Category B) where the potential AMR risk to public health is high, selection pressure could be significantly increased if these classes were to be used outside the terms of the marketing authorisation to treat groups of animals by mass oral administration.

To help mitigate the AMR risks associated with administration of an authorised formulation by an unauthorised route (in any species), or administration of extemporaneous formulations, a condition could be proposed to allow use in individual animals only or to restrict use to certain routes of administration.

Although the full importance of aquaculture as a source of antimicrobial-resistant bacteria and resistance genes of relevance to public and animal health and to environmental ecosystems has yet to be determined, there is evidence to suggest that aquaculture potentially plays a particularly significant role as a reservoir contributing to the dissemination of AMR. In the EU, antibiotics are mostly

administered to aquatic species in medicated feed, of which up to 30% may remain unconsumed and enter directly into the environment [16]. Further, it is estimated that 70-80% of antibiotic administered is excreted in active forms, which then persist for prolonged periods in aquatic sediment [16, 17]. Hence, in addition to selecting for AMR genes in treated fish, antibiotic residues may also select for resistance in environmental bacteria. These bacteria are present in biofilms on sediment and aquaculture facilities and also contain high concentrations of bacteriophages, conditions that favour the dissemination of AMR [18, 19]. Genetic determinants of antimicrobial resistance have been described in aquaculture environments and are commonly found on mobile genetic elements which are recognized as the primary source of antimicrobial resistance for important fish pathogens [20]. Based on existing studies, Caruso concluded that resistance in fish pathogens has been most frequently reported against oxytetracycline, tetracycline, ampicillin and florfenicol, all of which are representatives of classes already authorised in aquaculture in the EU [21]. Cabello identified that the same AMR genes coding for resistance to quinolones and beta-lactams can be found in fish pathogens, human pathogens and aquatic bacteria [16]. It has also been suggested that the use of antimicrobials in aquaculture, notably the use of colistin in Asian aquaculture, could be correlated with the emergence of the plasmid-encoded mobile colistin resistance (MCR) determinants and their ongoing transmission to humans [22-24].

These findings highlight that aquatic systems represent a potentially important setting (a 'hotspot') for driving emergence, release, transmission, persistence and spread of AMR bacteria and resistance genes. At the same time, there is a general lack of marketing authorisations for medications for fish, especially species other than salmonids, with the only antibiotics identified as authorised for use in food-production aquaculture in the EU being amoxicillin, enrofloxacin, florfenicol, flumequine, oxolinic acid, chlor-/oxytetracycline and sulfonamide-trimethoprim, authorised to be administered in-feed only [25]. In addition, FVE has indicated that for certain aquaculture diseases it would be difficult to implement measures that would reduce the need for antimicrobials e.g. strawberry disease in trout, furunculosis in farmed trout and Streptococcosis in sturgeon [26].

There is a need to ensure that antimicrobials already authorised for use in aquaculture remain available for treatment of minor aquatic species and indications in accordance with Article 114 of the Regulation. However, considering the high potential for the aquaculture environment to disseminate AMR, **pursuant to Article 107(6)(b) of the Regulation, certain conditions are proposed, as appropriate, to reduce the risk of the use outside the marketing authorisation of antimicrobials of high importance and not already authorised in food-production aquaculture**.

(iv) Use of medicinal products authorised for human use (HMP)

In accordance with Article 107(5), medicinal products containing antimicrobials designated as reserved for human use under Article 37(5) shall not be used in accordance with Articles 112, 113 and 114. The remaining human-only authorised antimicrobials include certain antibiotics (e.g. amdinopenicillins, ketolides, pseudomonic acids, rifamycins, streptogramins, riminofenazines, sulfones, other TB drugs) and various antifungals (e.g. echinocandins, amphotericin B), antivirals (e.g. antiretrovirals) and antiprotozoals.

Human medicinal products may be used outside the marketing authorisation in animals either because there is no VMP available in a suitable formulation to treat the disease, or because they contain an antimicrobial not authorised for veterinary use. Note that the considerations above regarding unauthorised indications, species, routes of administration and dose duration may equally apply to human medicinal products (HMPs). The CVMP previously recommended [9] that in particular when

prescribing AMEG Category 3 (now Category A) antibiotics, account should be taken of their importance in human medicine and the risk of AMR transfer from animals.

Conditions to help mitigate the AMR risk associated with the use of HMPs in animals could include a requirement for target pathogen identification and AST, restriction to specific indications and to allow use in individual animals only.

(v) Use of VMPs authorised in third countries

As the final option, Articles 112(2), 113(2) and 114(4) allow use of VMPs authorised in third countries for the same animal species and same indication. In this case, no AMR risk assessment will have been conducted according to the EU circumstances. Prohibitions still apply to antimicrobials that are designated for human use only under Article 37(5) and to use in food-producing species of antimicrobials that are not allowed in accordance with the MRL Regulations. It should be noted that there may be some variability in levels of resistance between target pathogen isolates from the EU and those from third countries.

To help mitigate the AMR risk associated with the use on VMPs authorised in third countries, a condition could require target pathogen identification and AST.

Summary of potential conditions to be considered for different types of use outside the terms of the marketing authorisation

In summary, the following conditions have been considered for use of certain antimicrobial classes outside the terms of the marketing authorisation, with specific adjustments as needed:

Summary Table 1. Conditions to be considered for certain antimicrobial classes according to the circumstances of use outside the terms of the marketing authorisation

Type/Nature of use	**Potential conditions to be considered**
Unauthorised indications	Target pathogen identification and susceptibility testing* for indications not included in the SPC Prohibit use for treatment of certain zoonotic pathogens associated with public health risk
Unauthorised animal species	Prohibit use in major animal species (excluding sheep) for which the antimicrobial is not yet authorised
Unauthorised route of administration or formulation	Restrict to certain routes of administration Restrict use of extemporaneous formulations Restrict use to individual animals only Restrict from use in food-production aquaculture
Use of human medicinal products	Target pathogen identification and susceptibility testing* Restrict to named indications Restrict to use in individual animals only
Third country VMPs	Target pathogen identification and susceptibility testing*

*A discussion of the practicability of different methods used for target pathogen identification and susceptibility testing is presented in Annex 1. , where the condition is elaborated in more detail to take account of specific circumstances e.g. the availability of reliable testing methods for certain pathogens or antimicrobials, or to allow exemptions for particular animal species.

3.1.3. Consideration of the need to prohibit use outside the terms of the marketing authorisation

If conditions alone, as discussed above, would not be sufficient in the context of the aim of Article 107(6), then it should be recommended that the antimicrobial should not be used in accordance with Articles 112, 113 and 114.

3.1.4. Other legal provisions in relation to use in accordance with Articles 112, 113 and 114 that were taken into consideration

The scope of this advice is limited in line with the legal provisions for use of medicinal products under Articles 112, 113 and 114, as outlined above (Section 1.2. 'Legislative background'), including:

- **That antimicrobials not listed in Table 1 of the Annex to Regulation (EU) 37/2010 cannot be used in food-producing species**. Articles 113(4) and 114(6) require that '*Pharmacologically active substances included in the medicinal product used in accordance with* [the quoted Articles] *shall be allowed in accordance with Regulation (EC) No 470/2009 and any acts adopted on the basis thereof*.' In addition, the **'Other provisions'** laid out in the MRL Regulation (EU) 37/2010 (e.g. restricting substances from use in animals producing milk or eggs for consumption, or on the route of administration) are understood equally to apply to use outside the terms of the marketing authorisation.
- The antimicrobials/indications listed in **Regulation (EC) 1950/2006 (in force at this time) as essential for the treatment of equine species**, or that bring added clinical benefit compared with other treatment options, should not be assessed against the criteria laid down in Article 107(6) of Regulation (EU) 2019/6 in the context of this advice (See Section 1.2).

3.1.5. Grouping of antimicrobials

In most instances, antibiotics, antifungals and antiprotozoals have been reviewed in groups according to their pharmacological (sub)class. Antiviral substances have been reviewed individually.

To assist with the identification of individual substances that belong to each group of antimicrobials considered, the related WHO ATC codes and ATC vet codes were included in the Annex 6 of the Article 37(5) Human Reserved List report [3].

Based on this, in this advice tables are included at the beginning of the monograph for each antibiotic class (see Section 4.) containing examples of substances in the class that are authorised in veterinary and human medicine in the EU.

The ATC classification [27] groups substances according to chemical, pharmacological and/or therapeutic groups. It should be noted that some substances appear in more than one ATC grouping and have different codes if they are included in different pharmaceutical forms (e.g. for systemic or topical use) or have different therapeutic uses (e.g. nitroimidazoles, used as antibacterials or antiprotozoals).

The tables may not be complete and in some cases it is not possible to verify the authorisation status due to the absence of a comprehensive database at the time of preparation of this advice.

3.2. Uncertainties and data gaps

Information on EU-authorised veterinary medicines has been extracted from SPCs contained in the Union Product Database. Since the database was under development and not completely populated or fully functional during the time of preparation of this advice, some authorised products/indications and alternative treatment options may have been overlooked. In addition, there is no comprehensive and easily searchable database for human medicinal products authorised in the EU or veterinary medicinal products authorised in third countries; therefore, some potential uses under Articles 112, 113 and 114 may not have been considered.

The key data gap is the lack of research or official collection of data on the use of antimicrobials in animals outside the terms of a marketing authorisation. Despite extensive searches performed, certain important related uses may not have come to light in this advice.

The extent/volume of use of antimicrobials for different species/indications outside the terms of a marketing authorisation is also unknown. An assumption has been made that antimicrobial exposure through use outside the marketing authorisation of pharmaceutical formulations intended for group administration is likely to be overall higher than that through formulations for individual administration; this is uncertain.

There is a lack of reliable published studies investigating the efficacy or safety of antimicrobials when used outside the terms of a marketing authorisation in animals, and specifically its impacts on AMR. Many published studies are isolated case reports and some evidence for use in certain species/indications derives from textbooks in which the original source is not always clear. Lack of, or poor quality, data can lead to uncertainty in conclusions. However, the standard of evidence for the present scientific advice is different from that which would be required in a regulatory application when a body of data are purposefully generated by an applicant with the aim of supporting a claim on, for example, the safety and efficacy of a given medicinal product.

Several limitations were identified in the Agency's 'open call for data on the use of antimicrobials' and are documented in the report in Annex 4 to the advice for the Human Reserved List [3]. The respondents to the survey were self-selected and their understanding of the 'cascade' was not assessed. It cannot be assumed that the findings of the call are fully representative of antimicrobial use across all sectors of use of veterinary medicines across the EU. Despite the shortcomings of the open call, the information gathered provides insight into use of antimicrobials outside the terms of a marketing authorisation and has been used as supportive evidence.

Information on the prevalence of antibiotic resistance in isolates of public health importance from food-producing animals in the EU has been extracted from joint EFSA/ECDC EU Summary Reports [28]; however, monitoring follows a protocol targeting specific animal categories, bacterial species and antimicrobial classes and these data can only be partially extrapolated to the evaluation of AMR relating to veterinary uses outside the marketing authorisation. There is presently no similar EU-wide programme for systematic monitoring of resistance in target animal pathogens or companion animal species or for other types of antimicrobials. Otherwise, information on the mechanisms and occurrence of AMR in pathogens/animal species and to different antimicrobial classes is limited to isolated studies and reports identified in literature.

At present, there is very little published information on the burden of animal diseases in the EU and hence it has been difficult to assess the impact on aquaculture and farming if certain conditions could no longer be treated.

The data gaps and uncertainties identified above, depending on circumstances, could lead to either under- or over-estimation of the need for conditions on use under Articles 112, 113 and 114; however, the proposed conditions are considered by the CVMP to be justified based on the available evidence (Sections 2.1 and 2.2) and expert judgement.

3.3. Detailed methodology used to evaluate antibiotics

Background information has been compiled for each antibiotic class that has potential veterinary use in the EU, and for classes authorised in third countries. The most frequently used sources are listed in Annex 3.

This information includes details relating to the availability of different formulations authorised in VMPs for use in different animal species, the MRL status of individual substances in the class and the formulations authorised in human medicinal products (HMPs).

For each class of veterinary antibiotic, the main authorised indications have been identified from SPCs of VMPs. Considering the number of VMPs and differences in indications across Member States, not all indications may have been identified.

Recommendations relating to WOAH, WHO and AMEG classifications for the class and any previous CVMP assessments relating to AMR risk (e.g. in referral procedures) have been documented.

Information has been included from published literature, standard textbooks and from the 'open call for data' relating to uses of substances from the class for indications and target species that, to the best of the experts' knowledge, are not in accordance with EU-authorised SPCs.

This background information is presented in the first part of the monograph for each antibiotic class. The second part of the monograph provides the evaluation of the class against the criteria of Article 107(6) and the consideration of any conditions to be recommended. The evaluation was performed as follows:

The scope of the evaluation of the class or substances within it has been clarified according to the legal restrictions on use in food-producing animals under the MRL Regulation (EU) 37/2010 and any listing in Regulation (EC) 1950/2006 ('equine list') [29, 30].

Step 1: Assessment against the criteria (b), (c) and (d) of Article 107(6)

The aim of Article 107(6) is to reduce the risk to public and animal health due to AMR that is associated with use of antimicrobials outside the marketing authorisation, whilst also acknowledging the need for availability of antimicrobials for limited markets and exceptional circumstances relating to animal health.

Taking account of this objective and the discussion of the criteria above (Section 3.1.1.), it was considered that for some classes of antibiotics, based on the evaluation of criteria (b), (c) and (d), a decision could be made to recommend that no additional legal restrictions should be placed on use under Articles 112, 113 and 114. For these classes, the evaluation stopped at the end of Step 1.

Step 2: Consideration of the conditions to be placed on use of the antibiotic outside the marketing authorisation

Section 3. of this advice includes a detailed discussion of the general use of antimicrobials according to the steps in Articles 112, 113 and 114 (e.g. use to treat unauthorised indications, use to treat unauthorised species, use of a different formulation), the additional AMR risk that may be associated with this use and possible conditions that could be applied to help mitigate this risk. For each antibiotic class, the suitability of the potential conditions discussed in Section 3.1.2. was considered according to an evaluation of identified uses outside the SPC and reasonably anticipated uses that could adversely impact the AMR selection pressure.

Step 3: Consideration of Criteria (a) and (e) in view of proposed conditions on use of the antibiotic outside the marketing authorisation

Criteria (a) and (e) were then evaluated considering use of the antibiotic outside of the marketing authorisation and in the context of the proposed conditions. Section 3.1.1. provides an explanation of how criteria (a) and (e) were applied.

Step 4: Final conclusion

In the light of the evaluation in Steps 1, 2 and 3, the conditions were concluded. As part of this exercise, its was also considered if conditions alone would be sufficient in the context of the aim of Article 107(6). If this was not the case, then it would be recommended that the antibiotic should not be used in accordance with Articles 112, 113 and 114.

3.4. Detailed methodology used to evaluate antivirals

To the best of knowledge, there are currently no direct-acting antiviral substances authorised in veterinary medicinal products in the EU, and none are compliant with the requirement of Articles 113(4) and 114(6) (i.e. 'allowed' in accordance with Table 1 of the Annex to the Regulation (EC) 470/2009 [4]). Therefore, direct-acting antivirals can only be used under Article 112, in non-food-producing animals, including non-food-producing equines.[10] Consequently, uses in accordance with Articles 113 and 114 were not considered.

A review of antiviral substances was undertaken to identify potential therapeutic uses in non-food-producing animals in the EU. Antiviral substances included in the Annex to Regulation (EU) 2022/1255, to be reserved for treatment of certain infections in humans, were excluded from the review. Substances were identified mainly through textbooks and bibliographic data. Owing to the nature of the use, some reports are not from peer-reviewed journals but are cited as they provide evidence for use of the antivirals in veterinary practice. It cannot be excluded that some antiviral substances have been overlooked.

Regarding species treated, one report was made to the 'open call for data' relating to the use of famciclovir to treat viral infections in pinnipeds; otherwise, although there are experimental studies on the use of antivirals in laboratory animals, very little published evidence was found to support their therapeutic use in species other than horses, cats and dogs.

The findings of the review are presented in Section 5.1. of the advice, including a conclusion on whether there is evidence of 'widespread' use of the antiviral to treat specific diseases. The following antivirals were identified as having widespread use for treatment of animals under Article 112: cidofovir, famciclovir, idoxuridine, remdesivir and valacyclovir/acyclovir.

[10] An exception is acyclovir and idoxuridine, which are out of scope of this advice when used for topical treatment of ocular ulcers in equines, being listed in Regulation (EC) 1950/2006 (amended by Commission Regulation (EU) 122/2013).

These antivirals were then evaluated against the criteria of Article 107(6) using the same step-wise process, as outlined above for the antibiotics.

In respect of criterion (b), it is important to note that those antivirals reported as used to treat zoonotic infections that are frequent or endemic in the EU or that, due to their spectrum of activity, may be active against such zoonotic viruses, and for which there is a risk of transmission of antiviral-resistant organisms from animals to humans, were recommended by CVMP for designation under Article 37(5) to be reserved for human use only and are included in the Annex to Regulation (EU) 2022/1255 [2]. The only exception was remdesivir. Therefore, for the remaining antivirals that have been reviewed in this exercise, it has already been concluded that there is no significant risk for human health due to drug-resistance related to their use in animals in the EU.

3.5. Detailed methodology used to evaluate antifungals and antiprotozoals

All antiprotozoal and antifungal drugs that were found to have potential veterinary use in the EU have been assessed. Evidence relating to authorised use in human and veterinary medicine and use outside a marketing authorisation in animals was identified from EU-authorised SPCs, reported uses in standard textbooks, from the 'open call for data' (see 2.2.5.) and from references and guidelines identified for the Article 37(5) Human Reserved List report [3]. Note that most veterinary uses outside the marketing authorisation are based on a very reduced evidence base. The MRL status of individual substances in the class and availability of authorised VMPs for use in different animal species was also documented. It was noted that certain substances/indications in equines were out of scope due to listing in Regulation (EC) 1950/2006 [30].

Each class was then evaluated against each of the Article 107(6) criteria (a) to (e), where found applicable. In particular, regarding criterion (b), for many antiprotozoal and antifungal drugs, information on resistance mechanisms, the prevalence of resistance and evidence for transmission of resistant organisms from animals to humans and other animals is much more limited compared with that for antibiotics. This lack of evidence in some cases makes it more difficult to perform an assessment of the potential risk to animal health and public health due to antiprotozoal or antifungal drug-resistance than it is to do the assessment for antibiotics. However, for some classes/substances, more certain conclusions can be drawn regarding the AMR risks to public health if the class is not related to drugs used in human medicine or where it is used to treat diseases in humans or animals that are not zoonotic/contagious and hence there is no obvious transmission pathway for drug resistance. In addition, several protozoal and fungal diseases are not endemic or are of very low prevalence in animals in the EU, and hence resistance due to use of antimicrobials outside a marketing authorisation could be associated with a low overall risk to animal health. Some of these diseases may only be seen in animals imported into the EU.

Based on criteria (b), (c) and (d), it was considered if conditions should be placed on use under Articles 112, 113 and 114. Conditions were proposed only for use of echinocandins and amphotericin B in accordance with Article 112. In regard to criterion (e), these substances cannot be used in food-producing animals in the absence of MRL status and there is no evidence for need for use in other farmed animals. In the light of the conclusions for the criteria, it was then considered if conditions alone would be sufficient in the context of the aim of Article 107(6). As this was the case for both echinocandins and amphotericin B, no antiprotozoals or antifungals have been recommended to be prohibited from use under Articles 112, 113 and 114.

4. Evaluation of antibiotics

4.1. Natural, narrow spectrum penicillins (beta-lactamase-sensitive penicillins)

4.1.1. Background information

Examples of substances included in the class that are used in veterinary and human medicine in the EU

Examples of substances authorised for veterinary use	**Examples of ATCvet codes**
Benethamine penicillin	QJ01CE91
Benzathine benzylpenicillin	QJ01CE08
Benzylpenicillin (Penicillin G)	QJ01CE01
Penethamate hydriodide	QJ01CE90
Phenoxymethylpenicillin	QJ01CE02
Procaine benzylpenicillin (Penicillin V)	QJ01CE09
Examples of substances authorised for human use	**Examples of ATC codes**
Benzathine benzylpenicillin	J01CE08
Benzathine phenoxymethylpenicillin	J01CE10
Benzylpenicillin	J01CE01
Pheneticillin	J01CE05
Phenoxymethylpenicillin	J01CE02
Procaine benzylpenicillin	J01CE09

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

Substance	**Species**	**MRL tissues**	**MRL milk**	**MRL eggs**	**Relevant 'Other provisions'**
Benzylpenicillin	All food-producing species	Yes	Yes	-	Not for use in animals from which eggs are produced for human consumption.
Penethamate	All mammalian food-producing species	Yes	Yes	-	-
Phenoxymethylpenicillin	Porcine Poultry	Yes	-	-	Not for use in animals from which eggs are produced for human consumption

EU-authorised VMP formulations, based on sales reported ESVAC

Species		**Route of administration**						
		Group		**Individual**				
		In-feed	**In-water**	**Injection**	**Oral e.g. tablet, paste**	**Topical/local (incl. intrauterine)**	**Intra-mammary**	**Oral powder**
Major	**Cattle**		BP	BP, PH		BP	BP, PH	
	Sheep (for meat)		BP	BP				
	Pigs	PMP	BP	BP		BP		
	Chickens		BP, PMP	BP				BP
	Dogs			BP				
	Cats			BP				
Limited market species As listed in SPCs	**Turkeys**		BP, PMP	BP				BP
	Goats		BP	BP				
	Horses			BP, PH				
	Fur animals							

Summary of main indications and contra-indications for EU-authorised VMPs, based on selected SPCs

Main indications	This class is very important in the treatment of many diseases in a broad range of animal species e.g. septicaemias, respiratory and urinary tract infections. SPC indications are often non-specific e.g. treatment of systemic infections caused by or associated with organisms susceptible to penicillin. Some specified indications are as follows: **Cattle, sheep, goats, pigs:** For the treatment of systemic infections caused by or associated with organisms susceptible to penicillin. Treatment of diseases e.g. erysipelas; navel/joint-ill; respiratory tract infections including pneumonia and atrophic rhinitis; listeriosis; septicaemia; urogenital tract infections and the control of secondary bacterial invaders in diseases of primary viral origin. Susceptible ensitive organisms include: *Streptococcus* and *Staphylococcus* spp., some *E. coli* and some *Salmonella* spp. **Cattle:** For treatment of subclinical and clinical mastitis and the prevention of new infections during the dry period, caused by bacteria susceptible to penicillin. **Horse:** Infections associated with *Streptococcus* spp. and *Staphylococcus* spp. **Chicken:** For the treatment and metaphylaxis of necrotic enteritis caused by *Clostridium perfringens*. **Turkeys**: In combination with streptomycin it is used to treat erysipelas. **Dogs, cats**: For the treatment of wounds, skin infections, tooth abscesses and bladder infections.
Contraindications	Do not use in known cases of hypersensitivity to penicillins. Do not administer by the intravenous route. Do not use in case of severe renal dysfunction with anuria and oliguria. Not to be used on very small herbivores such as guinea pigs, gerbils and hamsters. Do not use in the presence of beta-lactamase producing pathogens.

Examples of EU-authorised HMP formulations, from Article 57 database

Substance	Route of administration		
	Injection	Oral e.g. tablet, liquid	Topical/local
Benzathine benzylpenicillin	x		
Benzathine phenoxymethylpenicillin		x	
Benzylpenicillin	x		
Pheneticillin		x	
Phenoxymethylpenicillin	x	x	
Procaine benzylpenicillin	x		

Existing recommendations

WOAH recommendations

Natural penicillins (as part of the Penicillins class) are categorised VCIA by WOAH (formerly OIE). *Specific comments:* Penethamate (hydroiodide) is currently only used in animals. The wide range of applications and the nature of the diseases treated make penicillins extremely important for veterinary medicine. This class is used in the treatment of septicaemias, respiratory and urinary tract infections. This class is very important in the treatment of many diseases in a broad range of animal species. Few economical alternatives are available.

WHO classifications

WHO: HIA

- (C1: No) In certain geographic settings, Criterion 1 may be met: the class may be one of limited therapies for streptococcal infections, yaws and syphilis.
- (C2: Yes) May result from transmission of penicillin-resistant *Staphylococcus aureus*, from nonhuman sources.

WHO AWaRe: Access: Benzylpenicillin, Phenoxymethylpenicillin, Penamecillin, Clometocillin, Benzathine benzylpenicillin, Procaine benzylpenicillin; Watch: Pheneticillin

AMEG and CVMP recommendations

Narrow-spectrum penicillins are included in the AMEG Category D. There are alternative treatments in human and veterinary medicine for their indications and that do not select for resistance to Category A substances through specific multiresistance genes.

These antibiotics are not devoid of negative impact on resistance development and spread. To keep the risk from use of these antibiotic classes as low as possible it is important that responsible use principles are complied with in everyday practice. Unnecessary use and unnecessarily long treatment periods should be avoided and group treatment restricted to situations where individual treatment is not feasible.

Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Disclaimer: The information in this section reflects reported use of antimicrobials outside the terms of a marketing authorisation. No evaluation is made in this section by the working group on the efficacy or safety of the reported uses, or on their potential impact on development and dissemination of AMR.

Information from published sources

Considering the broad range and often non-specific indications stated in many SPCs and the wide range of animal species for which this class is authorised, uses outside the SPC many times relate to exotic or limited market species or use of human authorised formulations.

Information from the open call for data on use of antimicrobials in animals

Substance	Species	Indication	Alternatives	Consequences of unavailability
Benzylpenicillin (Penicillin G)	equine	foal septicaemia, threatening infections with Gram-positive bacteria	procaine penicillin	delay in treatment, muscular pain, increased use of ceftiofur
Benzylpenicillin (Penicillin G)	cattle	mastitis, pneumonia, footrot, acute metritis	tetracycline, macrolides florfenicol enrofloxacin	treatment with broader spectrum and in many cases less efficient substances
Benzylpenicillin (Penicillin G)	cattle, sheep	listeriosis		
Benzylpenicillin (Penicillin G)	swine	Respiratory disease caused by bacteria such as *Actinobacillus pleuropneumoniae* and *Pasteurella multocida* with wildtype minimal inhibitory concentrations where labelled doses result in treatment failure or suboptimal treatment outcome	Labelled dose has poor efficacy. Alternatives are more broad-spectrum i.e. cephalosporins, enrofloxacin, tetracyclines, and long-acting macrolides such as gamithromycin, tulathromycin, and tildipirosin	Use of more broad-spectrum antibiotics
Benzylpenicillin (Penicillin G)	dogs, cats	sepsis, pneumonia, complicated wounds	other beta-lactams	necessity to use beta-lactam molecules with

				larger spectrum even if unnecessary
Benzylpenicillin (Penicillin G)	rabbits	abscesses	amoxicillin-clavulanic acids	disease progression, pain, death
Benzylpenicillin (Penicillin G)	ornamental birds	Infection with bacteria that are only sensitive to this antibiotic like Infection with Clostridium perfringens	none	animals could not be treated adequately, which would cause a serious violation of animal welfare
Penethamate hydriodide	cattle	mastitis, pneumonia Hoof infections		
Penethamate hydriodide	goat	acute mastitis with impaired general condition caused by *Staphylococcus aureus*		
Phenoxymethylpenicillin	pig	*Clostridium perfringens* type C diarrhoea before prevention by vaccinations provides immunity.	None as no aminopenicillins (oral suspension) approved for food-producing animals in our country	Death or euthanasia of piglets
Phenoxymethylpenicillin	poultry	necrotic enteritis (*Clostridium perfringens*), arthritis and tendosynovitis (*Staph. aureus*), erysipelas	amoxicillin in drinking water (cascade use of vet med; no suitable product with marketing authorisation in our country)	
Phenoxymethylpenicillin	fur animals	infections caused by streptococci or staphylococci	lincomycin	
Procaine benzylpenicillin (Penicillin V)	equine	respiratory infection, wound infection	ampicillin	increased use of ampicillin with possible digestive side effects, or ceftiofur
Procaine benzylpenicillin (Penicillin V)	cattle	Mastitis		
Procaine benzylpenicillin (Penicillin V)	swine	Respiratory disease caused by bacteria such as *Actinobacillus pleuropneumoniae* and *Pasteurella multocida* with wildtype minimal inhibitory concentrations where labelled doses result in treatment failure or suboptimal treatment outcome.	Labelled dose has poor efficacy. Alternatives are more broad-spectrum i.e. cephalosporins, enrofloxacin, tetracyclines, and long-acting macrolides such as gamithromycin, tulathromycin, and tildipirosin.	Use of more broad-spectrum antibiotics see the adjacent "Existing alternatives".
Procaine benzylpenicillin (Penicillin V)	horse	Severe (systemic) infections		
Procaine benzylpenicillin (Penicillin V)	goat	first choice antibiotic in case of infections caused by Gram-positive bacteria, knowledge about the resistance situation assumed		

Procaine benzylpenicillin (Penicillin V)	rabbits	Infectious disease and abscesses		Rabbits die or get euthanized

4.1.2. Evaluation

Scope of permitted use according to the MRL Regulation

Narrow-spectrum penicillins (i.e. Benzylpenicillin, Penethamate, Phenoxymethylpenicillin) are included in Table 1 (allowed substances) of the Annex to Regulation (EU) 37/2010 and hence can be used in all food-producing species in accordance with Articles 113 and 114 of Regulation (EU) 2019/6. 'Other provisions' restrict certain narrow-spectrum penicillins from use in animals producing eggs for human consumption.

Narrow-spectrum penicillins can be used in non-food-producing species in accordance with Article 112.

Examples of veterinary-authorised formulations/species

Various narrow-spectrum penicillins are available for group administration in-water and/or in-feed to all major food-producing animals and some limited market species e.g. turkeys, goats, fur animals. They are also available for injection for treatment of individual food-producing species and for intramammary administration to cattle.

For dogs and cats, injectable products are available.

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Importance for human health

Penicillins belong to a large group of beta-lactam antibiotics, which share a common structural feature – the beta-lactam ring. Penicillins are further classified based on their spectrum of activity to penicillins, aminopenicillins (evaluated separately), antistaphylococcal penicillins (evaluated separately). Natural, narrow-spectrum penicillins (benzylpenicillin, benzathine benzylpenicillin, procaine benzylpenicillin, pheneticillin etc.) are evaluated here.

Penicillins are active against Gram-positive cocci, such as *Streptococcus pyogenes* and other beta-haemolytic streptococci, *S. pneumoniae*, *S.* viridans, and non-beta-lactamase-producing *Staphylococcus aureus.* Some Gram-negative bacteria such as *Neisseria meningitidis* and penicillin-sensitive *N. gonorrhoeae* are susceptible. Non-beta-lactamase-producing *Haemophilus influenzae* is moderately resistant, and all other aerobic, and aero-anaero facultative Gram-negative bacilli are highly resistant. Many organisms that were originally highly susceptible have now developed resistance, which limits the usefulness of these antibiotics in clinical settings [31].

Penicillin G remains a very effective treatment for infections caused *S. pyogenes*, such as pharyngitis, scarlet fever, cellulitis, necrotizing fasciitis, septic arthritis, uterine infection, and septicaemia [31].

The recent data from the EARS-Net showed that in EU/EEA (population-weighted mean) in 2019, 12.1% of *S. pneumoniae* isolates were resistant to penicillin and 15.5% of *S. aureus* isolates were MRSA [32].

Penicillins are nationally approved in the EU member states for indications that include the treatment of wound infections, pyogenic infections of the skin, soft tissue infections and infections of the nose, throat, nasal sinuses, respiratory tract and middle ear; they are also indicated for the following

infections caused by penicillin-sensitive microorganisms: generalised infections, septicaemia and pyaemia from susceptible bacteria; acute and chronic osteomyelitis, sub-acute bacterial endocarditis and meningitis caused by susceptible organisms; suspected meningococcal disease; gas gangrene, tetanus, actinomycosis, anthrax, leptospirosis, rat-bite fever, listeriosis, severe Lyme disease, and prevention of neonatal group B streptococcal infections; complications secondary to gonorrhoea and syphilis (e.g. gonococcal arthritis or endocarditis, congenital syphilis and neurosyphilis); diphtheria, brain abscesses and pasteurellosis.

Importance for animal health

The wide range of applications in a broad range of animal species and the nature of the diseases treated make penicillins extremely important for veterinary medicine. Narrow spectrum penicillins are used in the treatment of septicaemias, respiratory tract and urogenital infections, amongst others. According to ESVAC, sales of benzylpenicillin and its derivatives made up 10.6% of the sales of all penicillins for food-producing animals in the EU in 2021, equivalent to 3.3% of overall antibiotic usage (mg/PCU).

Activity of narrow spectrum penicillins is mainly limited to Gram-positive bacteria and Gram-negative cocci. Benzylpenicillin (penicillin G) and phenoxymethylpenicillin (penicillin V) have outstanding activity against many Gram-positive bacteria, notably beta-hemolytic streptococci, non-resistant staphylococci, *Actinomyces* spp*., Bacillus* spp*., Clostridium* spp*., Corynebacterium* spp*.,* and *Erysipelothrix rhuseopathiae*. Susceptible Gram-negative species include anaerobes such as Clostridium spp., some Bacteroides spp. and Fusobacterium spp. Penicillin V is used for oral administration as it resists hydrolysis by gastric acid.

Cattle, sheep, goat: The preferred medication for treating illnesses caused by susceptible bacteria, such as clostridial infections, *Corynebacterium renale* infections, *H. somni* infections, and pneumonic pasteurellosis, is penicillin G administered parenterally [33].

Swine: Penicillin may be administered parenterally for erysipelas, streptococcal, clostridial, and corynebacterial infections. Penicillin V may be administered orally for metaphylaxis of acute *Streptococcus suis* infections which can spread rapidly in piglets causing septicaemia, meningitis, arthritis and endocarditis and resulting in high mortality [33, 34].

Horses: Penicillin G is used to treat beta-hemolytic streptococci, in neonatal foals for *S. zooepidemicus* polyarthritis and meningitis, and in adult animals for infections of wounds, lower respiratory and urinary tracts, and the uterus [33].

Dogs and Cats: For actinomycosis, streptococcal and clostridial infections, as well as infections caused by susceptible Gram-negative bacteria such *P. multocida*, penicillin G may be used; however, due to poor oral absorption, amoxicillin is used instead [33, 35].

Poultry: Penicillin V is administered orally for the treatment of intestinal spirochetosis (*Brachyspira* spp.), and clostridial diseases (necrotic enteritis and ulcerative enteritis). It is also used in combination with streptomycin to treat erysipelas in turkeys [33, 36].

Considering the broad range and often non-specific indications stated in many SPCs and the wide range of animal species for which this class is authorised, uses published in literature that might be considered outside a marketing authorisation mostly relate to exotic or limited market species or use of human authorised formulations. According to the open call for data, penicillins are used in goats,

reindeer, fur animals and ornamental birds. Human formulations of sodium penicillin suitable for intravenous administration are used for treatment serious acute infections e.g. septicaemia and peritonitis in foals. Individual minor indications may not be included in the SPC for the specific product used, e.g. Tyzzer's disease (*Clostridium piliforme*) in rabbits.

Selection and development of resistance

The most important mechanisms of resistance to the beta-lactam antimicrobials in Gram-positive and Gram-negative bacteria is the production of beta-lactamase enzymes that catalyse the hydrolysis of the beta-lactam ring. There is a very wide variety of different beta-lactamases with varying substrate specificity [37]. Beta-lactamases are encoded by genes located on the bacterial chromosome or on mobile genetic elements (e.g. plasmids, transposons) [38]. A certain amount of cross-resistance exists between the different beta-lactam antibiotics and therefore the use of one beta-lactam antibiotic may select for resistance to other beta-lactam antibiotics. Generally, beta-lactam antibiotics with a narrow spectrum of action will exert a narrower selection pressure than beta-lactams with a more broad-spectrum such as aminopenicillins.

In Gram-positive bacteria, acquisition of PBPs with lower affinity for beta-lactams is another important resistance mechanism. This type of mechanism is common in staphylococci and is mediated by *mec* genes (e.g*. mec*A or *mecC* in MRSA) [38-40]. *Mec*A and *mec*C genes are part of the mobile staphylococcal chromosomal cassette, SCC*mec*. Staphylococci of animal origin carrying the *mec*A gene can be considered resistant to all beta-lactams except ceftobiprole and ceftaroline. Modification of PBPs and/or acquisition of PBPs is also a cause of beta-lactam resistance in *Streptococcus* spp., *Enterococcus* spp., *Neisseria* spp. and *Haemophilus* spp., although the genes conferring resistance are dependent on the bacterial species in question [41].

There is no monitoring of resistance specifically to narrow-spectrum penicillins under EFSA/ECDC mandatory EU surveillance of AMR in zoonotic and indicator bacteria from animals – Enterobacterales and most *Campylobacter* spp. are intrinsically resistant. Data on resistance to ampicillin can be found in the monograph on aminopenicillins; generally, bacteria that are resistant to aminopenicillins are also resistant to narrow-spectrum penicillins.

In the context of the Animal Health Law, Regulation (EU) 2016/429, EFSA has conducted an extensive review of literature studies to determine the global state of play of selected resistant bacteria that constitute a threat to the health of specified animal species. Information on resistance in target pathogens pooled from the European studies has been extracted from EFSA's reports for the purpose of this advice:

In dairy cattle in Europe (predominantly mastitis cases), the mean level of resistance to penicillin in *S. aureus* was 32.1% [range 4% - 63.1%] with levels being substantially higher in S. European countries compared with N. Europe. In staphylococci from pigs, the mean level of resistance to penicillin was 71.2%.

Susceptibility to narrow-spectrum penicillins in respiratory pathogens from food-producing species is variable. In *Pasteurella multocida* and *Mannheimia haemolytica* from cattle, the mean levels of resistance to penicillin were 1.7 and 21.0% respectively. In respiratory pathogens from pigs, *P. multocida*, *Glaeserella haemophilus* and APP, the mean resistance levels were 30.7, 20.0 and 7.8% resp.

In *Streptococcus suis* from pigs, mean levels of resistance to beta-lactams remain low (2.5% for penicillin). Similarly, there is generally low resistance to penicillin in streptococci from horses (mean level in *S. zooepidemicus* 8.9% and *S. equi* 0%).

Levels of resistance to beta-lactams are also very low in *Enterococcus* spp. from poultry in Europe and international data suggest that *Clostridium perfringens* also retains good susceptibility (See Annex 3. EFSA Animal Health Law Scientific opinions).

Resistance to penicillins is widespread in staphylococci from dogs and cats and is mainly due to production of penicillinases encoded by *bla*Z [42, 43].

Transmission of resistance

Beta-lactamase mediated narrow-spectrum penicillin resistance is very common and extensively distributed in several commensal bacterial species of human and animal origin, and therefore the route and direction of resistance transfer between animals and humans can be very challenging to investigate. Nevertheless, there are several examples demonstrating that drug-resistant bacteria can be transmitted between animals and humans.

There is direct and indirect evidence of animal to human transmission of livestock associated MRSA CC398, human to animal transmission of human associated MRSA strains [44]. The most remarkable livestock associated clone is ST398, which was initially found among pigs, and subsequently was detected in several companion and food-producing animals as well as in humans [45]. MRSA can be transmitted between pet animals and humans, horses and humans, and livestock and humans and the risk for MRSA carriage is higher in humans professionally exposed to animals [45].

Resistance can be spread vertically due to multiplication of resistant isolates or horizontally through the acquisition of mobile genetic elements (e.g. plasmids). The magnitude of the risk of resistance transfer from animals to humans and vice versa may depend on several factors related to the host animal and bacterial features (such as the amount of bacteria and the ability to colonize or cause infections in humans). Also the length and closeness of contact and route of transfer (via skin contact or contaminated food) may affect the magnitude of the risk of resistance transfer from animals to humans and vice versa. There is evidence that humans who have contact with livestock have a higher chance of carrying multi-drug resistant bacteria, such as ESBL-producing *E. coli* or LA-MRSA, compared to humans with no animal contact, whilst the risk for resistance transfer by consumption of food of animal origin is considered low, especially if good food hygiene practices are followed.

In conclusion for the criterion (b) in relation to risk for animal or public health in case of development of antimicrobial resistance,

- Narrow-spectrum penicillins are important antibiotics, used for a wide range of infections in both human and veterinary medicine but have a spectrum of activity limited to Gram-positive bacteria and some Gram-negative cocci.
- The main mechanisms of resistance to narrow-spectrum penicillins are the production of beta-lactamases and the acquisition of PBPs with lower affinity for beta-lactams.
- Resistance to narrow spectrum penicillins in many veterinary target bacteria (e.g. staphylococci) is widespread; however some important pathogens retain good susceptibility e.g. *Streptococcus suis* in pigs and enterococci and *C. perfringens* in poultry.
- Resistance to narrow-spectrum penicillins can be transmitted from animals to humans and other animals via zoonotic and target pathogenic bacteria and commensals organisms.

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to Narrow-spectrum penicillins.

Criterion (c) – availability of other treatments for animals

Narrow-spectrum penicillins are in the AMEG Category D and **in general there are alternative antimicrobials** dependent on the specific disease, pathogen and target animal species under treatment. However, alternatives in Category D may be less favoured in terms of AMR selection due to a broader spectrum of activity e.g. aminopenicillins, TMPS, or there may be high levels of resistance in target pathogens. In these cases, alternatives may only be available from a higher AMEG category.

Criterion (d) – availability of other antimicrobial treatments for humans

There are several alternative treatment options for each indication.

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

- Narrow-spectrum penicillins are regarded as first-line antimicrobials in human medicine. They are indicated for a wide range of infections, some of which are serious; however, sufficient alternative antibiotics are available.
- In veterinary medicine, narrow-spectrum penicillins are also a first-line choice (AMEG Category D), used to treat a wide range of infections in a variety of animal species.
- Resistance to narrow spectrum penicillins in many veterinary target bacteria (e.g. staphylococci) is widespread; however some important pathogens retain good susceptibility e.g. *Streptococcus suis* in pigs and enterococci and *C. perfringens* in poultry.
- Alternative antibiotics are available for veterinary indications and may also be from category D; although they may be less favoured in terms of AMR selection due to a broader spectrum of activity.
- Narrow-spectrum penicillins are authorised as local, parenteral and orally administered VMPs, including for group oral administration. Also considering the broad and often non-specific indications and the wide range of animal species in which this class is authorised, uses outside a marketing authorisation are not expected to contribute substantially to AMR beyond authorised use.

Therefore, considering the points above relevant to criteria (b), (c) and (d), it is recommended that no conditions should be placed on the use of Narrow-spectrum penicillins outside the terms of the marketing authorisation, although responsible antimicrobial use principles should be applied.

4.2. Antistaphylococcal penicillins (beta-lactamase-resistant penicillins)

4.2.1. Background information

Examples of substances in the class that are authorised in veterinary and human medicine in the EU

Examples of substances authorised for veterinary use	**Examples of ATCvet codes**
Cloxacillin	QJ01CF02
Dicloxacillin	QJ01CF01
Nafcillin	QJ01CF06
Oxacillin	QJ01CF04
Examples of substances authorised for human use	**Examples of ATC codes**
Cloxacillin	J01CF02
Dicloxacillin	J01CF01
Flucoxacillin	J01CF05
Oxacillin	J01CF04

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

Substance	**Species**	**MRL tissues**	**MRL milk**	**MRL eggs**	**'Other provisions'**
Cloxacillin, Dicloxacillin, Oxacillin	All food-producing species	Yes	Yes	No	Not for use in animals from which eggs are produced for human consumption
Nafcillin	All ruminants	Yes	Yes	No	For intramammary use only

EU-authorised VMP formulations, based on sales reported to ESVAC

Species		**Route of administration**					
		Group		**Individual**			
		In-feed	**In-water**	**Injection**	**Oral e.g. tablet, paste, powder**	**Topical/local (incl. intrauterine)**	**Intra-mammary**
Major	**Cattle**			CLOX, DCX		CLOX	CLOX, DCX, NAF, OXA
	Sheep (for meat)					CLOX	CLOX, NAF
	Pigs			DCX			
	Chickens						
	Dogs			DCX		CLOX	
	Cats			DCX		CLOX	
Limited market species As listed in SPCs	**Horses**			DCX		CLOX	
	Goats						CLOX, NAF

CLOX (cloxacillin), DCX (dicloxacillin), NAF (nafcillin), OXA (oxacillin)

Summary of main indications and contra-indications for EU-authorised VMPs, based on selected SPCs

Main indications	Treatment of intramammary infections at the point of drying off or during lactation in cows, sheep and goats mainly caused by *Staphylococcus aureus* (including penicillin resistant) as well as *Streptococcus agalactiae*, *Streptococcus uberis*, *Streptococcus dysgalactiae* and *Corynebacterium pyogenes*. There is limited availability of injectable products containing (di)cloxacillin in combination with amoxicillin, for treatment of cattle, pigs, horses, dogs and cats for intestinal, respiratory, urogenital infections; mastitis, endometritis, polyarthritis and traumatic reticulitis caused by Gram-positive bacteria (in particular *Streptococcus* spp., *Staphylococcus* spp. - including penicillinase producing strains - *Clostridium* spp. and *Bacillus* spp.).

	Topical treatment of eye infections in cattle, sheep, horses, dogs and cats caused by Gram-positive bacteria, including *Staphylococcus* spp. and *Bacillus* spp., and *Moraxella bovis*. An intrauterine tablet is available containing cloxacillin (± ampicillin), for treatment of endometritis due to susceptible pathogens in cattle.
Contraindications	Do not use in cases of known hypersensitivity to penicillins.

Examples of EU-authorised HMP formulations, from Article 57 database

Substance	Route of administration			
	Injection	Oral e.g. tablet, liquid	Topical/local	Inhalation
Cloxacillin	x	x		
Dicloxacillin		x		
Flucoxacillin	x	x		x
Oxacillin	x	x		

Existing recommendations

WOAH recommendations

Antistaphylococcal penicillins (as part of the Penicillins class) are categorised as VCIA by WOAH (formerly OIE). *Specific comments:* The wide range of applications and the nature of the diseases treated make penicillins extremely important for veterinary medicine. This class is used in the treatment of septicaemias, respiratory and urinary tract infections. This class is very important in the treatment of many diseases in a broad range of animal species. Few economical alternatives are available.

WHO classifications

WHO: HIA

- (C1: No) In certain geographic settings, Criterion 1 may be met: the class may be one of limited therapies for staphylococcal infections (*S. aureus*).
- (C2: Yes) May result from transmission of *S. aureus*, including MRSA, from nonhuman sources.

WHO AWaRe: Access: cloxacillin, dicloxacillin, nafcillin, oxacillin, flucloxacillin

AMEG and CVMP recommendations

Antistaphylococcal penicillins are included in the AMEG Category D. There are alternative treatments in human and veterinary medicine for their indications and that do not select for resistance to Category A substances through specific multiresistance genes.

These antibiotics are not devoid of negative impact on resistance development and spread. To keep the risk from use of these antibiotic classes as low as possible it is important that responsible use principles are complied with in everyday practice. Unnecessary use and unnecessarily long treatment periods should be avoided and group treatment restricted to situations where individual treatment is not feasible.

Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Disclaimer: The information in this section reflects reported use of antimicrobials outside the terms of a marketing authorisation. No evaluation is made in this section by the working group on the efficacy or safety of the reported uses, or on their potential impact on development and dissemination of AMR.

Information from published sources

A potential use of antistaphylococcal penicillins outside of a marketing authorisation is treatment of staphylococcal skin infections in dogs by oral administration; however, utility is reduced by availability of authorised alternatives [33, 46].

Information from the open call for data on use of antimicrobials in animals

The information below is summarised from the open call for data. Inclusion in the table does not endorse use or imply that it is consistent with use according to legislative provisions in Articles 112 to 114.

Substance	Species	Indication	Alternatives	Consequences of unavailability
Cloxacillin	Horses	Corneal disease (ulcerative keratitis)		
Cloxacillin intramammary formulation	Cattle	Keratoconjunctivitis caused by *Moraxella bovis*	Tulathromycin, but cannot be used in dairy cows	Animal suffering and economic losses

4.2.2. Evaluation

Scope of permitted use according to the MRL Regulation

Cloxacillin, dicloxacillin, oxacillin and nafcillin are included in Table 1 (allowed substances) of the Annex to Regulation (EU) 37/2010 and hence can be used in all food-producing species in accordance with Articles 113 and 114 of Regulation (EU) 2019/6. However, there are 'Other provisions' that restrict cloxacillin, dicloxacillin and oxacillin from use in animals producing eggs for human consumption and nafcillin is restricted to intramammary use only.

Antistaphylococcal penicillins can be used in non-food-producing species in accordance with Article 112.

Examples of veterinary-authorised formulations/species

The majority of formulations of antistaphylococcal penicillins are intramammary preparations for use in cattle, sheep and goats. However, topical ocular formulations are available for cattle, sheep, horses, dogs and cats, and intrauterine formulations for use in cattle. Injectable formulations of antistaphylococcal penicillins are available for use in cattle, pigs, horses, dogs and cats.

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Importance for human health

Antistaphylococcal penicillins are active against Gram-positive cocci (e.g., *Staphylococcus aureus, S. epidermidis, Streptococcus pyogenes, S. pneumoniae*). They have less intrinsic activity than penicillin G, and are ineffective for enterococci, *Listeria*, and *Neisseria* spp. They have no activity against Gram-negative bacteria [47]. They are used for treatment of penicillin-resistant methicillin susceptible staphylococcal infections such as bacteraemia, skin and soft tissue infections (SSTIs), bone and joint infections, endocarditis, severe pneumonia and meningitis [48].

Antistaphylococcal penicillins are nationally approved in the EU, both alone and in combinations. Approved indications include the treatment of the following infections in adults and children: osteomyelitis, endocarditis and the treatment of patients with bacteraemia that occurs in association with, or is suspected to be associated with, any of the infections listed above. Flucloxacillin may also be

used in the peri-operative prophylaxis for surgical procedures when appropriate, for example cardiothoracic or orthopaedic surgery.

Importance for animal health

Antistaphylococcal penicillins are authorised in VMPs in the EU for use in cows, goats and sheep for local treatment of intramammary infections (IMI) due to *Staphylococcus aureus*, *Streptococcus* spp. and *Trueperella pyogenes*. Mastitis due to IMI is one of the most common diseases in dairy cows, having well recognised effects on health and welfare and frequently requiring antimicrobial treatment [49-51]. Mastitis in sheep and goats is also recognised as a significant welfare issue [52]. Severe IMI due to *S. aureus* or *T. pyogenes* can result in potentially fatal sepsis. In addition, IMI due to *S. aureus* are contagious and if not treated promptly may become chronic, transmit within the herd and result in loss of yield and culling of animals [53, 54]. Antistaphylococcal penicillins are used for the treatment of penicillinase-producing strains of *S. aureus*, which are common in certain EU regions [55]. Antistaphylococcal penicillins are a narrow-spectrum treatment option.

Injectable formulations of (di)cloxacillin in combination with amoxicillin have limited availability for treatment cattle, pigs, horses, dogs and cats for intestinal, respiratory and genitourinary infections, mastitis, polyarthritis and trauma reticulitis, for infections including penicillinase-producing *Staphylococcus* spp.

Cloxacillin is authorised as a topical treatment for eye infections due to *Staphylococcus* spp., *Bacillus* spp. and *Moraxella bovis* in food-producing and companion animals.

According to standard textbooks, a potential use for antistaphylococcal penicillins is for oral treatment of staphylococcal skin infection in dogs. In the absence of an oral veterinary formulation in the EU, this would entail use of human-authorised medicines, although alternative VMPs containing beta-lactams with more reliable bioavailability are available.

No authorised VMPs containing antistaphylococcal penicillins were found for use in aquaculture in the EU, and no evidence was found for use in these species outside a marketing authorisation.

Reports to the Open call for data indicated the use of cloxacillin to treat ulcerative keratitis in horses; it is not clear what aspect of this use is outside the terms of a marketing authorisation. In addition, intramammary formulations of cloxacillin were reported to be used for treatment of keratoconjunctivitis caused by *Moraxella bovis*; this use may be related to lack of local availability of the authorised ocular preparation.

Development, selection and transmission of resistance

Antistaphylococcal penicillins are stable to staphylococcal penicillinase. The most common mechanism of resistance to these antibiotics in staphylococci is through acquisition of a *mec* gene that encodes a penicillin-binding protein (PBP) with lower affinity for most beta-lactams, (including antistaphylococcal penicillins), except to the staphylococcal cephalosporins, ceftobiprole and ceftaroline. The *mec* gene is located on the mobile genetic element, SCCmec ([39, 40, 56]. The SCC*mec* might carry resistance to other antimicrobials (e.g. aminoglycosides and macrolides), and can spread between different staphylococci species that are part of normal microbiota or potential pathogens. Methicillin-resistant staphylococci is usually transmitted clonally.

Cross-resistance between antistaphylococcal penicillins and other beta-lactams, with exception of ceftobiprole and ceftaroline, is commonly observed in staphylococci carrying *mec* genes. The animal origin of isolates carrying different *mec* genes has been suggested [39, 57, 58].

There is no statutory monitoring of resistance to antistaphylococcal penicillins in animal isolates in the EU. Monitoring of MRSA under EFSA/ECDC surveillance in food-producing species is voluntary and data are provided by few member states. Most isolates are LA-MRSA. The prevalence ranges from 0% to 100% depending on animal production type and country [59]. There is little reporting on prevalence of MRSA/P in companion animals, which appears to vary across the EU based on studies available [60-62].

With respect to target pathogens from animals, in dairy cattle in Europe (predominantly mastitis samples) the mean proportion of *Staph. aureus* resistant to methicillin (MRSA) was 9.9% [range 0 – 27.1%]. In *S. aureus* from horses the mean level of methicillin resistance was 7.3% [0-27.1%]. In dogs and cats, a mean of 5.8% [range 0-41.4%] of *Staph. pseudintermedius* and 17.5% [range 0-35.9%] of *S. aureus* were reported to be methicillin resistant (See Annex 3. EFSA Animal Health Law Scientific opinions).

Food is generally not considered to be a significant source of MRSA in humans [63, 64]. MRSA is mainly transmitted by direct contact from food-producing animals [65]. In geographical areas with high density of farms, livestock associated MRSA (LA-MRSA) could contribute significantly to the burden of MRSA disease in humans [62, 66, 67]. There is evidence for rare zoonotic transmission of MRSA/P from companion animals to persons in contact [62, 66, 67]. MRSA and MRSP may also be transmitted between animals [44, 68].

In conclusion, there is evidence for the selection and transmission of resistance to antistaphylococcal penicillins between animals and from animals to humans via zoonotic pathogens or commensal bacteria capable of transferring resistance to human pathogens.

In conclusion,

- In human medicine, antistaphylococcal penicillins are important for treatment of a variety of serious penicillin-resistant methicillin-susceptible staphylococcal infections and for surgical prophylaxis. In veterinary medicine, they are, primarily used in ruminants for intramammary treatment of mastitis due to penicillinase-producing staphylococcal and other Gram-positive organisms and are important for treatment of eye infections in cattle due to *Moraxella bovis*. When used alone, they are a narrow-spectrum treatment option.
- There is little evidence for use of antistaphylococcal penicillins in animals outside the terms of the marketing authorisation in the EU.
- The main mechanism of resistance in Staphylococci is through acquisition of *mec* genes, which confer cross-resistance to almost all beta-lactams. MRSA/P can be transmitted between animals and from animals to humans. The risk is highest for humans or animals in direct contact with infected livestock/pets.

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to Antistaphylococcal penicillin.

Criterion (c) – availability of other treatments for animals

Alternatives for intramammary treatment of IMI caused by *S. aureus* are TMPS and novobiocin [69], although only available in combination with other antibiotics, or substances from a higher AMEG category with a broader spectrum (e.g. lincosamides, amoxicillin-clavulanate, aminoglycosides, cephalosporins). Cloxacillin and nafcillin are one of few antibiotics authorised as a VMP for intramammary use in sheep in the EU.

Criterion (d) – availability of other antimicrobial treatments for humans

Alternative treatment for invasive staphylococcal infections (i.e., bacteraemia and endocarditis) include 1st-generation cephalosporins but the antistaphylococcal penicillins are the preferred option. Vancomycin can be used for patients with allergy to penicillin but is less efficient [70, 71].

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

- In human medicine, antistaphylococcal penicillins are important for treatment of a variety of serious penicillin-resistant methicillin-susceptible staphylococcal infections e.g. SSTI, bone and joint infections, endocarditis, bacteraemia and pneumonia. They are also used for surgical prophylaxis. Alternatives are available.
- In veterinary medicine, antistaphylococcal penicillins are primarily administered locally to ruminants, for intramammary treatment of mastitis due to penicillinase-producing staphylococci and other Gram-positive organisms. They are also important for topical treatment of eye infections various species, including *Moraxella bovis* in cattle. There is limited availability of injectable products and little information on use of this class in companion animals in the EU.
- Antistaphylococcal penicillins are regarded as a first-choice narrow-spectrum (AMEG Category D) antibiotic. There are alternatives for the main indications in veterinary medicine, but these mostly have a broader spectrum of activity and may be from a higher AMEG category.
- The main mechanism of resistance in staphylococci is through acquisition of *mec* genes, which confer cross-resistance to almost all beta-lactams. The prevalence of LA-MRSA food-producing animals is variable depending on animal production type and country. LA-MRSA may transfer to human handlers through direct contact on farms/abattoirs, but food is generally not considered to be a significant source of MRSA in humans. There are rare reports of zoonotic transmission of MRSA/P from companion animals to persons in contact.
- There is little evidence for use of antistaphylococcal penicillins outside the terms of a marketing authorisation in animals in the EU.

Therefore, considering the points above relevant to criteria (b), (c) and (d), it is recommended that no conditions should be placed on the use of Antistaphylococcal penicillins outside the terms of the marketing authorisation, although responsible antimicrobial use principles should be applied.

4.3. Aminopenicillins, without beta-lactamase inhibitors

4.3.1. Background information

Examples of substances in the class that are authorised in veterinary and human medicine in the EU

Examples of substances authorised for veterinary use	Examples of ATCvet codes
Amoxicillin	QJ01CA04
Ampicillin	QJ01CA01
Metampicillin	QJ01CA14
Examples of substances authorised for human use	**Examples of ATC codes**
Amoxicillin	J01CA04
Ampicillin	J01CA01
Bacampicillin	J01CA06
Pivampicillin	J01CA02

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

Substance	Species	MRL tissues	MRL milk	MRL eggs	Other provisions
Amoxicillin	All food-producing species	Yes	Yes	-	Not for use in animals from which eggs are produced for human consumption.
Ampicillin	All food-producing species	Yes	Yes	-	Not for use in animals from which eggs are produced for human consumption.

EU-authorised VMP formulations, based on sales reported to ESVAC

Species		Route of administration						
		Group		Individual				
		In-feed	In-water	Injection	Oral e.g. tablet, paste, powder	Topical/local (incl. intrauterine)	Intra-mammary	Oral powder
Major	**Cattle**	AMX, AMP	AMX, AMP	AMX, AMP	AMX	AMX, AMP	AMX, AMP	AMX
	Sheep (for meat)		AMX, AMP	AMX, AMP	AMX	AMX	AMX, AMP	
	Pigs	AMX, AMP, MAMP	AMX, AMP	AMX, AMP	AMX	AMX		AMX
	Chickens	AMX, AMP	AMX, AMP	AMP	AMX			AMX
	Dogs		AMX, AMP	AMX, AMP	AMX, AMP	AMX		AMX
	Cats		AMX, AMP	AMX, AMP	AMX, AMP			AMX
Limited market species As listed in SPCs	**Turkeys**	AMX, AMP	AMX, AMP	AMP	AMX			AMX
	Ducks		AMX	AMP	AMX			AMX
	Geese		AMX	AMP				
	Horses		AMX	AMX, AMP	AMX	AMX		
	Goats		AMX, AMP	AMX, AMP			AMX, AMP	
	Fish	AMX			AMX			AMX
	Guinea fowls		AMX	AMP				
	Quails		AMX	AMP				
	Pheasants			AMP				
	Racing pigeons		AMX, AMP	AMP	AMP			AMX
	Partridges		AMX					
	Ornamental birds	AMP		AMP				

AMX (amoxicillin), AMP (ampicillin), MAMP (metampicillin)

Summary of main indications and contra-indications for EU-authorised VMPs, based on selected SPCs

Main indications	Ampicillin and amoxicillin are authorised in many different formulations as indicated above, for use in all major food-producing and companion animal species, and many minor species. Disease indications and target pathogens are frequently not specified in SPCs beyond 'infections caused by bacteria susceptible to …'. Where mentioned, indications are broad including gastrointestinal, respiratory and urogenital infections, septicaemia, meningitis, arthritis, intramammary infections, SSTI, secondary infections. Target pathogens, if listed, include e.g. *Streptococcus* spp., *Staphylococcus* spp., *Trueperella pyogenes*, *Erysipelothrix rhusiopathiae*, *Actinomyces* spp., *Clostridium* spp., *Mannheimia haemolytica*, *Pasteurella multocida*, *Actinobacillus* spp., *E. coli*, *Salmonella* spp., *Proteus* spp., *Leptospira* spp., *Aeromonas salmonicida*.
Contraindications	Do not use in case of hypersensitivity to penicillin or other beta-lactams. Do not administer to rabbits, hamsters, guinea pigs or other small herbivores. Some products include a contraindication from use in *Equidae*. Do not use in animals with serious kidney dysfunction.

Examples of EU-authorised HMP formulations, from Article 57 database

Substance	**Route of administration**		
	Injection	**Oral e.g. tablet, liquid**	**Topical/local**
Amoxicillin	x	x	
Ampicillin	x	x	
Bacampicillin		x	
Pivampicillin		x	

Existing recommendations

WOAH recommendations

Aminopenicillins (as part of the Penicillins class) are categorised VCIA by WOAH (formerly OIE). *Specific comments:* The wide range of applications and the nature of the diseases treated make penicillins extremely important for veterinary medicine. This class is used in the treatment of septicaemias, respiratory and urinary tract infections. This class is very important in the treatment of many diseases in a broad range of animal species. Few economical alternatives are available.

WHO classifications

WHO: CIA

- (C1: Yes) Limited therapy for Listeria and *Enterococcus* spp. (aminopenicillins)
- (C2: Yes) May result from transmission of *Enterococcus* spp., Enterobacteriaceae, including *E. coli* from non-human sources
- (P1: No) In certain geographic settings, this factor may be met: there may be a high absolute number of people affected by diseases for which the antimicrobial is the sole or one of few therapies available.
- (P2: Yes) High frequency of use in human medicine.
- (P3: Yes) Transmission of resistant *Enterococcus* spp. and Enterobacteriaceae (including *Salmonella* spp. and *E. coli*).

WHO AWaRe: Access: e.g. Ampicillin, Amoxicillin, Hetacillin

AMEG and CVMP recommendations

Aminopenicillins are included in the AMEG Category D. There are alternative treatments in human and veterinary medicine for their indications and that do not select for resistance to Category A substances through specific multiresistance genes.

These antibiotics are not devoid of negative impact on resistance development and spread. To keep the risk from use of these antibiotic classes as low as possible it is important that responsible use principles are complied with in everyday practice. Unnecessary use and unnecessarily long treatment periods should be avoided and group treatment restricted to situations where individual treatment is not feasible.

A CVMP reflection paper on aminopenicillins and their beta-lactamase inhibitor combinations noted that aminopenicillins are important in both human and veterinary medicine as first-line options for a variety of infections. It highlighted that resistant organisms, such as MRSA and those producing ESBL and AmpC beta-lactamases, may be transferred between animals and humans. Also, concerns were raised about the adequacy of dosing regimens for certain veterinary pathogens. For these reasons, it is recommended that susceptibility testing be conducted prior to treatment of infections due to Enterobacterales due to the high levels of resistance.

Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Disclaimer: The information in this section reflects reported use of antimicrobials outside the terms of a marketing authorisation. No evaluation is made in this section by the working group on the efficacy or safety of the reported uses, or on their potential impact on development and dissemination of AMR.

Information from published sources

Considering the broad range and often non-specific indications stated in many SPCs and the wide range of animal species for which this class is authorised, uses outside the SPC mostly relate to exotic or limited market species or use of human authorised formulations. In particular mention is made of use of the human formulations for intravenous infusion e.g. for treatment of horses and serious infections in companion animals [38, 72].

Information from the open call for data on use of antimicrobials in animals

The information below is summarised from the open call for data. Inclusion in the table does not endorse use or imply that it is consistent with use according to legislative provisions in Articles 112 to 114.

Substance	Species	Indication	Alternatives	Consequences of unavailability
Amoxicillin (oral powder)	Trout and other fish	Rainbow Trout Fry Syndrome, Furuculosis	Florfenicol	
Amoxicillin (injection, oral powder)	Mink	Enteritis, pre-weaning diarrhoea, pneumonia, mastitis, pyometra, UTI, wounds	None	Increased mortalities
Amoxicillin	Pheasant, partridges	Protozoal infections, bacterial infections, dysbacteriosis	Doxycycline	Mortalities
Amoxicillin	Cetaceans, pinnipeds	Susceptible bacterial infections		
Amoxicillin (human product for IV infusion)	Dogs, cats, horses	(Pleuro)pneumonia, septicaemia, bacteraemia, septic arthritis	None	Inadequate treatment, welfare issues

Ampicillin (human product for IV infusion)	Dogs, cats Horses	Septicaemia Surgical prophylaxis Severe systemic infections, septic arthritis	None	Mortalities

4.3.2. Evaluation

Scope of permitted use according to the MRL Regulation

Amoxicillin and ampicillin are included in Table 1 (allowed substances) of the Annex to Regulation (EU) 37/2010 and hence can be used in all food-producing species in accordance with Articles 113 and 114 of Regulation (EU) 2019/6. 'Other provisions' state that they are not for use in animals from which eggs are produced for human consumption.

Aminopenicillins can be used in non-food-producing species in accordance with Article 112.

Examples of veterinary-authorised formulations/species

Amoxicillin and ampicillins are authorised in VMPs intended for group administration, in-feed and/or in-drinking water, for all major food-producing species and fish. Formulations for administration in drinking water are also authorised for use in limited market poultry and game species (e.g. turkeys, ducks, geese, pheasant, quail).

Aminopenicillins are also available in injectable formulations for individual administration to all major food-producing species and many limited market species e.g. horses, goats, poultry. They are available in intra-uterine formulations for cattle, sheep, pigs and horses, and intramammary formulations for ruminants and horses.

In cats and dogs, aminopenicillins are available for administration by injectable and oral routes, amongst others.

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Importance for human health

Aminopenicillins are active against many Gram-positive bacteria (e.g., *Streptococcus pyogenes*, *S. pneumoniae*, *S. viridans*, *Enterococcus* spp., *Corynebacterium diphtheriae*, *Bacillus anthracis*, *Clostridium tetani*, *C. perfringens*, *C. botulinum*, and other *Clostridium* spp., *Listeria monocytogenes*). Many Gram-negative strains previously susceptible (e.g. *E. coli*, *Salmonella*) are nowadays frequently resistant to ampicillin and amoxicillin, but some remain susceptible (or infrequently resistant) (e.g., *Brucella* spp. and *Helicobacter pylori*).

Aminopenicillins are recommended for a wide range of infections which makes ampicillin one of the most prescribed antibiotics. However, an increasing prevalence of beta-lactamase producing organisms has resulted in reduced use of aminopenicillins as monotherapy.

Ampicillin can be used for upper and lower respiratory tract infections (RTIs) caused by *S. pneumoniae*, beta-haemolytic streptococci, and non–beta-lactamase–producing strains of *H. influenzae*. It is also used in the treatment of meningitis caused by group *B. streptococci*, *L. monocytogenes*, *Neisseria meningitidis*, and penicillin-susceptible strains of *S. pneumoniae*. In many countries, ampicillin has been replaced by amoxicillin, especially in oral therapy. Amoxicillin is used to

treat group A streptococcal pharyngitis, otitis media and acute sinusitis, urinary tract infections (UTIs), typhoid fever, gonorrhoea, uncomplicated mild community-acquired pneumonia (CAP) and for more severe cases can be used in combination with macrolides or doxycycline. Amoxicillin is one of the treatments of choice for erythema migrans as part of Lyme disease. Amoxicillin is now recommended to treat *E. faecalis* endocarditis (combination of intravenous ampicillin–amoxicillin plus either low-dose gentamicin or ceftriaxone). Amoxicillin or ampicillin can be used for neonatal septicaemia (usually combined with either gentamicin or amikacin, to provide treatment for aminopenicillin-resistant Gram-negative bacilli, such as *E. coli*, *Klebsiella* spp., and *P. aeruginosa*) [73].

Ampicillin and amoxicillin are nationally approved in the EU Member States for the treatment of ear, nose and throat infections, bronchitis, pneumonia, urinary tract infections, gonorrhoea, gynaecological infections, septicaemia, peritonitis, endocarditis, meningitis, enteric fever, gastro-intestinal infections etc. They are also indicated for the prophylaxis of endocarditis.

Importance for animal health

Ampicillin and amoxicillin have been widely used for decades for the treatment of infections in food-producing and companion animals in the EU. In 2021, penicillins (including aminopenicillins), were the most used antibiotic class in the EU, comprising 31.2% of the sales in mg/PCU [15]. There are numerous aminopenicillin products available for cattle, sheep, pigs, poultry, dogs and cats and limited market species including goats and fish. Formulations are available for group oral treatment/metaphylaxis (in-feed/drinking water) and for individual parenteral, oral, intrauterine or intramammary administration. Disease indications and target pathogens are frequently not specified in the SPC beyond 'infections caused by bacteria susceptible to …'. The target pathogens include genera such as *Actinobacillus* spp., *Pasteurella* spp., *Bibersteinia* spp., *Haemophilus* spp., *Histophilus* spp., *Mannheimia* spp., *Streptococcus* spp., *Enterococcus* spp., *Staphylococcus* spp., *Moraxella* spp., *Trueperella* spp., *Erysipelothrix* spp., *Clostridium* spp., *Escherichia coli* , *Salmonella* spp., *Bordetella bronchiseptica* and *Aeromonas salmonicida*. Of these, the four last mentioned are inherently less susceptible to aminopenicillins compared to other genera [38].

In pigs aminopenicillins are used for the treatment of respiratory infections, GI-tract infections, meningitis, arthritis, and skin and soft tissue infections. In cattle and calves, indications include respiratory tract, gastrointestinal, soft tissue and urogenital infections. In ruminant species, intramammary formulations are authorised for treatment and prevention of intramammary infections. In poultry, indications include respiratory and GI-tract infections. Amoxicillin is also authorised for the treatment of furunculosis caused by *A. salmonicida* in Atlantic salmon.

Aminopenicillins, mainly ampicillin, have been mentioned in the textbooks as an option for treating various equine infections [74]. Oral formulations have poor systemic bioavailability in horses and are associated with diarrhoea; therefore, they are administered by IM or IV injection. Injectable amoxicillin products authorised for use in horses have limited availability in the EU and human authorised intra-venous ampicillin formulations are used. Target pathogens for aminopenicillins in horses include streptococci, enterococci, *Pasteurellaceae* (incl. Actinobacillus), *Listeria* spp., and Enterobacterales (including *Salmonella* spp.) in various organ systems. Aminopenicillins may be combined with an aminoglycoside when treating neonatal infections or severe polymicrobial infections in adult horses [74].

Infections treated with aminopenicillins in dogs and cats include respiratory tract infections, urinary tract infections, genital infections, wound infections, skin and soft tissue infections, and enteric conditions [75]. A wide range of Gram-positive and Gram-negative bacterial species are mentioned as

target pathogens in SPCs of aminopenicillin products, such as staphylococci, streptococci, *Pasteurella* spp., *Clostridium* spp., *Proteus* spp., *E. coli*, and *Bordetella bronchiseptica*.

Considering the broad range and often non-specific indications stated in many SPCs and the wide range of animal species for which this class is authorised, uses outside the SPC are likely to be minor by comparison. According to the open call for data, quoted uses of aminopenicillins outside the terms of a marketing authorisation related mostly to their use in minor species for which they are not authorised e.g. food-producing fish species, mink, zoo/exotic species, or to use of human products for intravenous infusion, which may be used for horses and companion animals that are severely, acutely ill or for surgical prophylaxis.

Development and selection of resistance

Aminopenicillins have greater activity against Gram-negative bacteria compared with Penicillin G. Main mechanisms of bacterial resistance to aminopenicillins are i) alterations in penicillin-binding proteins (PBP) mediated by the *mec* genes, ii) hydrolysis by beta-lactamases, iii) presence of efflux pumps/ alterations in expression of outer membrane proteins.

Enzymatic degradation of beta-lactams by beta-lactamases

The most important mechanisms of resistance to the extended-spectrum penicillins are the beta-lactamase enzymes that catalyse hydrolysis of the beta-lactam ring. There is a very wide variety of different beta-lactamases with varying substrate specificity [37]. Beta-lactamases are generally encoded by genes located on mobile, extrachromosomal genetic elements (e.g. plasmids) responsible for the wide dissemination of these enzymes, or in the bacterial chromosome.

Aminopenicillins are liable to hydrolysis by all clinically relevant beta-lactamases, including the staphylococcal penicillinases and narrow broad spectrum beta-lactamases (e.g.TEM-1), ESBLs (e.g. TEM, SHV, CTX-M), AmpC and carbapenemases of Gram-negative bacilli.

Modification of the target site

Another important mechanism of beta-lactam resistance is alterations in penicillin binding proteins, PBPs. This type of mechanism is found in staphylococci and is mediated by *mec* genes [39, 40, 76, 77]. The result of the *mec*-gene is a modified penicillin binding protein with low affinity to nearly all beta-lactams except to the staphylococcal cephalosporins, ceftobiprole and ceftaroline. *mec* gene-harbouring staphylococci are known as methicillin-resistant staphylococci (MRS). Today, methicillin resistance is a common feature in *Staphylococcus aureus*, *Staphylococcus pseudintermedius* and in many coagulase negative staphylococci [78]. The *mec* genes locate in a chromosomal genetic element called Staphylococcal Cassette Chromosome mec (SCCmec). There is evidence suggesting that *mec* genes or SCC *mec* elements are transferrable between different staphylococcal species [78, 79]. *mec*B can also be plasmid encoded [76]. Methicillin-resistant staphylococci usually spread clonally.

Other resistance mechanisms

A third mechanism of beta-lactam resistance is decreased expression of outer membrane proteins. Another mechanism of beta-lactam resistance is due to non-selective multi-drug efflux pumps (either acquired or intrinsic) which remove a wide range of substrates from the periplasmic space to the surrounding environment. These types of pumps exist commonly in Gram-negative species.

A certain amount of cross-resistance exists between the different beta-lactam antibiotics and therefore the use of one beta-lactam antibiotic may select for resistance to other beta-lactam antibiotics. Generally, beta-lactam antibiotics with a broader spectrum of action, such as aminopenicillins will exert

a broader selection pressure than beta-lactams with a more narrow-spectrum such as narrow-spectrum penicillins or antistaphylococcal penicillins.

Prevalence of resistance in public health and target pathogens

Monitoring of *Salmonella* spp. under EFSA/ECDC mandatory EU surveillance in food-producing animals showed that resistance to ampicillin was observed at overall high levels in *Salmonella* spp. isolates from humans in 2019–2020 and ranging from moderate to very high in isolates from animals.

Ampicillin resistance was reported at overall moderate levels in both broiler carcasses and turkey carcases (18.8% and 19.1%, respectively). Among *Salmonella* spp. recovered from carcass swabs of pigs and calves in 2019, the highest levels of resistance were noted to ampicillin, sulfamethoxazole and tetracycline considering all reporting MSs. High to extremely high levels of resistance to these antimicrobials were recorded in pig carcases, while resistance to these compounds generally ranged from high to very high among isolates from calf carcases (overall resistance in pig carcases: 48.9%, 52.1% and 52.7%, respectively; overall resistance in calf carcases: 22%, 31.9% and 41.8%, respectively).

Among indicator *E. coli* isolates collected from animals during the 2019-20 EU monitoring, resistance to ampicillin ranged from moderate to very high in most MSs. In *E. coli* isolates from fattening pigs, the average level of resistance to ampicillin was 40.5% (range 9.2 – 74.7%), in isolates from broilers 49.8% (range 4.1 – 86.0%), in isolates from calves under 1 year of age 30.6% (range 6.4- 66.9) and for fattening turkeys 57.4 (range 9.1- 93.5%) [28].

Monitoring of MRSA under EFSA/ECDC surveillance in food-producing species is voluntary and data are provided by few member states. Most isolates are LA-MRSA. The prevalence ranges from 0% to 100% depending on animal production type and country [59].

The literature reviews performed by EFSA in the context of the Animal Health Law, which considered publications since 2010 and national AMR monitoring reports, identified levels of resistance (based on clinical breakpoints, as available) to aminopenicillins in key target animal pathogens in the EU.

In *Streptococcus suis* from pigs, mean levels of resistance to beta-lactams remain low (0.5% for aminopenicillins). Levels of resistance to beta-lactams are also very low in *Enterococcus* spp. from poultry in Europe and international data suggest that *Clostridium perfringens* retains good susceptibility.

In staphylococci from animals, resistance to aminopenicillins is widespread due to production of beta-lactamases [38, 60, 80, 81].

In cattle and pigs in Europe, the mean level of resistance to aminopenicillins in *E. coli* from mainly gastrointestinal infections was very high at 79.7% and 63.9% resp. It was lower, but still high in *E. coli* mastitis cases from cattle at 31.1%. In *E. coli* from infections in broiler chickens, the mean level of resistance to aminopenicillins was 28.1% [range 7-82%] and in turkeys it was 45.7%. In *E. coli* from horses mean resistance was 32.7%. In cats and dogs, a high proportion of *E. coli* infections were UTI and the mean level of resistance was 33.1% [range 12.1 – 100%]

Resistance to aminopenicillins in respiratory pathogens from food-producing species generally remains low-moderate, although increasing rates have been demonstrated e.g. in isolates from pigs in Spain and Italy and cattle in Germany. In *Pasteurella multocida* and *Mannheimia haemolytica* from cattle the mean levels of resistance to aminopenicillins were 15.3 and 12.3% respectively. In the respiratory pathogens from pigs *P. multocida* and *Glaeserella haemophilus* the mean resistance levels were 10.4, and 0.1 % resp. (See Annex 3. EFSA Animal Health Law Scientific opinions).

Transmission of resistance

Beta-lactamase mediated aminopenicillin resistance is very common and extensively distributed in several commensal bacterial species of human and animal origin, and therefore the route and direction of resistance transfer between animals and humans can be very challenging to investigate. Nevertheless, there are several examples demonstrating that drug-resistant bacteria can be transmitted between animals and humans. Transmission of the multidrug-resistant, aminopenicillin resistant *Salmonella* Typhimurium (ASSuT phenotype) or its monophasic variant is an example of animal to man transmission of *Salmonella* serotypes [82]. This is also an example of a multi-drug resistant organisms in food-animal populations that could be selected by different antibiotics, including aminopenicillins, raising concerns that livestock are a source of these bacteria or their resistance determinants for humans.

There is also direct and indirect evidence that humans and animals share identical ESBLs/AmpC/carbapenemase-producing Enterobacterales, suggesting interspecies transfer [62, 83-85].

Enterobacterales can be transferred from food-producing animals to humans via the foodborne route [82, 86]. Transfer of resistant zoonotic pathogens is demonstrated for *Salmonella* spp. and certain *E. coli* strains (e.g. STEC, EHEC). Moreover, the same or similar beta-lactam resistance genes (including ESBLs) have been isolated in bacteria of human and animal origin, and molecular studies support the potential for transfer of mobile genetic elements (MGEs) from animal to human enteric commensals, contributing to the spread of antibiotic resistance genes and resistant bacteria in the human intestinal tract [38, 87, 88]. A statistically significant association was found between aminopenicillin resistance in indicator *E. coli* and *Salmonella* spp. isolates from food-producing animals and humans in the JIACRA III analysis [89].

Companion animals may also be a reservoir for beta-lactamase resistance that can be transferred between animals and humans via Enterobacterales that are zoonotic pathogens or commensal bacteria, and by direct and indirect transmission, although there are few studies investigating these pathways [90-92].

There is direct and indirect evidence of animal to human transmission of livestock associated MRSA CC398 and human to animal transmission of human associated MRSA strains [44]. The most remarkable livestock associated clone is CC398, which was initially found among pigs, and subsequently was detected in other food-producing animals, in companion animals as well as in humans [45]. MRSA can be transmitted between companion animals and humans, horses and humans, and livestock and humans and the risk for MRSA carriage is higher in humans professionally exposed to animals [45].

Food is generally not considered to be a significant source of MRSA in humans [63, 93]. MRSA is mainly transmitted by direct contact from food-producing animals [65]. In geographical areas with high density of farms, livestock associated MRSA (LA-MRSA) contribution to the burden of MRSA disease could be significant [94, 95]. There is evidence for rare zoonotic transmission of MRSA/P from companion animals to persons in contact [62, 66, 67, 90].

In conclusion for the criterion (b) in relation to risk for animal or public health in case of development of antimicrobial resistance,

- Aminopenicillins are important broad-spectrum antibiotics, used as first-line therapy for a wide range of infections in both human and veterinary medicine.

- The main mechanism of resistance to aminopenicillins is production of beta-lactamase enzymes, encoded by genes located on mobile genetic elements (plasmids, transposons) and in the bacterial chromosome. In Enterobacterales, resistance to aminopenicillins is present at moderate to high levels in isolates from food-producing animals in most EU member states.
- Resistance to aminopenicillins due to production of beta-lactamases in staphylococci from animals is widespread. Resistance in *Staphylococcus* spp. also occurs due to alteration of penicillin binding proteins, mediated by *mec* genes (e.g. LA-MRSA, MRSP).
- Certain target bacteria e.g. streptococci, respiratory pathogens from pigs and cattle and *Clostridium perfringens* in poultry retain good susceptibility to aminopenicillins.
- Resistance to aminopenicillins can be transmitted from animals to humans and other animals via zoonotic and target pathogenic bacteria and commensals organisms.

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to Aminopenicillins.

Criterion (c) – availability of other treatments for animals

Aminopenicillins are included in the AMEG Category D, acknowledging that in general there are alternative treatments in veterinary medicine for their indications. For respiratory disease due to Pasteurellaceae in cattle and pigs, alternatives include e.g. tetracyclines, amphenicols, macrolides or TMPS. Loss of efficacy of aminopenicillins due to resistance in Enterobacterales may often necessitate use of antibiotics from a higher AMEG category (e.g. aminoglycosides, colistin, fluoroquinolones) in food-producing species. In companion animals, the amoxicillin-clavulanate combination or cephalosporins may be an alternative for infections due to beta-lactamase-producing Enterobacterales or *Staphylococcus* spp. Fewer alternatives are available for horses [38].

Criterion (d) – availability of other antimicrobial treatments for humans

For the above-mentioned indications there are generally other effective alternative antibiotics available. Other drugs that can be used for pneumococcal infections include, for example, macrolides, tetracyclines or a trimethoprim-sulphonamide combination; although acquired resistance to these drugs is common. For *Haemophilus influenzae* and *Moraxella catarrhalis*, aminopenicillin resistance is often due to beta-lactamase production and amoxicillin-clavulanic acid is an alternative [38].

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

- Aminopenicillins are important broad-spectrum antibiotics, used as first-line therapy and one of the most commonly prescribed classes for a wide range of diseases e.g. RTI, UTI, gastrointestinal infections, in both human and veterinary medicine.
- Resistance to aminopenicillins is mostly due to production of beta-lactamase enzymes encoded by genes located on mobile genetic elements and in the bacterial chromosome. This resistance is widespread in Enterobacterales and staphylococci from animals. In *Staphylococcus* spp. resistance may also be mediated by acquisition of *mec* genes (MRSA/P). Despite this, some target pathogens from animals retain good susceptibility to aminopenicillins. animals retain good susceptibility to aminopenicillins.
- There is cross-resistance with other beta-lactam classes depending on their individual susceptibility e.g. to specific beta-lactamase enzymes.

- Resistance to aminopenicillins can be transmitted from animals to humans and other animals via zoonotic and target pathogenic bacteria and commensals organisms.
- In general, there are alternatives to aminopenicillins for indications in both human and veterinary medicine; however, for infections due to multi-drug-resistant Enterobacterales in animals, these are likely to be from a higher AMEG category.
- In veterinary medicine, aminopenicillins are authorised for use in all major and several limited market species, in formulations for group and individual animal administration. Considering the breadth and extent of authorised indications for aminopenicillins in VMPs, uses outside of the SPC are likely to be limited and mostly concern administration to minor species or use of human IV formulations that are unavailable as VMPs. It seems unlikely that use in outside the marketing authorisation would contribute substantially to the AMR risk to public and animal health beyond the risk relating to authorised use.

Therefore, considering the points above relevant to criteria (b), (c) and (d), it is recommended that no conditions should be placed on the use of Aminopenicillins outside the terms of the marketing authorisation, although responsible antimicrobial use principles should be applied.

4.4. Aminopenicillins in combination with beta-lactamase inhibitors

4.4.1. Background information

Examples of substances in the class that are authorised in veterinary and human medicine in the EU

Examples of substances authorised for veterinary use	**Examples of ATCvet codes**
Amoxicillin and beta-lactamase inhibitor	QJ01CR02
Examples of substances authorised for human use	**Examples of ATC codes**
Amoxicillin and beta-lactamase inhibitor	J01CR02
Ampicillin and beta-lactamase inhibitor	J01CR01
Sultamicillin	J01CR04

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

Substance	**Species**	**MRL tissues**	**MRL milk**	**MRL eggs**	**Other provisions**
Amoxicillin	All food-producing species	Yes	Yes	-	Not for use in animals from which eggs are produced for human consumption.
Clavulanic acid	Bovine, porcine	Yes	Yes (bovine)	-	

EU-authorised VMP formulations, based on sales reported to ESVAC

Species		**Route of administration**						
		Group		**Individual**				
		In-feed	**In-water**	**Injection**	**Oral e.g. tablet, paste**	**Topical/local (incl. intrauterine)**	**Intra-mammary**	**Oral powder**
Major	**Cattle**			AMX+BLI	AMX+BLI		AMX+BLI	
	Pigs		AMX+BLI	AMX+BLI				
	Dogs		AMX+BLI	AMX+BLI	AMX+BLI			AMX+BLI
	Cats		AMX+BLI	AMX+BLI	AMX+BLI			AMX+BLI

AMX+BLI (amoxicillin + beta lactamase inhibitor)

Summary of main indications and contra-indications for EU-authorised VMPs, based on selected SPCs

Main indications	VMPs containing amoxicillin-clavulanate (amoxiclav) are authorised for cattle, pigs, dogs and cats for treatment of infections due to a range of Gram-positive (*Actinomyces bovis, Bacillus anthracis*, clostridia, corynebacteria, *Peptostreptococcus* spp., staphylococci, streptococci) and Gram-negative bacteria (*Actinobacillus* spp., *Bacteroides* spp., *Bordetella bronchiseptica, Campylobacter* spp., *Escherichia coli, Fusobacterium necrophorum, Haemophilus* spp., *Klebsiella* spp, *Moraxella* spp., *Pasteurella* spp., *Proteus* spp., *Salmonella* spp.), including beta-lactamase-producing strains. In <u>cattle</u> – injectable formulations are available for respiratory infections, soft tissue infections (e.g. joint-ill/navel-ill, abscesses, metritis and mastitis). In calves, oral formulations of amoxiclav are available for treatment of enteritis and navel ill. Intramammary preparations are authorised for treatment of clinical mastitis in cows due to staphylococci, streptococci and *E. coli*. In <u>pigs</u> - injectable and drinking water formulations are available for respiratory and gastrointestinal infections and colibacillosis. Injections are also authorised for periparturient infections in sows (e.g. mastitis, metritis and agalactia.) <u>Cats and dogs</u> Injectable and oral formulations of amoxiclav are authorised for a wide range of infections including skin diseases (including deep and superficial pyodermas); urinary tract infections; respiratory diseases (upper and lower respiratory tract); gastroenteritis; soft tissue infections (abscesses and anal sacculitis); dental infections (e.g. gingivitis).

Contraindications	Not to be used in animals with hypersensitivity to beta-lactam antibiotics. Not to be used in serious renal dysfunction accompanied by anuria and oliguria. Not be given to rabbits, guinea pigs, hamsters or gerbils.

Examples of EU-authorised HMP formulations, from Article 57 database

Substance	Route of administration		
	Injection	Oral e.g. tablet, liquid	Topical/local
Amoxicillin + clavulanic acid	x	x	
Ampicillin + sulbactam	x		
Sultamicillin		x	

Existing recommendations

WOAH recommendations

Aminopenicillins + BLIs (as part of the Penicillins class) are categorised VCIA by WOAH (formerly OIE). *Specific comments:* The wide range of applications and the nature of the diseases treated make penicillins extremely important for veterinary medicine. This class is used in the treatment of septicaemias, respiratory and urinary tract infections. This class is very important in the treatment of many diseases in a broad range of animal species. Few economical alternatives are available.

WHO classifications

WHO: CIA

- (C1: Yes) Limited therapy for Listeria and *Enterococcus* spp. (aminopenicillins).
- (C2: Yes) May result from transmission of *Enterococcus* spp., Enterobacteriaceae, including *E. coli* from non-human sources.
- (P1: No) In certain geographic settings, this factor may be met: there may be a high absolute number of people affected by diseases for which the antimicrobial is the sole or one of few therapies available.
- (P2: Yes) High frequency of use in human medicine.
- (P3: Yes) Transmission of resistant *Enterococcus* spp. and Enterobacteriaceae (including *Salmonella* spp. and *E. coli*).

WHO AWaRe: Access: e.g. Amoxicillin-Clavulanic acid, Ampicillin-Sulbactam

AMEG and CVMP recommendations

Aminopenicillins, in combination with beta lactamase inhibitors are included in the AMEG Category C: this category includes antibiotics for which there are alternatives in human medicine for their indications but which comply with one or both of the following criteria:

- For the veterinary indication under treatment, there are few or no alternatives belonging to Category D. Some examples of these indications are given in Table 4 of the AMEG advice [8], alongside the relevant (sub)class.
- The antibiotic selects for resistance to a substance in Category A through specific multiresistance genes.

Antibiotics placed in this category present a higher AMR risk for human and/or animal health than antibiotics placed in Category D. These antibiotics should only be used when there is no available substance in Category D that would be clinically effective.

Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Disclaimer: The information in this section reflects reported use of antimicrobials outside the terms of a marketing authorisation. No evaluation is made in this section by the working group on the efficacy or safety of the reported uses, or on their potential impact on development and dissemination of AMR.

Information from published sources

Considering the broad and non-specific authorised indications for VMPs containing amoxiclav, it was difficult to identify further indications from standard textbooks that would be strictly identified as use outside the terms of the marketing authorisation. FECAVA/FVE mention specifically use of amoxiclav to treat pyothorax, hepatic disease and septic arthritis in companion animals [72]. Use of human preparations of ampicillin-sulbactam is reported in companion animals, but the combination is generally regarded as being less effective than amoxicillin-clavulanate [96].

Information from the open call for data on use of antimicrobials in animals

The information below is summarised from the open call for data. Inclusion in the table does not endorse use or imply that it is consistent with use according to legislative provisions in Articles 112 to 114.

Substance	Species	Indication	Alternatives	Consequences of unavailability
Amoxicillin-clavulanate	Ovine	Metritis, joint infections		
Amoxicillin-clavulanate	Mink	Enteritis, pre-weaning diarrhoea, greasy kit syndrome, skin disease, mastitis, pneumonia	None	Increased mortality
Amoxicillin-clavulanate	Ornamental birds, breeding hens, cetaceans, pinnipeds	Susceptible bacterial infections		Severe disease and mortalities
Amoxicillin-clavulanate (human formulation for IV administration)	Dogs and cats	Septicaemia, endocarditis, acute pneumonia, peritonitis, pancreatitis, UTI Surgical antibiotic prophylaxis	None	Mortalities
Amoxicillin-clavulanate (human formulation to treat animals < 10 Kg bodyweight)	Dogs and cats	UTI and Respiratory disease		
Amoxicillin-clavulanate tablets	Horses (foals)	Bacterial infection		Mortalities

4.4.2. Evaluation

Scope of permitted use according to the MRL Regulation

Clavulanate is the only beta-lactamase inhibitor included in Table 1 (allowed substances) of the Annex to Regulation (EU) 37/2010. Hence clavulanate can be used in all food-producing species in accordance with Articles 113 and 114 of Regulation (EU) 2019/6. It is usually used in combination with the aminopenicillin, amoxicillin, in the EU. There are no 'Other provisions' for clavulanate that would be important for use outside a marketing authorisation; however, amoxicillin is not for use in animals from which eggs are produced for human consumption.

Aminopenicillin-BLI combinations can be used in non-food-producing species in accordance with Article 112.

Examples of veterinary-authorised formulations/species

Amoxicillin-clavulanate is the only aminopenicillin-BLI combination authorised as a VMP in the EU. It is authorised for group treatment of pigs by administration in the drinking water, and for administration to cattle and pigs by injection. It is also authorised for intramammary administration in cattle. In cats and dogs, amoxicillin-clavulanate is authorised for administration by injection and orally through tablets and oral powder.

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Importance for human health

Aminopenicillins (evaluated separately) in clinical practice are combined with beta-lactamase inhibitors (BLIs) such as clavulanic acid and sulbactam to broaden their spectrum of activity. Aminopenicillin-BLIs are well established in therapy of a wide range of infections. Use of beta-lactamase inhibitors restores the activity of aminopenicillins on beta-lactamase-producing strains and allows for successful inhibition of beta-lactamases produced by Gram-positive (e.g., *Staphylococcus aureus*, excluding MRSA) and Gram-negative bacteria (*H. influenzae*, *Neisseria gonorrhoeae*, *Moraxella catarrhalis*, *Bacteroides fragilis* and some Enterobacterales). Aminopenicillin-BLIs are extensively used for a wide range of indications such as RTIs including otitis media, pharyngitis, sinusitis, UTIs and surgical prophylaxis including mainly abdominal and gynaecological surgeries. Amoxicillin-clavulanic acid (with or without macrolide) is recommended as one of several first-line treatment options for mild to moderate CAP [97, 98].

Moreover, they are used for treatment of mixed aerobic and anaerobic infections such as pelvic inflammatory disease or intra-abdominal infections. Sulbactam is the main treatment for MDR *Acinetobacter baumannii*, due to its intrinsic activity against *A. baumannii*, not due to inhibition of beta-lactamases, but it is not commercialised alone, only as ampicillin-sulbactam [99].

Medicines that contain aminopenicillin and BLI are nationally approved in the EU. The approved indications include the treatment of the following infections in adults and children: acute bacterial sinusitis, acute otitis media, acute exacerbations of chronic bronchitis, CAP, cystitis, pyelonephritis, SSTIs in particular cellulitis, animal bites, severe dental abscess with spreading cellulitis, bone and joint infections, in particular osteomyelitis, intraabdominal infections, bacteraemia.

Importance for animal health

VMPs containing amoxicillin-clavulanate are authorised for treatment of infections affecting the gastrointestinal, genitourinary and respiratory tracts and various skin and soft tissue infections. Target pathogens include a broad spectrum of Gram-positive and Gram-negative bacterial species. The indications are not always specified in detail in the SPC.

Companion animals

In dogs and cats beta-lactams are probably the most commonly used antimicrobials, particularly aminopenicillins and their inhibitor combinations [75, 100, 101], although there is lack of systematic data collection for these species. Of veterinary authorised tablets containing extended spectrum penicillins, beta-lactamase inhibitor combinations were the most sold agents [15].

In dogs and cats, guidelines advise that amoxicillin-clavulanate is important as first tier for the treatment of SSTI caused by beta-lactamase-producing staphylococci. Skin infections are one of the

most common reasons for antibiotic prescribing in dogs and cats in the EU and are serious when recurrent or progressing to cellulitis [49, 72, 102, 103].

Amoxiclav is also recommended in ISCAID guidelines for dogs and cats as empiric treatment for bacterial cystitis due to staphylococci, *E. coli* and *Klebsiella* spp. where regional antimicrobial susceptibility testing (AST) data suggest resistance to amoxicillin alone [104]. Guidelines also recommend amoxiclav as a first tier alternative to doxycycline (if not tolerated) or amoxicillin for acute and chronic upper respiratory tract infections in cats and bacterial canine infectious respiratory disease complex (infectious tracheobronchitis), for treatment of bacterial pneumonia associated with e.g. *E. coli*, *Klebsiella* spp., MSSA or *Bordetella bronchiseptica* and for pyothorax. These diseases can have high morbidity and result in mortalities, particularly in vulnerable animals in rescue shelters [72, 105-107]. Amoxiclav may also be used for treatment of sepsis in cats and dogs [33].

Food-producing species

Although aminopenicillins are one of the most important antibiotic classes used in food-producing species, aminopenicillin-BLI combinations make up only 2% of their total use [15]. In the absence of MRLs for clavulanic acid in other species, amoxicillin-clavulanate is only authorised in VMPs for cattle and pigs. In ruminants and pigs, prevalence of resistance to first-line antimicrobials in respiratory pathogens is generally low (other than to tetracyclines) but amoxiclav is important for treatment of resistant infections e.g. *Mannheimia*, *Pasteurella*, and in particular *Actinobacillus* spp., the latter causing severe bronchopneumonia with high morbidity and mortality in young pigs [108]. Amoxiclav is also authorised for treatment of gastrointestinal infections in calves and pigs, with approval of formulations for group administration in drinking water to pigs being especially relevant considering the high level of resistance observed in *Escherichia coli* for amoxicillin. It is also authorised for oral treatment of *Salmonella* spp. infections in calves and pigs. Where susceptibility testing supports use, amoxiclav may be an alternative to AMEG Category B substances for treatment of enteritis due to *E. coli* in juvenile animals [33] and *Clostridium perfringens* in piglets [96]. In cows and sows, amoxiclav is used for systemic treatment of mastitis and metritis, with intramammary formulations also available for cattle.

All species

Amoxiclav is one of few options for treatment of anaerobic infections, including *Bacteroides* and *Prevotella* spp., producing beta-lactamases. Anaerobes may be a component of serious mixed infections e.g. cholecystitis, peritonitis following surgery and soft tissue infections.

According to the Open call for data, amoxicillin-clavulanate is used to treat bacterial infections in (limited market) species for which it is not authorised (e.g. mink and exotic/zoo species such as ornamental birds, cetaceans and pinnipeds). Human formulations that are suitable for IV infusion are often used to treat acute infections in dogs and cats (e.g. septicaemia, acute pneumonia) and for surgical prophylaxis.

Development and selection of resistance

The most important mechanisms of resistance to the beta-lactam antimicrobials are the beta-lactamase enzymes that catalyse the hydrolysis of the beta-lactam ring. There is a very wide variety of different beta-lactamases with varying substrate specificity. Aminopenicillins are prone to hydrolysis by all clinically relevant beta-lactamases**,** including the staphylococcal penicillinases and broad-spectrum beta-lactamases such as ESBLs, AmpC and carbapenemases of Gram-negative bacilli. Clavulanic acid and sulbactam are beta-lactam compounds that can inactivate several class A beta-lactamases (e.g. TEM-1, SHV-1, SHV-5, CTX-M, but not KPC-2). They also do not inhibit class B (e.g. NDM), class C

(e.g. AmpC, CMY-2) and class D (e.g. OXA-48 and OXA-23) beta-lactamases. Additionally, sulbactam has inherent antibacterial activity against a few bacterial species, e.g. in *A. baumannii*, through PBP-binding.

Beta-lactamases are generally encoded by genes located on mobile, extrachromosomal genetic elements (e.g. plasmids) responsible for the wide dissemination of these enzymes, or in the bacterial chromosome.

In Gram-positive bacteria, PBP mutation or acquisition of PBPs with lower affinity for beta-lactams is another important resistance mechanism. This type of mechanism is common in staphylococci and is mediated by *mec* genes (e.g. mecA or mecC in MRSA) [39]. Modification of PBPs is also a cause of beta-lactam resistance in *Streptococcus* spp., *Enterococcus* spp., *Neisseria* spp. and *Haemophilus* spp., although the genes conferring resistance are dependent on the bacterial species in question [41].

A certain amount of cross-resistance exists between the different beta-lactam antibiotics and therefore the use of one beta-lactam antibiotic may select for resistance to other beta-lactam antibiotics. Generally, beta-lactam antibiotics with a broader spectrum of action, such as aminopenicillin-BLI, will exert a broader selection pressure than beta-lactams with a more narrow-spectrum such as narrow-spectrum penicillins or antistaphylococcal penicillins.

There is no monitoring of resistance specifically to aminopenicillin-BLI under EFSA/ECDC mandatory EU surveillance in food-producing animals; however, monitoring of *Salmonella* spp. and *E. coli* shows that the prevalence of extended-spectrum beta-lactamases (ESBL) and AmpC producers is low in the EU overall, but varies greatly between animal production type and country.

In broilers and broiler carcasses, ESBL and/or AmpC producing *Salmonella* spp. were identified in 2.1% and 0.3% of isolates; in fattening pigs and pig carcasses 0.8% and 0.5% respectively; in isolates from fattening turkeys 0.4%, in laying hens 0.2% and in bovine animals under 1 year of age 0% [28]. In 2019 and 2020, none of the Salmonella isolates recovered from any of the animal or carcass origins exhibited 'microbiological' resistance to the carbapenem, meropenem [28].

Presumptive ESBL-producing *Salmonella* spp. from humans were identified in 0.6% of the tested isolates (range 0.3% - 2.0%). AmpC was less frequent, identified in 0.2% of tested isolates (range 0.1-0.6%). One isolate (0.02%) was reported as both presumptive AmpC- and ESBL-producing. ESBL was reported most commonly in *S*. Infantis, *S*. Kentucky and *S*. Saintpaul. Presumptive ESBL-production was more frequent in *S*. Typhimurium and monophasic *S*. Typhimurium 1,4,[5],12:i:- (both 0.6%) than in *S.* Enteritidis (0.1%). Two Salmonella isolates were reported as resistant to meropenem in 2020 [28].

Among *C. jejuni* from humans resistance to amoxiclav was 0.1% and among *C. coli* 1.1% [28].

The proportion of ESC-resistant indicator *E. coli* isolates collected within the routine monitoring was generally low in 2019 and 2020 (ranging between 1.2% and 1.7% of the investigated isolates), depending on the animal population. The occurrence of presumptive ESBL/AmpC-producing indicator *E. coli* in food-producing animals is much higher when using specific monitoring (selective culturing): 39.6% in broilers, 31.5% in broiler meat, 42.7% in pigs, 6.7% in pig meat. 34.2% in turkeys, 36.4% in bovines under the age of 1 year and 4.9% in bovine meat [28]. One *E. coli* isolate with carbapenem-resistance-phenotype from broilers was detected. This isolate harboured the metallo-betalactamase resistance gene *bla*VIM-1.

Monitoring of MRSA under EFSA/ECDC surveillance in food-producing species is voluntary and data are provided by few member states. Most isolates are LA-MRSA. The prevalence ranges from 0% to 100%

depending on animal production type and country. There is no recent mandatory EU monitoring for enterococci.

Amoxicillin-clavulanate has a wider spectrum of activity and thus it is likely that it has higher chance to select multidrug resistant organisms, several ESBLs and all AmpC-producers compared with aminopenicillins alone.

Enterobacterales producing ESBLs and AmpC and MRSA/P have also been isolated from companion animals.

Resistance in target pathogens

The literature reviews performed by EFSA in the context of the Animal Health Law, which considered publications since 2010 and national AMR monitoring reports, identified levels of resistance (based on clinical breakpoints, as available) to amoxicillin-clavulanate in key target animal pathogens in the EU (See Annex 3. EFSA Animal Health Law Scientific opinions).

In mainly gastrointestinal *E. coli* infections from calves and lambs in Europe, the mean levels of resistance to amoxi-clav were 49.1% and 26.2%, respectively. It was lower in *E. coli* from mastitis in cows, at 16.8%. In *E. coli* from pigs and horses, the mean levels of resistance were 15.7% and 24.6%, respectively.

In *E. coli* from cats and dogs, a high proportion of which were from UTI, the mean level of resistance to amoxi-clav was 18.6% [range 0 – 100%]. In *E. coli* from horses mean resistance was 24.6%.

As would be expected, levels of resistance in *E. coli* are significantly lower to combinations with BLI compared with levels of resistance to aminopenicillins alone.

In respiratory pathogens from pigs, *P. multocida, Glaeserella haemophilus* and *A. pleuropneumoniae*, the mean resistance levels to amoxi-clav were very low at 0.4, 0 and 1.3% respectively.

Other publications

In isolates collected from cattle with acute mastitis in 8 EU countries in 2015-16 under the VetPath programme, in *E. coli* (n=225) 2.7% of isolates were resistant to amoxicillin/clavulanate and 3.7% of Enterobacterales were harbouring an ESBL/AmpC gene. For streptococcal isolates, susceptibility to aminopenicillins remained high [109].

Transmission of resistance

Enterobacterales can be transferred from food-producing animals to humans via the foodborne route [82, 86]. Transfer of resistant zoonotic pathogens is demonstrated for *Salmonella* spp. and certain *E. coli* strains (STEC, EHEC). Moreover, the same or similar beta-lactam resistance genes (including ESBLs) have been isolated in bacteria of human and animal origin, and molecular studies support the potential for transfer of mobile genetic elements (MGEs) from animal to human enteric commensals, contributing to the spread and persistence of antibiotic resistance genes and resistant bacteria in the human intestinal tract [38, 87, 88].

Companion animals may also be a reservoir for beta-lactamase resistance that can be transferred between animals and humans via Enterobacterales that are zoonotic pathogens or commensal bacteria, and by direct and indirect transmission, although there are few studies investigating these pathways [90-92].

Food is generally not considered to be a significant source of MRSA in humans [63, 93]. MRSA is mainly transmitted by direct contact from food-producing animals [65]. In geographical areas with

high density of farms, livestock associated MRSA (LA-MRSA) contribution to the burden of MRSA disease could be significant [94, 95]. There is evidence for rare zoonotic transmission of MRSA/P from companion animals to persons in contact [62, 66, 67, 90].

Resistance may also be transmitted between animals both vertically via target pathogens and horizontally via commensal bacteria, through direct and indirect contact [110, 111].

In conclusion, there is evidence for the selection and transmission of resistance to aminopenicillin-BLI combinations from animals to humans and other animals via zoonotic/target pathogens or commensal bacteria capable of transferring resistance to pathogens.

In conclusion for the criterion (b) in relation to risk for animal or public health in case of development of antimicrobial resistance,

- Aminopenicillin-BLI combinations have well established use in human medicine for treatment of a wide range of infections, for example, amoxicillin-clavulanate is used for otitis media, pharyngitis, sinusitis, pneumonia, SSTI and UTI.
- Likewise, in cats and dogs amoxicillin-clavulanate is used to treat a range of infections, including as first-line treatment for skin infections due to beta-lactamase-producing staphylococci, respiratory and urinary tract infections.
- In food-producing species, authorised amoxicillin-clavulanate VMPs are available only for cattle and pigs. Use of this combination constitutes only 2% of the overall aminopenicillin use in food-producing species. The combination is used in these species for treatment of respiratory and enteric infections, STI, mastitis and metritis.
- In both human and veterinary medicine, aminopenicillin-BLI combinations are important for treatment of infections due to beta-lactamase-producing bacteria and for therapy of serious polymicrobial e.g. intra-abdominal infections including anaerobes.
- The main mechanism of resistance to aminopenicillin-BLIs is production of broad-spectrum beta-lactamase not inhibited by the BLI (e.g. AmpC and metalo-beta-lactamases) or due to overproduction of beta-lactamases inhibited by BLIs (e.g. co-production of TEM-1 and ESBL).
- The prevalence of extended-spectrum beta-lactamases (ESBL) and AmpC producers in indicator *E. coli* from food-producing animals varies greatly between animal production type and country. Levels of resistance in animal pathogenic *E. coli* are significantly lower to aminopenicillin combinations with BLI compared with levels of resistance to aminopenicillins alone.
- Resistance in *Staphylococcus* spp. mainly occurs due to the acquisition of an altered penicillin binding proteins, mediated by *mec* genes (e.g. LA-MRSA, MRSP).
- There is cross-resistance with other beta-lactam antibiotics classes depending on their individual susceptibility e.g. to specific beta-lactamase enzymes. Beta-lactam antibiotics with a broader spectrum of action, such as aminopenicillin-BLI will exert a broader selection pressure. MRS are resistant to almost all beta-lactams.
- Resistance to aminopenicillins can be transmitted from animals to humans and other animals via zoonotic and target pathogenic bacteria and commensals organisms.

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to Aminopenicillin-BLIs.

Criterion (c) – availability of other treatments for animals

In dogs, TMPS is an alternative first-line antibiotic for bacterial cystitis and TMPS or clindamycin could be alternatives for the treat of pyoderma . However, pathogens causing UTI (e.g. Enterobacterales) and SSTI (e.g. *S. pseudintermedius*) in dogs and cats are increasingly resistant to first-line antibiotics. For resistant and other serious infections, e.g. septicaemia, it may be necessary to resort to fluoroquinolones.

Amoxiclav is also one of few options for treatment of anaerobic infections, including *Bacteroides* and *Prevotella* spp., producing beta-lactamases. Anaerobes may be a component of serious mixed infections e.g. cholecystitis, peritonitis following surgery and soft tissue infections. There are few alternatives, e.g. metronidazole or clindamycin for companion animals only, or for food-producing species, either 3rd-generation cephalosporins (AMEG category B) or potentially certain macrolides depending on the disease/target pathogen [33].

In cattle and pigs, amoxiclav may be used for treatment of respiratory infections resistant to first-line alternatives e.g. unpotentiated amoxicillin, tetracyclines, TMPS; hence alternatives to amoxiclav may be limited to amphenicols, macrolides or fluoroquinolones, dependent on pathogen/susceptibility [33].

Potential alternatives to amoxiclav for resistant *E. coli* infections in food-producing animals are often restricted to AMEG Category B substances, i.e. colistin or fluoroquinolones, or, depending on patient/disease suitability, aminoglycosides (Category C).

Criterion (d) – availability of other antimicrobial treatments for humans

Alternative treatment options include carbapenems to treat Enterobacterales, ceftobiprole and ceftaroline to treat some Gram-positive infections (MRSA), 3rd-generation cephalosporines to treat *H. influenzae*, *M. catarrhalis*, *N. gonorrhoeae*, and carbapenems, cefiderocol and colistin to treat MDR *A. baumannii* [112-114].

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

- In human medicine, aminopenicillin-BLIs are extensively used for a wide range of indications including otitis media, pharyngitis, sinusitis, SSTI, UTIs and for surgical prophylaxis. They are used as first-line treatment for CAP.
- Likewise, in cats and dogs amoxicillin-clavulanate is used to treat a range of infections, including as first-line treatment for skin infections due to beta-lactamase-producing staphylococci, respiratory and urinary tract infections.
- In food-producing species, authorised amoxicillin-clavulanate VMPs are available only for cattle and pigs. The combination is used in these species for treatment of respiratory and enteric infections, soft tissue infections, mastitis and metritis.
- In both human and veterinary medicine, aminopenicillin-BLI combinations are important for treatment of infections due to beta-lactamase-producing bacteria and for therapy of serious polymicrobial e.g. intra-abdominal infections including anaerobes.
- The most important mechanism of resistance to aminopenicillin-BLI in Gram-negative bacteria is due to plasmid-borne extended-spectrum beta-lactamases (ESBLs) or AmpC genes. The prevalence of ESBL and AmpC producers in indicator *E. coli* from food-producing animals varies between animal production type and country.
- There is cross-resistance with other beta-lactam antibiotics classes depending on their individual susceptibility e.g. to specific beta-lactamase enzymes. Beta-lactam antibiotics with a broader spectrum of action, such as aminopenicillin-BLI, will exert a broader selection pressure.

- Resistance in *Staphylococcus* spp. also occurs due to alteration of penicillin binding proteins, mediated by *mec* genes (e.g. LA-MRSA, MRSP). This mechanism confers resistance to almost all betalactam antibiotics.
- Resistance to aminopenicillin-BLIs can be transmitted from animals to humans, including via the foodborne route, and to other animals. For certain important uses in veterinary medicine e.g. UTI due to *E. coli*, canine staphylococcal pyoderma, septicaemia, fluoroquinolones may be the only alternative due to high levels of resistance to first-line antibiotics. Alternatives in human medicine are also likely to be of higher importance and may be last resort antibiotics.
- Although otherwise authorised for use in VMP formulations for individual animal administration only, amoxicillin-clavulanate is also available as a formulation for administration in drinking water to groups of pigs.
- A broad range of non-specific indications are authorised for use of amoxicillin-clavulanate in veterinary medicine. Due to this it is difficult to identify if some uses mentioned in publications are outside the marketing authorisation. The extent of this use is unknown but reports to the 'open call' relate mostly to use in minor and exotic/zoo species and to use of human-authorised intravenous formulations for the treatment of severe acute infections in companion animals.

Therefore, considering the points above relevant to criteria (b), (c) and (d), it should be considered if conditions or a prohibition should be placed on the use of Aminopenicillin-BLIs outside the terms of the marketing authorisation.

Step 2. Considerations of conditions to be placed on use outside the terms of a marketing authorisation

Please refer to Section 3.1.2. of the main report for the general rationale behind the proposed conditions.

(i) Use for unauthorised indications

Condition: *For those indications not included in the SPC of the concerned product, use must be based on target pathogen identification and antimicrobial susceptibility testing that demonstrates that aminopenicillin-BLI are likely to be effective and that antimicrobials from a lower AMEG category would not be effective, unless it can be justified that this is not possible.*

Rationale: See Section 3.1.2.(i) of the advice.

(ii) Use for unauthorised target species

Aminopenicillin-BLI combinations are authorised in VMPs intended for use in cattle, pigs, dogs and cats. Considering the 'Other provisions' in Regulation (EU) 37/2010, they cannot be used in animals laying eggs for human consumption, but they could be used outside a marketing authorisation in breeder and broiler poultry.

Condition: *Not to be used in poultry.*

Rationale: As identified from EFSA mandatory surveillance of AMR in food-producing animals [28], poultry and poultry products are most frequently reported to carry ESBL and/or AmpC-producing Salmonella and *E. coli*. Although decreasing trends have now been observed in some member states, the prevalence of ESBL/AmpC producing *E. coli* in meat samples from poultry is still high (based on culture of samples on selective media) when considering the mean across member states. Based on the CVMP's reflection paper on the use of 3rd- and 4th-generation cephalosporins in food-producing animals in the EU [115] and the EFSA Scientific Opinion on the public health risks of bacterial strains

producing extended-spectrum beta-lactamases and/or AmpC beta-lactamases in food and food-producing animals [84], a subsequent CVMP referral and Commission Decision issued in January 2012 determined that VMPs containing 3rd- and 4th-generation cephalosporins should include in the SPCs a contraindication from use in poultry. Considering the potential for aminopenicillin-BLI combinations to select for similar mechanisms of resistance as the 3rd- and 4th-generation cephalosporins, it should be considered to apply the same condition relating to use of the former in poultry. See also Section 3.1.2.(ii) of this advice.

(iii) Administration by an unauthorised route or use of extemporaneous formulation

Authorised VMPs containing Aminopenicillin-BLI combinations (amoxicillin-clavulanate) are available for administration in formulations for individual animal use, via injection for cattle, pigs, dogs and cats and intramammary administration in cattle. Oral formulations are authorised for individual administration to dogs, cats and calves and use in drinking water in groups of pigs.

Condition: *Not to be used in food-producing aquaculture*

Rationale: Although EFSA does not monitor for antimicrobial resistance in aquaculture food production, ESBLs have been detected in isolates from fish and other species reared in aquaculture systems globally [116, 117]. Considering that aquaculture systems are regarded as potential hotspots for driving emergence, release, transmission and persistence and spread of AMR bacteria and resistance genes [18, 19] as discussed in Section 3.1.2(iii) of this advice, and the high importance to human and animal health of this antimicrobial class, it is recommended that its use in aquaculture should be restricted [118].

No further conditions proposed.

(iv) Use of a human medicinal product

HMPs containing aminopenicillin-BLI are available for administration by injection or orally.

No further conditions proposed to those mentioned above.

Rationale: See Section 3.1.2.(iv) of this advice.

(v) Use of a third country veterinary medicinal product

According to Articles 112(2), 113(2) and 114(4), third country VMPs may only be used in the same species and for the same indication. No further conditions proposed to those mentioned above.

Rationale: See Section 3.1.2.(v) of this advice.

Step 3. Consideration of Criteria (a) and (e) in view of proposed conditions to be placed on use outside the terms of a marketing authorisation

Criterion (a) – risk to animal health or public health if the antimicrobial is used in accordance with Articles 112, 113 and 114

SPCs recommend that amoxicillin-clavulanate should not be used in small herbivores (clostridial overgrowth) or in animals with severe renal dysfunction or hypersensitivity to beta-lactam antibiotics. Target animal safety warnings in the SPCs of authorised VMPs should be followed. Amoxicillin is usually well tolerated but oral administration may cause diarrhoea/enteritis in horses and ruminants associated with alterations of gut flora. Neurotoxicity may be observed at high doses or with prolonged use in dogs. Clavulanate may be associated with vomiting [46, 119].

Consumer safety is mitigated through the application of the statutory withdrawal period in accordance with Article 115.

Criterion (e) Impact on aquaculture and farming if the animal affected by the condition receives no treatment

Proposed condition	Potential impact on aquaculture and farming if animal affected by the condition receives no treatment
For those indications not included in the SPC of the concerned product, use must be based on target pathogen identification and antimicrobial susceptibility testing that demonstrates that aminopenicillin-BLI are likely to be effective and that antimicrobials from a lower AMEG category would not be effective, unless it can be justified that this is not possible.	This condition does not preclude treatment. See Annex 1 of report for further discussion.
Not to be used in poultry	Considering the 'Other provisions' in Regulation (EU) 37/2010, aminopenicillin-clavulanate combinations could be used outside a marketing authorisation in breeder and broiler poultry only. There was one report to the 'open call for data' that mentioned use in breeding hens to treat 'susceptible bacteria'. Without further information, the impact of loss of this treatment on poultry farming is difficult to foresee, but is not anticipated to be large considering that no evidence was found for use of aminopenicillin-BLI combinations in the standard textbooks.
Not to be used in food-producing aquaculture	No evidence was found for the use of aminopenicillin-BLI combinations in food-production aquaculture in the EU; therefore, although impact on aquaculture cannot be fully foreseen, it is not expected to be significant under current circumstances.

Step 4. Final conclusion - recommendations made for conditions to be placed on use outside the terms of a marketing authorisation

Based on the discussion above, the following conditions are proposed for use under Articles 112, 113 and 114:

- For those indications not included in the SPC of the concerned product, use must be based on target pathogen identification and antimicrobial susceptibility testing that demonstrates that aminopenicillin-BLI are likely to be effective and that antimicrobials from a lower AMEG category would not be effective, unless it can be justified that this is not possible.
- Not to be used in poultry
- Not to be used in food-producing aquaculture

4.5. Amdinopenicillins

Amdinopenicillins are authorised in human medicinal products in the EU. At present they are not authorised in veterinary medicinal products in the EU.

4.5.1. Background information

Examples of substances included in the class that are authorised in human medicine only

Examples of substances authorised for human use	Examples of ATC codes
Mecillinam	J01CA11
Pivmecillinam	J01CA08

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

Substance	Species	MRL	Other provisions
Mecillinam	bovine	No MRL required	For intrauterine use only

According to the MRL summary report [120], it was intended that mecillinam would be used in combination with a 1st-generation cephalosporin as a uterine bolus for treatment of endometritis in cows.

Examples of EU-authorised HMP formulations, from Article 57 database

Substance	Route of administration		
	Injection	Oral e.g. tablet, liquid	Topical/local
Mecillinam	x		
Pivmecillinam		x	

Existing recommendations

WOAH recommendations

Amdinopenicillins (as part of the Penicillins class) are categorised VCIA by WOAH (formerly OIE). *Specific comments:* The wide range of applications and the nature of the diseases treated make penicillins extremely important for veterinary medicine. This class is used in the treatment of septicaemias, respiratory and urinary tract infections. This class is very important in the treatment of many diseases in a broad range of animal species. Few economical alternatives are available.

WHO classifications

WHO: HIA

- (C1: No) In certain geographic settings, Criterion 1 may be met: the class may be one of limited therapies for infections with MDR *Shigella* spp.
- (C2: Yes) May result from transmission of Enterobacteriaceae, including *E. coli*, from non-human sources.

WHO AWaRe: Access: mecillinam, pivmecillinam

AMEG recommendations

Amdinopenicillins are included in the AMEG Category A: these classes are not authorised in veterinary medicine but are authorised in human medicine in the EU. These antibiotic classes may only be used exceptionally in individual companion animals in compliance with the prescribing "cascade". Substances in these classes cannot be used for food-producing animals in the absence of established maximum residue limits.

Amdinopenicillins, mainly pivmecillinam, have been used extensively in European Nordic countries with few problems, but, despite this, these antimicrobials are not widely used in other European countries.

Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Disclaimer: The information in this section reflects reported use of antimicrobials outside the terms of a marketing authorisation. No evaluation is made in this section by the working group on the efficacy or safety of the reported uses, or on their potential impact on development and dissemination of AMR.

Information from published sources

No evidence could be found for the use of, or specific need for, amdinopenicillins to treat serious infections in animals in the EU or globally at the present time.

Information from the open call for data on use of antimicrobials in animals

No information on use outside the terms of a marketing authorisation was provided in the open call for data.

4.5.2. Evaluation

Scope of permitted use according to the MRL Regulation

Amdinopenicillins are not authorised for use in VMPs in the EU. However, mecillinam is included in Table 1 (allowed substances) of the Annex to the MRL Regulation (EU) 37/2010. Other provisions state that it is for intrauterine use only; hence mecillinam can be used in food-producing species in accordance with Article 113 of Regulation (EU) 2019/6.

According to the MRL summary report [120], it was intended that mecillinam would be used in combination with a 1st-generation cephalosporin as a uterine bolus for treatment of endometritis in cows.

Amdinopenicillins can be used in non-food-producing animals in accordance with Article 112.

Examples of veterinary-authorised formulations/species

No authorised VMPs identified.

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Importance for human health

In humans, amdinopenicillins are mainly used for treatment of uncomplicated urinary tract infections (UTIs) due to Enterobacterales. These infections are not considered life-threatening. Due to the relative stability of mecillinam to some ESBLs, it could be an alternative treatment in certain systemic infections due to Enterobacterales, in combination with aminoglycosides.

Pivmecillinam shows activity against *Salmonella* spp. and preliminary studies in a limited number of patients suggest that it may be a useful alternative antibiotic in the treatment of acute typhoid fever and in some carriers of *Salmonella*. However, efficacy data are limited due to the small number of patients and few clinical studies, so caution is recommended.

Importance for animal health

No evidence could be found for the use of amdinopenicillins in veterinary medicine in the EU. The 'open call for data' did not receive any report of the use of amdinopenicillins in animals.

Development, selection and transmission of resistance

Resistance to amdinopenicillins in Enterobacterales is mainly due to chromosomal mutations but the mechanisms of mecillinam resistance in clinical isolates remain poorly understood. They can also be hydrolysed by some ESBLs and carbapenemases e.g. OXA, KPC, MBLs, but are generally more stable than other penicillins. Indeed, several studies, most of them *in vitro*, highlighted that a majority of ESBL-producing Enterobacterales are susceptible to mecillinam. Recently, it has been shown that a mutation in *cys*B, preventing production of cysteine is the major mechanism of mecillinam resistance in clinical isolates [121].

There is no monitoring of resistance specifically to amdinopenicillins under EFSA/ECDC mandatory EU surveillance of AMR in zoonotic and indicator bacteria from animals. No specific studies were identified relating to the monitoring of susceptibility to amdinopenicillins in target pathogens from animals.

Although evidence on prevalence and mechanisms of resistance specifically to amdinopenicillins is limited at present, ESBLs have been detected in Enterobacterales from food-producing animals and companion animals in the EU and may be transferred from animals to humans (See Section 4.7. on 3rd- and 4th-generation cephalosporins). Use of amdinopenicillins in animals, would have the potential to select for resistant bacteria that could result in transfer of resistance to humans and to other animals.

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to Amdinopenicillins.

Criterion (c) – availability of other treatments for animals

No evidence could be found for the use of, or specific need for, amdinopenicillins to treat serious infections in animals in the EU or globally at the present time; therefore, alternatives cannot be proposed.

Criterion (d) – availability of other antimicrobial treatments for humans

Sufficient alternatives are usually available for treatment of uncomplicated UTIs, including those caused by ESBL-producing Enterobacterales.

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

- In human medicine, amdinopenicillins are mainly used for treatment of uncomplicated urinary tract infections (UTIs) due to Enterobacterales. These infections are not considered life-threatening and alternative antibiotics are available for their treatment.
- No authorised VMP containing amdinopenicillins were identified in the EU, and no evidence for their use in animals was found in published literature or reports to the 'open call for data'.
- Resistance to amdinopenicillins is mainly due to chromosomal mutations. There is little evidence available specifically on the prevalence of resistance to amdinopenicillins in animal isolates; however, their use could potentially select for certain ESBLs.
- Amdinopenicillins can only be used outside the terms of the marketing authorisation in non-food-producing animals or mecillinam may be used by the intrauterine route in food-producing animals. It is considered that possible use outside the terms of the authorisation would be very rare.

Therefore, considering the points above relevant to criteria (b), (c) and (d), it is recommended that no conditions should be placed on the use of Amdinopenicillins outside the terms of the marketing authorisation, although responsible antimicrobial use principles should be applied.

4.6. Evaluation of 1st- and 2nd-generation cephalosporins, and cephamycins

4.6.1. Background information

Examples of substances in the class that are authorised in veterinary and human medicine in the EU

Examples of substances authorised for veterinary use		Examples of ATCvet codes
1st-gen	Cefacetrile	QJ01DB10 QJ51DB10
	Cefadroxil	QJ01DB05
	Cefalexin	QJ01DB01 QJ51DB01
	Cefalonium	QJ51DB90
	Cefapirin	QJ01DB08 QJ51DB08
	Cefazolin	QJ01DB04 QJ51DB04
Examples of substances authorised for human use		**Examples of ATC codes**
1st-gen	Cefalexin	J01DB01
	Cefalotin	J01DB03
	Cefazolin	J01DB04
	Cefadroxil	J01DB05
	Cefatrizine	J01DB07
	Cefradine	J01DB09
2nd-gen	Cefoxitin*	J01DC01
	Cefuroxime	J01DC02
	Cefamandole	J01DC03
	Cefaclor	J01DC04
	Cefonicid	J01DC06
	Cefotiam	J01DC07
	Cefmetazole*	J01DC09
	Cefminox*	J01DC12
	Cefprozil	J01DC10
	Ceforanide	J01DC11

*Also known as cephamycins

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

Substance	Species	MRL tissues	MRL milk	MRL eggs	Other provisions
Cefacetrile	Bovine	-	Yes	-	Intramammary use only
Cefalexin	Bovine	Yes	Yes	-	-
Cefalonium	Bovine	No MRL required	Yes	-	Intramammary use and eye treatment only
Cefapirin	Bovine	Yes	Yes	-	-
Cefazolin	bovine, ovine, caprine	No MRL required	Yes	-	For intramammary use, except if the udder may be used as food for human consumption.

EU-authorised VMP formulations, based on sales reported to ESVAC

Species		Route of administration						
		Group		Individual				
		In-feed	In-water	Injection	Oral e.g. tablet, paste	Topical/local (incl. intrauterine)	Intra-mammary	Oral powder
Major	**Cattle**			CFX		CEPR	CFC, CFX, CNM, CEPR, CFZ	

	Sheep (for meat)						CFZ	
	Pigs							
	Chickens							
	Dogs		CFX	CFX	CDX, CFX			
	Cats		CFX	CFX	CDX, CFX			
Limited market species As listed in SPCs	**Goats**						CFZ	
	Buffaloes						CFC	

CFX (cefalexin), CEPR (cefapirin), CDX (cefadroxil), CFC (cefacetrile), CFZ (cefazolin), CNM (cefalonium)

Examples of EU-authorised HMP formulations, from Article 57 database

Substance	Route of administration		
	Injection	Oral e.g. tablet, liquid	Topical/local
Cefaclor		x	
Cefadroxil		x	
Cefalexin		x	
Cefalotin	x		
Cefamandole	x		
Cefatrizine		x	
Cefazolin	x		
Cefmetazole	x		
Cefminox	x		
Cefonicid	x		
Ceforanide	x		
Cefotiam		x	
Cefoxitin	x		
Cefprozil		x	
Cefradine		x	
Cefuroxime	x	x	

Summary of main indications and contra-indications for EU-authorised VMPs, based on selected SPCs

Main indications	Cefalexin injection, tablets, paste and oral suspension are authorised for dogs and cats for treatment of respiratory, urogenital, skin and soft tissue and gastrointestinal infections. In cattle, injectable cefalexin is authorised for various indications including mastitis, metritis, pododermatitis, respiratory, urogenital, skin and soft tissue and gastrointestinal infections caused by various Gram-positive and Gram-negative pathogens including Enterobacterales and certain anaerobes. 1st-generation cephalosporins are available by intramammary route to treat mastitis in lactating dairy cows and for dry cow treatment. They are also authorised for treatment of mastitis in lactating sheep, buffalo, bison and goats. Cefapirin is authorised for intrauterine administration to treat endometritis in cattle.
Contraindications	Do not use in rabbits and rodents. Do not use in case of hypersensitivity to beta-lactams.

Existing recommendations

<u>WOAH recommendations</u>

1st- and 2nd-generation cephalosporins are categorised VHIA by WOAH (formerly OIE). *Specific comments:* Cephalosporins are used in the treatment of septicaemias, respiratory infections, and mastitis.

<u>WHO classifications</u>

WHO: HIA

- (C1: No)

- (C2: Yes) May result from transmission of Enterobacteriaceae, including *E. coli*, from non-human sources.

WHO AWaRe: Access (First-generation cephalosporins): e.g. Cefacetrile, Cefadroxil, Cefalexin, Cefapirin, Cefatrizine, Cefazolin; Watch (Second-generation cephalosporins): e.g. Cefaclor, Cefonicid, Ceforanide, Cefotiam, Cefoxitin, Cefprozil, Cefuroxime.

AMEG and CVMP recommendations

1st- and 2nd-generation cephalosporins are included in the AMEG Category C: this category includes antibiotics for which there are alternatives in human medicine for their indications but which comply with one or both of the following criteria:

- For the veterinary indication under treatment, there are few or no alternatives belonging to Category D. Some examples of these indications are given in Table 4 of the AMEG advice [8], alongside the relevant (sub)class.
- The antibiotic selects for resistance to a substance in Category A through specific multiresistance genes.

Antibiotics placed in this category present a higher AMR risk for human and/or animal health than antibiotics placed in Category D. These antibiotics should only be used when there is no available substance in Category D that would be clinically effective.

Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Disclaimer: The information in this section reflects reported use of antimicrobials outside the terms of a marketing authorisation. No evaluation is made in this section by the working group on the efficacy or safety of the reported uses, or on their potential impact on development and dissemination of AMR.

Information from published sources

Most published reports of use outside the marketing authorisation relate to use of human formulations of cefazolin that can be administered intravenously in the perioperative period for surgical prophylaxis in companion animals [122-124]. Cefazolin (1st-generation) is somewhat more active against Enterobacterales compared with other 1st-generation cephalosporins and shows good penetration into bone, hence its use during orthopaedic surgery and for treatment of osteomyelitis in dogs [125]. Cefoxitin (2nd-generation) may also be used for treatment of infections such as septic peritonitis due to mixed infections including anaerobic bacteria (e.g. *Bacteroides* spp.) and Gram-negative bacilli [125].

Information from the open call for data on use of antimicrobials in animals

The information below is summarised from the open call for data. Inclusion in the table does not endorse use or imply that it is consistent with use according to legislative provisions in Articles 112 to 114.

Substance	Species	Indication	Alternatives	Consequences of unavailability
Cefazolin (human IV formulation)	Dogs and cats	Septicaemia, peritonitis, bone infections Surgical prophylaxis e.g. for orthopaedic, bowel surgery	Lack of availability of veterinary IV formulations	Need to resort to a higher category antibiotic for IV formulation. Prophylactic use of e.g. fluoroquinolones is prohibited in some MSs.

Cefuroxime (human IV formulation)	Dogs, cats, horses and other species	Sepsis, severe infections Surgical prophylaxis e.g. eye surgery	No IV formulations available	Inability to manage severe infections. Increased use of IV marbofloxacin.
Cefalexin	Elasmobranchs	Bacterial infections	ceftazidime	Severe disease, mortalities

4.6.2. Evaluation

Scope of permitted use according to the MRL Regulation

Several first generation cephalosporins are included in Table 1 (allowed substances) of the Annex to Regulation (EU) 37/2010 and hence can be used in all food-producing species in accordance with Articles 113 and 114 of Regulation (EU) 2019/6. However, 'Other provisions' state that cefazolin and cefacetrile are for intramammary use only, whilst cefalonium is for intramammary and ocular use only. There are no 'Other provisions' relating to use of cefalexin and cefapirin.

There are no 2nd-generation cephalosporins with MRL status.

First and second generation cephalosporins can be used in non-food-producing species in accordance with Article 112.

Examples of veterinary-authorised formulations/species

Cefalexin is authorised as an injectable formulation for use in cattle, dogs and cats. There are also oral formulations of 1st-generation cephalosporins authorised for use in dogs and cats.

Intramammary formulations are available for cattle and limited market ruminants (sheep, goats, buffalo). An intra-uterine formulation of cefapirin is also authorised for cattle.

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Importance for human health

In general, the 1st- and 2nd-generation cephalosporins are active against a range of Gram-positive bacteria (e.g., *Staphylococcus* spp. except MRSA, *Streptococcus* spp.) and some Gram-negative bacteria (e.g. *Escherichia coli* and *Haemophilus influenzae* presenting the intrinsic phenotype of resistance). 1st-generation cephalosporins generally have a narrow spectrum, being active against Gram-positive cocci, including MSSA but not *Enterococcus* spp. and non-beta-lactamase producing a Gram-negative rods. The 2nd-generation cephalosporins agents tended to have decreased potency against the Gram-positive bacteria, but improved antibacterial activity against Gram-negative pathogens (e.g. beta-lactamase producing *H. influenzae)* [126-130].

1st-generation cephalosporins, specifically cefazolin, are considered antibiotics of choice for perioperative surgical prophylaxis of infections (e.g. MSSA) in a wide variety of situations e.g. caesarean section, breast surgery, vaginal and abdominal hysterectomies [126]. 1st-generation cephalosporins are also used as chemoprophylaxis in preventing group B streptococcal disease in the new-born and may still be recommended for women with a history of penicillin allergy [126].

Cefazolin is, in addition, suggested as a treatment option for MSSA bacteraemia and MSSA endocarditis [70, 71].

2nd-generation cephalosporins are used to treat mild cases of pharyngitis, tonsillitis, sinusitis and bacterial bronchitis [129, 130]. They are also first empirical choice for UTI treatment in children [131, 132].

1st- and 2nd-generation cephalosporins are nationally approved in the EU. Indications for 1st-generation cephalosporins include streptococcal pharyngitis and tonsillitis, otitis media, bronchopneumonia, bacterial pneumonia, UTIs (pyelonephritis, cystitis), SSTIs (abscesses, furunculosis, impetigo, erysipelas, pyoderma, lymphadenitis), bone and joint infections. The approved indications for 2nd-generation cephalosporins include SSTIs as well as bone and joint infections caused by susceptible organisms, perioperative prophylaxis (for surgical operations with increased risk of infections with anaerobic pathogens, e.g. colorectal surgery, a combination with an appropriate substance with activity against anaerobes is recommended). Cefoxitin and cefotetan (both cephamycins) also have activity against anaerobes, but increasing resistance in anaerobic Gram-negative bacilli (e.g. Bacteroides) has been reported [133].

Importance for animal health

In cattle, cefalexin injection is authorised for various indications including mastitis, metritis, pododermatitis, respiratory, urogenital, skin and soft tissue and gastrointestinal infections caused by various Gram-positive and Gram-negative pathogens including Enterobacterales and certain anaerobes. Various 1st-generation cephalosporins are also authorised in intramammary preparations for treatment of IMI due to *Staphylococcus, Streptococcus, Trueperella* spp. and *E. coli* in ruminants (lactating and dry) and in an intrauterine formulation for endometritis in cattle.

No specific evidence was found for use of this class outside the terms of the marketing authorisation in food-producing species.

In dogs and cats, 1st-generation cephalosporins are authorised in the EU for treatment of respiratory, urogenital, skin and soft tissue and gastrointestinal infections caused by a range of Gram-positive and Gram-negative bacteria. There are no authorised VMPs containing 2nd-generation cephalosporins in the EU.

1st-generation cephalosporins are notable for high activity against Gram-positive bacteria, including penicillinase-producing *Staphylococcus pseudintermedius* (not MRS), and their good oral bioavailability in monogastrics and tolerability for infections requiring extended treatment duration e.g. for deep pyoderma in dogs. They are recommended as a first-line/empirical treatment option for SSTI in dogs and cats. Skin infections are one of the most common reasons for antibiotic prescribing in dogs and cats in the EU and become serious if recurrent or progressing to cellulitis.

Most published reports of use outside the marketing authorisation relate to use of human formulations of cefazolin (1st-generation) that are administered intravenously in the perioperative period for surgical prophylaxis in companion animals [33, 123, 124, 134]. Cefazolin shows good penetration into bone, hence its use during orthopaedic surgery and for treatment of osteomyelitis in dogs [33, 125]. Additional cover against Gram-negative bacteria e.g. aminoglycosides may be required in equines. Cefoxitin (2nd-generation) may also be used for treatment of infections such as septic peritonitis due to mixed infections including anaerobic bacteria (e.g. *Bacteroides* spp.) and Gram-negative bacilli [125].

Similarly, in the open call for data, it was reported that in the absence of suitable veterinary formulation for IV use, the human IV formulations of both 1st- and 2nd-generation cephalosporins are important both for treatment of serious infections in companion animals e.g. septicaemia, peritonitis,

and for surgical prophylaxis (also to avoid use of an antibiotic from a higher AMEG category). They are also used in exotic/zoo animal species.

Development and selection of resistance

Resistance to 1st- and 2nd-generation cephalosporins in Gram-negative bacilli is mainly due to production of beta-lactamases [135], either chromosomal or plasmid encoded. Cephamycins have improved resistance to some beta-lactamases produced by anaerobes, including CepA of *Bacteroides fragilis* [38]. 1st- and 2nd-generation cephalosporins remain active against staphylococci producing beta-lactamases. In staphylococci, production of an additional PBP (e.g. PBP2a), through the acquisition of *mec* genes, is the main mechanism of acquired resistance (e.g. MRSA/MRSP).

Cross-resistance between aminopenicillins, carboxypenicillins and 1st- and 2nd-generation cephalosporins is common. Cefuroxime and cefoxitin (2nd-generation cephalosporins) may retain activity against certain isolates resistant to 1st-generation cephalosporins (e.g. *E. coli* producing large spectrum beta-lactamases, such as TEM-1).

Gram-negative bacilli resistant to 3rd-, 4th- or 5th-generation cephalosporins are also usually resistant to 1st- and 2nd-generation cephalosporins. Staphylococci carrying *mec* genes commonly show cross-resistance to all beta-lactams, although usually ceftobiprole and ceftaroline remains active.

There is no monitoring of resistance specifically to 1st- and 2nd-generation cephalosporins under EFSA/ECDC mandatory EU surveillance in food-producing animals; however, resistance to aminopenicillins in Enterobacterales from all food-producing species is generally high [59] and these isolates would mostly also be resistant to 1st- and 2nd-generation cephalosporins owing to cross-resistance. Monitoring of MRSA under EFSA/ECDC surveillance in food-producing species is voluntary and data are provided by few member states. Most isolates are LA-MRSA. The prevalence ranges from 0% to 100% depending on animal production type and country [59].

Resistance to 1st- and 2nd-generation cephalosporins is reported in Enterobacterales and staphylococci isolates from companion animals [38, 136]. There is little reporting on prevalence of MRSA/P in companion animals, which appears to vary across the EU [60, 61]. Based on a literature review performed by EFSA [136] the mean percentage methicillin resistance in *S. pseudintermedius* isolates from 23 EU studies published since 2010 was 5.8% (range 4.2 to 41.4%)

Transmission of resistance

There is evidence for the transmission of pathogenic or commensal Enterobacterales resistant to 1st- and 2nd-generation cephalosporins between animals and from food-producing and companion animals to humans. Transfer of resistance genes from commensals to human pathogens might also occur.

Food is generally not considered to be a significant source of MRSA in humans [63, 64]. MRSA is mainly transmitted by direct contact from food-producing animals [65]. In geographical areas with high density of farms, livestock associated MRSA (LA-MRSA) could contribute significantly to the burden of MRSA disease in humans [62, 66, 67]. There is evidence for rare zoonotic transmission of MRSA/P from companion animals to persons in contact [62, 66, 67]. MRSA and MRSP may also be transmitted between animals [44, 68].

In conclusion, there is evidence for the selection and transmission of resistance to 1st- and 2nd-generation cephalosporins from animals to humans via zoonotic pathogens or commensals bacteria capable of transferring resistance to human pathogens.

In conclusion,

- 1st- generation cephalosporins are active against Gram-positive cocci and some Gram-negative bacilli; importantly they are resistant to staphylococcal beta-lactamases. The 2nd-generation cephalosporins have improved resistance to some beta-lactamases produced by Gram-negative bacteria including CepA of the anaerobe *Bacteroides fragilis*. 1st- and 2nd-generation cephalosporins are used in humans in particular for perioperative surgical prophylaxis, but have a variety of indications including RTI, UTI, SSTI and bone and joint infections.
- In cattle, 1st-generation cephalosporins are authorised for various indications including mastitis, pododermatitis, respiratory and urogenital infections. They have limited use in other food-producing species except for local treatment of intramammary infections in ruminants.
- 1st-generation cephalosporins are authorised for a range of indications in dogs and cats but are notably a first-line option for treatment of SSTI due to penicillinase-producing *Staphylococcus* spp. Use outside the marketing authorisation relates mostly to surgical prophylaxis especially for orthopaedic surgery and for treatment of serious infections e.g. septicaemia in companion animals.
- Little evidence was found for use of 2nd-generation cephalosporins in veterinary medicine.
- Resistance to 1st- and 2nd-generation cephalosporins is mostly due to production of beta-lactamases in Gram-negative bacilli and acquisition of *mec* genes in *staphylococcus* spp. There is cross-resistance with other beta-lactam antibiotics. This resistance can be transmitted from animals to humans and other animals via zoonotic and target pathogenic bacteria and commensals organisms.

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to 1st- and 2nd-generation cephalosporins.

Criterion (c) – availability of other treatments for animals

Alternatives for skin infections in dogs (and cats) include amoxiclav, clindamycin or TMPS; however, variably high levels of resistance are noted to the latter two classes in *S. pseudintermedius*. Otherwise, 3rd-generation cephalosporins or fluoroquinolones (AMEG category B) might be used as second-line [49, 72, 102, 103, 136, 137]. Alternatives for surgical prophylaxis are dependent on the nature of the procedure and organ system, but could include parenterally administered ampicillin (not alone in presence of Gram-negative bacteria), amoxiclav, 3rd-generation cephalosporins or metronidazole (anaerobes).

In ruminants, alternatives to 1st-generation cephalosporins for local treatment of IMI due to Gram-positive cocci include lincosamides, penicillin-novobiocin and antistaphylococcal penicillins, noting that these other beta-lactams will not be effective in the presence of cross-resistance [33].

Note that alternatives may be limited by resistance development or may be from a higher AMEG category.

Criterion (d) – availability of other antimicrobial treatments for humans

For surgical prophylaxis, alternatives exist but overall, in particular cefazolin is considered an antibiotic of choice and is therefore critical for surgical prophylaxis [138, 139]. For other indications, there are alternatives but some of them belong to classes that are classified by WHO as Critically Important Antimicrobials [6].

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

- 1st- and 2nd-generation cephalosporins are authorised in human medicine for treatment of a variety of indications including RTI, UTI and SSTI due to Gram-positive and some Gram-negative

bacteria. The 2nd-generation cephalosporins have improved resistance to some beta-lactamases produced by GNB, including CepA of *Bacteroides fragilis* group. 1st- and 2nd-generation cephalosporins are particularly important in human medicine for surgical prophylaxis. There are alternative antibiotics for the stated indications, but they may be substances of higher importance for human health.

- 1st-generation cephalosporins are authorised for various indications in cattle including mastitis, pododermatitis, respiratory and urogenital infections, but have limited authorised use in other food-producing species, except for local treatment of intramammary infections in ruminants. They have a broad range of indications in dogs and cats and are notably used as a first-line option for treatment of SSTI due to penicillinase-producing *Staphylococcus* spp.
- It is reported that human formulations for intravenous administration are used outside the terms of the marketing authorisation in companion animals for surgical prophylaxis, under defined conditions and for serious infections e.g. septicaemia. 1st-generation cephalosporins are also used in exotic/zoo animal species.
- Alternatives are available for the veterinary indications, but are likely to be substances from AMEG Categories B or C.
- Little evidence was found for the use of 2nd-generation cephalosporins in veterinary medicine.
- Resistance to 1st- and 2nd-generation cephalosporins is mostly due to production of beta-lactamases in Gram-negative bacilli and acquisition of *mec* genes in *Staphylococcus* spp. Although 1st- and 2nd-generation cephalosporins are stable to certain beta-lactamases, there is cross-resistance between these two classes and with many other commonly used beta-lactam antibiotics. This resistance can be transmitted from animals to humans and other animals via zoonotic and target pathogenic bacteria and commensals organisms.
- Based on the availability of authorised human and veterinary formulations and identified indications, treatment outside the SPC is likely to be predominantly to individual companion animals.

Therefore, considering the points above relevant to criteria (b), (c) and (d), it is recommended that no conditions should be placed on the use of 1st- and 2nd-generation cephalosporins outside the terms of the marketing authorisation, although responsible antimicrobial use principles should be applied.

4.7. 3rd- and 4th-generation cephalosporins, except combinations with beta-lactamase inhibitors

4.7.1. Background information

Examples of substances in the class that are authorised in veterinary and human medicine in the EU

Examples of substances authorised for veterinary use		**Examples of ATCvet codes**
3rd-gen	Cefoperazone	QJ01DD12 QJ51DD12
	Cefovecin	QJ01DD91
	Ceftiofur	QJ01DD90 QJ51DD90
4th-gen	Cefquinome	QJ01DE90 QJ51DE90
Examples of substances authorised for human use		**Examples of ATC codes**
3rd-gen	Cefditoren	J01DD16
	Cefixime	J01DD08
	Cefodizime	J01DD09
	Cefoperazone	J01DD12
	Cefotaxime	J01DD01
	Cefpodoxime	J01DD13
	Ceftazidime	J01DD02
	Ceftriaxone	J01DD04
	Ceftibuten	J01DD14
	Ceftizoxime	J01DD07
4th-gen	Cefepime	J01DE01

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

Substance	**Species**	**MRL tissues**	**MRL milk**	**MRL eggs**	**Other provisions**
Cefoperazone	Bovine	-	Yes	-	Intramammary use in lactating cows only
Cefquinome	Bovine, Porcine, Equidae	Yes	Yes	-	-
Ceftiofur	All mammalian food-producing species	Yes	Yes	-	-

EU-authorised VMP formulations, based on sales reported to ESVAC

Species		**Route of administration**					
		Group		**Individual**			
		In-feed	**In-water**	**Injection**	**Oral e.g. tablet, paste, powder**	**Topical/local (incl. intrauterine)**	**Intra-mammary**
Major	**Cattle**			CEF, CEQ			CFP, CEQ
	Sheep (for meat)			-			
	Pigs			CEF, CEQ			
	Chickens			-			
	Dogs			CVN			
	Cats			CVN			
Limited market species As listed in SPCs	**Horses**			CEF, CEQ			

CEF (ceftiofur), CEQ (cefquinome), CVN (cefovecin), CFP (cefoperazone)

Summary of main indications and contra-indications for EU-authorised VMPs, based on selected SPCs

Main indications	**Bovine –** Treatment of bacterial respiratory disease (*Mannheimia haemolytica, Pasteurella multocida, Histophilus somni*). Treatment of acute interdigital necrobacilosis (*Panaritium* or foot rot). Treatment of acute post-partum metritis.

	Treatment of clinical mastitis in the lactating cow (*Streptococcus uberis, Streptococcus dysgalactiae, Staphylococcus aureus, Escherichia coli*). Treatment of subclinical mastitis at drying off and the prevention of new bacterial infections of the udder during the dry period (*Streptococcus uberis, Streptococcus dysgalactiae, Streptococcus agalactiae, Staphylococcus aureus,* CNS). In calves, treatment of *E. coli* septicaemia. **Pigs** – Treatment of bacterial respiratory infections (*Actinobacillus pleuropneumoniae, Pasteurella multocida, Haemophilus parasuis, Streptococcus suis*). Treatment of septicaemia, polyarthritis or polyserositis (*Streptococcus suis*). In piglets, reduction of mortality in cases of meningitis caused by *Streptococcus suis*. Treatment of arthritis caused by *Streptococcus spp., E. coli*. Epidermitis caused by *Staphylococcus hyicus*. **Horses-** Treatment bacterial respiratory disease (*Streptococcus zooepidermicus, Streptococcus equi, Staphylococcus spp., Pasteurella spp.*) **Dogs and cats –** Treatment of skin and soft tissue infections including (*Staphylococcus pseudintermedius, Pasteurella multocida*) Treatment of urinary tract infections (*Escherichia coli, Proteus spp.*) Treatment of severe infections of the gingiva and periodontal tissues (*Porphyromonas spp. Prevotella spp.*) in dogs.
Contraindications	Do not use in known cases of hypersensitivity to cephalosporin antibiotics or to other beta-lactam antibiotics.

Examples of EU-authorised HMP formulations, from Article 57 database

Substance	Route of administration		
	Injection	Oral e.g. tablet, liquid	Topical/local
Cefditoren		x	
Cefepime	x		
Cefixime		x	
Cefodizime	x		
Cefoperazone	x		
Cefotaxime	x		
Cefpodoxime		x	
Ceftazidime	x		
Ceftibuten		x	
Ceftizoxime	x		
Ceftriaxone	x		

Existing recommendations

WOAH recommendations

3rd- and 4th-generation cephalosporins are categorised VCIA by WOAH (formerly OIE). *Specific comments:* The wide range of applications and the nature of the diseases treated make cephalosporin third and fourth generation extremely important for veterinary medicine. Cephalosporins are used in the treatment of septicaemias, respiratory infections, and mastitis. Alternatives are limited in efficacy through either inadequate spectrum or presence of antimicrobial resistance.

Additional WOAH recommendations for 3rd- and 4th-generation cephalosporins:

- Not to be used as preventive treatment applied by feed or water in the absence of clinical signs in the animal(s) to be treated;
- Not to be used as a first line treatment unless justified, when used as a second line treatment, it should ideally be based on the results of bacteriological tests; and
- Extra-label/off-label use should be limited and reserved for instances where no alternatives are available. Such use should be in agreement with the national legislation in force.

WHO classifications

WHO: HPCIA

- (C1: Yes) Limited therapy for acute bacterial meningitis and disease due to *Salmonella* spp. in children. Limited therapy for infections due to MDR Enterobacteriaceae, which are increasing in incidence worldwide. Additionally, 4th-generation cephalosporins provide limited therapy for empirical treatment of neutropenic patients with persistent fever.
- (C2: Yes) May result from transmission of Enterobacteriaceae, including *E. coli* and *Salmonella* spp., from non-human sources.
- (P1: Yes) High absolute number of people affected by diseases for which the antimicrobial is the sole or one of few therapies available.
- (P2: Yes) High frequency of use in human medicine.
- (P3: Yes) Transmission of extended spectrum beta-lactamase producing (ESBL) Enterobacteriaceae, including E. coli and Salmonella spp., from non-human sources.

WHO AWaRe: Watch (third-generation cephalosporins): e.g. Cefixime, Cefmenoxime, Cefodizime, Cefoperazone, Cefotaxime, Cefpiramide, Cefpodoxime proxetil, Ceftazidime, Ceftizoxime, Ceftriaxone, Latamoxef; Watch (fourth-generation cephalosporins): e.g. Cefepime, Cefozopran, Cefpirome

AMEG and CVMP recommendations

3rd- and 4th-generation cephalosporins are included in the AMEG Category B, for which there is a higher AMR risk to public health. For these antimicrobials, the risk to public health resulting from veterinary use needs to be mitigated by specific restrictions. These restricted antimicrobials should only be used for the treatment of clinical conditions when there are no alternative antimicrobials in a lower category that could be effective. Especially for this category, use should be based on the results of antimicrobial susceptibility testing, whenever possible.

The CVMP of the EMA has made recommendations on the use of 3rd- and 4th-generation cephalosporins [115]. Specific precautionary phrases have been included in the summary of product characteristics i.e. for systemically administered broad spectrum cephalosporins (3rd- and 4th-generation) it should be reflected that these are to be reserved for the treatment of clinical conditions which have responded poorly, or are expected to respond poorly, to more narrow spectrum antimicrobials. Increased use, including use of the product deviating from the instructions given in the SPC, may increase the prevalence of bacteria resistant to the 3rd- and 4th-generation cephalosporins. Official, national and regional antimicrobial policies should be taken into account when the product is used.

Due to concerns regarding misuse of the products for preventive group treatments in cattle, swine, horses, and particularly in day-old chicks, and associated concerns over the human health risk due to selection of ESBLs (extended-spectrum beta-lactamases), further warnings were added. These included a contraindication from use of the products in poultry and statements indicating that the products are intended for use in individual animals only, and should not be used for disease prevention [140].

Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Disclaimer: The information in this section reflects reported use of antimicrobials outside the terms of a marketing authorisation. No evaluation is made in this section by the working group on the efficacy or safety of the reported uses, or on their potential impact on development and dissemination of AMR.

Information from published sources

Textbooks refer to the use of 3rd-generation cephalosporins for last resort treatment of serious infections due Gram-negative bacteria, some of which may not be directly covered by the authorised indications in the SPC. For example, there are references to their use for treatment of *E. coli* (post-weaning colibacillosis) and *Salmonella* spp. associated with bacteraemia in calves, piglets and foals and for UTI in cattle [34, 53]. Use of 3rd- and 4th-generation cephalosporins for treatment of septicaemias, including with meningitis, has been reported in foals [141, 142].

In companion, zoo and exotic animals, ceftazidime has been used as one of few antibiotics effective for treatment of *Pseudomonas aeruginosa* infections and Enterobacterales resistant to other antibiotics [46, 125].

Information from the open call for data on use of antimicrobials in animals

The information below is summarised from the 'open call for data'. Inclusion in the table does not endorse use or imply that it is consistent with use according to legislative provisions in Articles 112 to 114.

In many cases, specific disease conditions and/or target pathogens were not reported and as such, information provided on alternative treatment classes and consequences if the antimicrobial or formulation would no longer be available for cascade use was not reliable.

Substance	Species	Indication	Alternatives
ceftiofur, cefquinome	Horses	Neonatal septicaemia, non-respiratory bacterial infections with limit susceptibility to other AM classes	
cefquinome	Dogs	Bacterial infections with limited susceptibility to other AM classes	
Ceftazidime (human formulation)	Dogs	otitis	Off-label use of marbofloxacin (injectable formulations used topically)
Ceftazidime (human formulation)	Reptiles	Sepsis, pneumonia	
Ceftazidime (human formulation)	Ornamental birds, reptiles	Dermatitis associated with bacteria with limit susceptibility to other AM classes, *Pseudomonas spp.*	
Ceftazidime (human formulation)	Ornamental fish	Septicaemia (*Aeromonas spp., Pseudomonas spp.) with multiresistant bacteria.*	
ceftiofur	Zoo and aquarium species (Penguins, waterbirds)	Susceptible bacterial diseases	
cefovecin, ceftriaxone (human formulation)	Zoo and aquarium species (cetaceans, pinnipeds)	Susceptible bacterial diseases	
Ceftazidime (human formulation)	Zoo and aquarium species (Teleosts, elasmobranchs)	Susceptible bacterial diseases	

4.7.2. Evaluation

Scope of permitted use according to the MRL Regulation

Cefoperazone, cefquinome and ceftiofur are included in Table 1 (allowed substances) of the Annex to Regulation (EU) 37/2010 and can be used accordingly in food-producing species in compliance with Articles 113 and 114 of Regulation (EU) 2019/6. 'Other provisions' state that cefoperazone is for intramammary use in lactating cows only.

All 3rd- and 4th-generation cephalosporins can be used in non-food-producing species in accordance with Article 112.

Examples of veterinary-authorised formulations/species

3rd-generation cephalosporins are available as injectable formulations for cattle, pigs, horses, dogs and cats. They are also authorised as intramammary preparations for cattle. There are no formulations authorised as VMPs for oral administration, or for administration to groups of animals.

The 4th-generation cephalosporins, cefquinome, is authorised as an injectable formulation for cattle, pigs and horses and as an intramammary formulation for use in cattle.

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Importance for human health

3rd- and 4th-generation cephalosporins are active against Gram-positive and Gram-negative bacteria, and are notably stable to many beta-lactamases, including the common beta-lactamases produced by staphylococci. In addition, ceftazidime, cefoperazone and fourth generation cephalosporins have activity against *Pseudomonas* spp.

In human medicine, 3rd-and 4th-generation cephalosporins are important to treat a high number of patients with severe infections including meningitis, community- and hospital-acquired pneumonia, bacteraemia, acute intra-abdominal infections, complicated UTI, skin and soft tissue and bone and joint infections, gonorrhoea and endocarditis. They are regarded as an essential component of the limited treatment alternatives available for management of serious, life-threatening infections.

Importance for animal health

In food-producing species, 3rd- and 4th-generation cephalosporins are authorised for use in cattle, pigs and horses only. In cattle, they are authorised for use by injection to treat respiratory diseases due to *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni*, interdigital necrobacillosis, septicaemia and acute mastitis with systemic involvement caused by *E. coli* and acute metritis. They are also authorised for use via the intramammary route for subclinical and clinical mastitis. In pigs, 3rd- and 4th-generation cephalosporins are authorised for treatment of respiratory infections (*Actinobacillus pleuropneumoniae, Pasteurella multocida, Glaesserella parasuis, Streptococcus suis*), mastitis-metritis-agalactia syndrome and septicaemia, arthritis, polyserostis, meningitis and epidermitis due to *Strep. suis*. In horses, they are authorised for administration by injection to treat respiratory diseases (*Streptococcus zooepidermicus, Streptococcus equi, Staphylococcus* spp.*, Pasteurella* spp).

No evidence was found for the use of 3rd- and 4th-generation cephalosporins in food-production aquaculture in the EU.

In dogs and cats, 3rd-generation cephalosporins (cefovecin only) are authorised by injection for treatment of skin and soft tissue infections, UTI and severe periodontal infections.

In line with good veterinary practice, 3rd- and 4th-generation cephalosporins are to be reserved for use in veterinary medicine when there are no alternatives from a lower AMEG category that could be clinically effective, and preferably on the basis of susceptibility testing.

The 'open call for data' received reports of use of 3rd- and 4th-generation cephalosporins outside the terms of the marketing authorisation to treat infections in various minor species including reptiles, ornamental birds, fish and zoo and aquarium species e.g. cetaceans and pinnipeds. Human formulations were also reported to be used in these species. In textbooks, it is noted that 3rd-generation cephalosporins have been used to treat septicaemia and meningitis in foals, for *Salmonella* Choleraesuis infections in pigs [33, 53] and various serious infections involving MDR Enterobacterales and *P. aeruginosa* (notably ceftazidime) in companion and zoo animals [125]. Ceftiofur and other cephalosporins are identified as important for treatment of zoo animals due to long dosing intervals. In addition, there are reports of use of human-authorised 3rd-generation cephalosporins (cefpodoxime) for oral treatment of SSTI and UTI in companion animals, although this may relate to availability of veterinary formulations in third countries [46].

Development and selection of resistance

The most important mechanisms of resistance to cephalosporins are the beta-lactamase enzymes (e.g. ESBL, AmpC) that catalyse hydrolysis of the beta-lactam ring. There is a very wide variety of different beta-lactamases with varying substrate specificity [37].Beta-lactamases are generally encoded by genes located on mobile, extrachromosomal genetic elements (e.g. plasmids) responsible for the wide dissemination of these enzymes, or in the bacterial chromosome.

Resistance can also be due to efflux pumps and decreased permeability of the cell membrane in Gram-negative bacilli.

Another important mechanism of beta-lactam resistance is alterations in penicillin binding proteins, PBPs. This type of mechanism is found in staphylococci and is mediated by *mec* genes [39, 40, 76, 77]. The result of the *mec*-gene is a modified penicillin binding protein with low affinity to nearly all beta-lactams except to the staphylococcal cephalosporins, ceftobiprole and ceftaroline. *mec* gene-harbouring staphylococci are known as methicillin-resistant staphylococci (MRS). Today, methicillin resistance is a common feature in *Staphylococcus aureus*, *Staphylococcus pseudintermedius* and in many coagulase negative staphylococci [78]. The *mec* genes locate in a chromosomal genetic element called Staphylococcal Cassette Chromosome mec (SCCmec). There is evidence suggesting that *mec* genes or SCC *mec* elements are transferrable between different staphylococcal species [78, 79]. *mec*B can also be plasmid encoded [76]. Methicillin-resistant staphylococci usually spread clonally.

Cross-resistance between 3rd- and 4th-generation cephalosporins is common.

Monitoring of *Salmonella* spp. and indicator *E. coli* under EFSA/ECDC mandatory EU surveillance in food-producing animals showed that resistance to 3rd-generation cephalosporins was seldom detected in 2019-20. The prevalence of resistance to 3rd-generation cephalosporins was generally very low in *Salmonella* spp. (1.1% of isolates from animals/carcasses), but with variability between MS which may be due to the presence of resistant clones of particular serovars in certain animal populations. No resistance to 3rd-generation cephalosporins was detected in *Salmonella* spp. isolates from calves or their carcases. The proportion of presumptive ESBL-and AmpC-producers was very low/low in *Salmonella* isolates, although higher in some serovars (e.g. S. *Infantis*, S. *Kentucky*) and in isolates from broilers. In *Salmonella* spp. from human cases of non-typhoidal salmonellosis in 2019-20, resistance to 3rd-generation cephalosporins was overall very low at 0.8%.

Resistance to 3rd-generation cephalosporins in indicator *E. coli* isolates was not detected by some MSs and median levels across the EU were very low/low in the four animal populations in 2019-20, ranging from 1.2 to 1.7% of isolates according to population. The proportion of presumptive ESBL-and AmpC-producers was low overall ranging from 1.0% of isolates from calves to 1.3% isolates from turkeys;

although with higher levels in some MSs, up to 5.6, 5.9, 6.3 and 7.1% in calves, pigs, turkeys and broilers, respectively [28].

Monitoring of MRSA in food-producing animals is voluntary and not harmonised, and data are provided by few member states to EFSA/ECDC surveillance. Most isolates are LA-MRSA. In 2019-20, the prevalence of MRSA varied widely depending on animal production type and country [28].

The EU mandatory surveillance programme does not monitor for AMR in isolates from food-producing aquatic species. High rates of resistance to 3rd- and 4th-generation cephalosporins have been identified in foodborne pathogens from aquatic food animals in Asia [116].

Monitoring for resistance to 3rd- and 4th-generation cephalosporins in target pathogens

In the context of the Animal Health Law, Regulation (EU) 2016/429, EFSA has assessed AMR bacteria responsible for animal transmissible diseases, with a view to such pathogens being listed for EU action. For this assessment, EFSA conducted an extensive literature review to determine the global state of play of selected resistant bacteria that constitute a threat to animal health and this was used by experts to identify those bacteria most relevant to the EU. Scientific opinions were developed separately for species including dogs and cats, horses, pigs, cattle, small ruminants and rabbits (See Annex 3. EFSA Animal Health Law Scientific opinions).

EFSA identified *E coli* as a relevant AMR pathogen in dogs and cats, horses, pigs, cattle, sheep and goats and rabbits in the EU. For dogs and cats, thirteen EU studies were reviewed which included *E. coli* isolates. The level of resistance to 3rd-generation cephalosporins ranged from 0.2 to 71.4%, with mean resistance of 6.5%. In horses, 7 EU studies were considered; resistance levels varied from 2.9 to 60%, with an average of 8.9%. Studies with the highest levels of resistance included isolates from hospitalised patients. For swine, EFSA reviewed 12 papers, together including > 8,000 isolates and derived a mean resistance to 3rd-generation cephalosporins of 4.2 % (range 0 to 15.5%). In cattle, the average level of resistance to 3rd-generation cephalosporins in *E. coli* isolates was 2.9% (3 studies) and in dairy cattle alone (mostly mastitis cases) it was 4.3% (14 studies). In most EU studies, the proportion of resistance to 3rd-generation cephalosporins in *E. coli* from sheep and goats was very low (≤ 3%). In rabbits, *E. coli* is mostly associated with neonatal and post-weaning colibacillosis and overall 1% of isolates were resistant to ceftiofur.

In cattle, EFSA also identified *Staphylococcus aureus* as a relevant AMR bacteria. *S. aureus* is an opportunistic pathogen of the skin and most importantly a cause of mastitis which can be transmitted between cows at milking. Review of four papers from Europe identified an average resistance of 13.7% to cefoperazone and of 6.9% to ceftiofur in samples originating mainly from mastitis cases. It was noted that in some studies that level of resistance to these two drugs was not the same as that for methicillin despite the mechanism of resistance being the same and that the ceftiofur clinical breakpoint (CBP) may not identify all MRSA infections. For *Streptococcus uberis*, the average levels of resistance were 5.7% for cefoperazone (3 studies) and 13% for ceftiofur (1 study), and for *Strep. dysgalactiae*, the average level of resistance was 4.2% for cefoperazone (1 study).

For companion animals, recent European studies have shown a diversity of $bla_{\text{CTX-M}}$ genes (e,g. $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-27}}$, $bla_{\text{CTX-M-55}}$, and $bla_{\text{SHV-12}}$) in *E.coli* isolates from dogs, cats and horses in the EU, and the presence of highly virulent human-related clones such as *E. coli* ST131. These isolates can be associated with both colonisation and infection in companion animals [143-145]. Increasing prevalence of MRSP infections in dogs is also of growing concern considering the limited therapeutic options [146].

Transmission of resistance

There is evidence for the transmission of resistance to 3rd- and 4th-generation cephalosporins from food-producing and companion to humans via Enterobacterales that are zoonotic pathogens or commensal bacteria. Enterobacterales are mainly transferred from food-producing animals to humans via the foodborne route [82, 86]. Transfer of resistant zoonotic pathogens is demonstrated for *Salmonella* spp. and certain *E. coli* strains [147-152]. Moreover, the same or similar beta-lactam resistance genes (including ESBLs) have been isolated in bacteria of human and animal origin, and molecular studies support the potential for transfer of MGEs from animal to human enteric commensals, contributing to the spread of antibiotic resistance genes and resistant bacteria in the human intestinal tract [38, 87, 88]. Companion animals may also be a reservoir for beta-lactamase resistance that can be transferred between animals and humans via Enterobacterales that are zoonotic pathogens or commensal bacteria, and by direct and indirect transmission, although there are few studies investigating these pathways [90-92].

Methicillin-resistant staphylococci may also be transmitted from food-producing and companion animals to humans. Food is generally not considered to be a significant source of MRSA in humans [59, 93]. MRSA is mainly transmitted by direct contact from food-producing animals [65]. In geographical areas with high density of farms, livestock associated MRSA (LA-MRSA) contribution to the burden of MRSA disease could be significant [94, 95, 153]. There is evidence for rare zoonotic transmission of MRSA/P from companion animals to persons in contact [62, 66, 67, 90].

In conclusion, there is evidence to support the selection and transmission of resistance to 3rd- and 4th-generation cephalosporins from animals to humans and other animals via zoonotic or target pathogens or commensals bacteria capable of transferring resistance to other pathogenic bacteria [3].

In conclusion,

- 3rd- and 4th-generation cephalosporins are essential antimicrobials in both veterinary and human medicine, used to treat serious, including life-threatening, infections.
- In veterinary medicine, 3rd- and 4th-generation cephalosporins are regarded as last resort antibiotics, used in particular to treat Gram-negative infections that are resistant to other veterinary-authorised beta-lactam antibiotics.
- Resistance to 3rd- and 4th-generation cephalosporins in Gram-negative bacteria is mainly due to plasmid-borne genes encoding ESBLs or chromosomal AmpC.
- The prevalence of extended-spectrum beta-lactamases (ESBL) and AmpC producers in indicator *E. coli* from food-producing animals varies greatly between animal production type and country.
- Resistance in Staphylococcus spp. also occurs due to alteration of penicillin binding proteins, mediated by mec genes (e.g. LA-MRSA, MRSP).
- There is cross-resistance with other beta-lactam classes depending on their individual susceptibility e.g. to specific beta-lactamase enzymes. Beta-lactam antibiotics with a broader spectrum of action, such as 3rd- and 4th-generation cephalosporins will exert a broader selection pressure. MRS are resistant to almost all beta-lactams.
- Resistance may be transferred from animals to humans via the foodborne route and to other animals by direct and indirect contact, through transmission of zoonotic and target pathogenic bacteria and commensals organisms.

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to 3rd- and 4th-generation cephalosporins.

Criterion (c) – availability of other treatments for animals

The incidence of infectious diseases in cattle and pigs can be reduced by adjusting management practices e.g. limiting travelling distances, quarantine, avoiding co-mingling of animals from different sources, improving housing, ventilation and general biosecurity, and also through use of vaccinations [12]. In pigs, vaccinations (sows or piglets) can be an effective way to reduce the occurrence of neonatal and post-weaning diarrhoea due to *E. coli*; however, it is necessary to use the appropriate vaccine for the most prevalent ETEC pathotype on the farm and to ensure that the vaccine is administered at the optimal time. In poultry, vaccination programmes for various viral diseases (e.g. ND, IB, IBD, Marek's) have greatly reduced the need for antibiotic treatments but there is a great diversity in APEC strains and fewer effective vaccines are available. Vaccines are also available against *Salmonella* Typhimurium and certain other serovars in pigs and cattle in the EU. However, these options cannot replace antibiotics when treatment is needed for sick animals.

Potential alternatives to 3rd- and 4th-generation cephalosporins for resistant *E. coli* infections are limited to AMEG Category B substances, i.e. colistin (not foals) or fluoroquinolones, or, depending on patient/disease suitability, aminoglycosides (Category C). 3rd- and 4th-generation cephalosporins may also be used for treatment of respiratory tract infections in horses (*Streptococcus zooepidermicus, Streptococcus equi, Staphylococcus* spp., *Pasteurella* spp.), cattle (e.g. *Mannheimia haemolytica*) and pigs (e.g. *Actinobacillus pleuropneumoniae*), metritis in cattle and pigs and interdigital necrobacillosis in cattle (e.g. *Fusobacterium* spp.). For these diseases, resistance to all first-line antimicrobials is uncommon in the EU [55, 108, 154]; however, in line with VMP authorisations, use of 3rd- and 4th-generation cephalosporins is restricted to infections in individual animals that have responded poorly or are unlikely to respond to first-line antimicrobials e.g. based on susceptibility testing [155]. Therefore, potential alternatives would be dependent on the findings of AST and are likely to be limited to other Category B antibiotics.

In dogs and cats, 3rd-generation cephalosporins are authorised for treatment of UTI, SSTI and severe periodontal infections with SPC restrictions as mentioned above. Pathogens causing UTI (e.g. Enterobacterales) and SSTI (e.g. *S. pseudintermedius*) in dogs and cats are increasingly resistant to first-line antibiotics. 3rd-generation cephalosporins are one of limited options for pyelonephritis which requires rapid empirical treatment; fluoroquinolones are the alternative. SSTI become serious if recurrent or progressing to cellulitis and 3rd-generation cephalosporins may be used as a second-line treatment; alternatives are fluoroquinolones or, according to patient suitability, aminoglycosides or rifampicin. 3rd-generation cephalosporins are one of few antibiotics available for treatment of anaerobic infections in companion animals with alternative options being clindamycin and metronidazole [3].

Criterion (d) – availability of other antimicrobial treatments for humans

Alternatives to treat infections in humans caused by resistant Enterobacterales such as *E. coli* and *K. pneumoniae* are limited and include 'last resort' antibiotics such as combinations of 3rd-generation cephalosporins with a beta-lactamase inhibitor (e.g. ceftazidime-avibactam), carbapenems and colistin, as well as monobactams, according to the specific mechanism of resistance and susceptibility to other classes [3].

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

- 3rd- and 4th-generation cephalosporins are essential antibiotics in both veterinary and human medicine for treatment of both Gram-positive and Gram-negative infections, being stable to many beta-lactamases. In human medicine, they are important due to use to treat a high number of

patients with severe infections, including e.g. pneumonia and meningitis, caused in particular by Enterobacterales and (4GCs) *Pseudomonas* spp.

- In veterinary medicine, 3rd- and 4th-generation cephalosporins are authorised for use in cattle, pigs, horses and companion animals. They are used as last resort, both in accordance with and outside the marketing authorisations to treat life-threatening infections such as septicaemia, urogenital and respiratory infections. They are also used outside of the marketing authorisation for treatment of animal species not listed in the SPCs, including 'exotic' and zoo species.
- The most important mechanism of resistance to 3rd- and 4th-generation cephalosporins in Gram-negative bacteria is due to plasmid-borne ESBLs or AmpC genes. Although prevalence of this resistance in animal isolates of indicator *E. coli* and *Salmonella* spp. is generally low on mandatory surveillance in food-producing species, it is variable in clinical isolates. There is evidence to support the selection and transmission of resistance to 3rd- and 4th-generation cephalosporins from animals to humans, including via the foodborne route, and to other animals.
- 3rd- and 4th-generation cephalosporins are included in the AMEG category B. According to SPCs and responsible use guidance, they should preferably be used in animals on the basis of susceptibility testing and only when antibiotics from a lower AMEG category would not be clinically effective; therefore, the availability of alternative treatments for animals in these circumstances is limited. In human medicine, the alternatives are often human-only last resort antibiotics.
- The extent of use of 3rd- and 4th-generation cephalosporins outside the marketing authorisation is unknown. Many examples were found in literature, relating to individual animal administration. There are no VMPs containing 3rd- and 4th-generation cephalosporins that are authorised in the EU for group administration.

Therefore, considering the points above relevant to criteria (b), (c) and (d), it should be considered if conditions or a prohibition should be placed on the use of 3rd- and 4th-generation cephalosporins outside the terms of the marketing authorisation.

Step 2. Considerations of conditions to be placed on use outside the terms of a marketing authorisation

Please refer to Section 3.1.2. of the main report for the general rationale behind the proposed conditions.

(i) Use for unauthorised indications

Condition: *For those indications not included in the SPC of the concerned product, use must be based on target pathogen identification and antimicrobial susceptibility testing that demonstrates that 3rd- and 4th-generation cephalosporins are likely to be effective and that antimicrobials from a lower AMEG category would not be effective, unless it can be justified that this is not possible.*

Rationale: See Section 3.1.2.(i) of the advice.

Condition: *Use 3rd- and 4th-generation cephalosporins under Article 113 to treat salmonellosis should be restricted to use of injectable products in individual animals with potentially life-threatening infection.*

Rationale: The primary mechanisms for controlling Salmonella in pigs in the EU are through elimination or control and reduction programmes [156], including use of vaccination and husbandry measures outlined above [157]. Despite these measures, Salmonella can be re-introduced onto the farm through contaminated feed and water or wildlife such as rodents, birds and foxes. Clinical salmonellosis

infection in pigs is usually due to host-adapted *S.* Choleraesuis (causing septicaemia) or non-host adapted *S*. Typhimurium (enterocolitis). Ubiquitous serotypes such as *S.* Typhimurium and *S.* Enteritidis generally cause human infections, but serious systemic illness in humans due to *S*. Choleraesuis is rare. Use of antibiotics has been justified to reduce severity of signs and prevent suffering in individual animals but does not reduce the prevalence or duration of shedding by sick or recovered animals, hence it has been concluded that use of antimicrobials for *Salmonella* control (metaphylaxis) in pigs should be discouraged due to the public health risk and use should be limited to individuals with life-threating salmonellosis (bacteriemia with high fever, depression and dyspnoea) [158, 159].

Salmonella infection in cattle can manifest as haemorrhagic enteritis, endotoxaemia, septicaemia, pneumonia and abortions. Host-adapted *S.* Dublin is the most common serotype in cattle and rarely causes infections in humans; *S.* Typhimurium is the second most common serotype. Control programmes are also implemented in some EU countries. Antimicrobial treatment is controversial due to the public health risk and possibility that cattle infected by *S*. Dublin may become chronic sub-clinical carriers that maintain infection in the herd. However, faecal shedding is a lesser problem in calves. Antimicrobial use in calves has been justified in case of enteritic salmonellosis to prevent development of bacteraemia and multiple organ disease, in which case systemic antimicrobial treatment is always needed [53, 160].

In terms of public health risk, most concern relates to serovars of Salmonella that have been associated with human foodborne diseases outbreaks. In the EU, data for 2020 show that most such outbreaks were due to S. Enteritidis (57.9%), *S.* Typhimurium, Monophasic *S.* Typhimurium, *S.* Infantis and *S.* Derby. S. Enteritidis was primarily linked to broilers and layers/eggs, *S.* Typhimurium to broilers and pig sources, Monophasic *S.* Typhimurium to pigs and broilers, *S.* Infantis to broilers and *S.* Derby to pigs and turkeys [28]. EFSA/ECDC monitoring data from 2019-2020 show overall high resistance levels to ampicillin, sulfonamides and tetracyclines (ASuT) in Salmonella spp. from human cases and moderate-very high ASuT resistance in Salmonella isolates from food-producing species in most member states, limiting first-line treatment options. Resistance to fluoroquinolones was also very high amongst Salmonella spp. from poultry and moderately high in isolates from human cases; whilst it was moderate-low in isolates from calves and pigs. Invasive salmonella infections in humans are treated by preference with 3rd-generation cephalosporins, fluoroquinolones or, in children, azithromycin. As noted above, resistance to 3rd-generation cephalosporins in Salmonella isolates from food-producing species in 2019-2020 was either not detected, or detected at very low levels in most reporting MSs, with combined resistance to cefotaxime and ciprofloxacin also very low in both animal and human isolates, with the exception of *S.* Kentucky and *S.* Infantis serovars.

Considering the zoonotic risk related to salmonella in poultry, antimicrobial use in national control programmes is already restricted in accordance with Commission Regulation EC 1177/2006 and the principal control strategy is elimination by testing and culling of infected flocks. See also proposed Condition under (ii), below.

Although there is lower potential for transmission of resistant salmonella clones from other food-producing animals to humans, this is an on-going public health concern [82]. In conclusion, there may be justification for antibiotic use to reduce severity of signs of salmonellosis and prevent suffering in individual animals with potentially life-threatening infection, considering that many member states do not have 'stamping out' policies for salmonellosis other than in poultry. It is proposed that 3rd- and 4th-generation cephalosporins should be prohibited for treatment or metaphylaxis of *Salmonella* spp. by use of oral group administration.

(ii) Use for unauthorised target species

3rd- and 4th-generation cephalosporins are authorised in VMPs intended for use in cattle, pigs, horses, dogs and cats.

Condition: *Not to be used in poultry.*

Rationale: As identified from EFSA mandatory surveillance of AMR in food-producing animals (EFSA/ECDC 2022), poultry and poultry products are most frequently reported to carry ESBL and/or AmpC-producing *Salmonella* and *E. coli*. Although decreasing trends have now been observed in some member states, the prevalence of ESBL/AmpC producing *E. coli* in meat samples from poultry is still high (based on culture of samples on selective media) when considering the mean across member states. Based on the CVMP's reflection paper on the use of 3rd- and 4th-generation cephalosporins in food-producing animals in the EU (EMEA CVMP, 2009) and the EFSA Scientific Opinion on the public health risks of bacterial strains producing extended-spectrum beta-lactamases and/or AmpC beta-lactamases in food and food-producing animals [84], a subsequent CVMP referral and Commission Decision issued in January 2012 determined that VMPs containing 3rd- and 4th-generation cephalosporins should include in the SPCs a contraindication from use in poultry.

Reference should also be made to Section 3.1.2.(ii) of the advice

(iii) Administration by an unauthorised route or use of extemporaneous formulation

Condition: *To be used in individual animals only*. *Exemption: Ornamental or conservation aquatic animals kept in closed water tanks.*

Rationale: Authorised VMPs containing 3rd- and 4th-generation cephalosporins are available for administration in formulations for individual animal use, via injection or intramammary routes. 3rd- and 4th-generation cephalosporins have not been authorised in veterinary medicines for administration as group treatments and therefore no formal AMR risk assessment has been conducted for associated routes of administration (see Section 3.1.2.(iii) of this advice).

Condition: *Not to be used in food-producing aquaculture*

Rationale: Although EFSA does not monitor for antimicrobial resistance in aquaculture food production, ESBLs have been detected in isolates from fish and other species reared in aquaculture systems globally [116, 117]. Considering that aquaculture systems are regarded as potential hotspots for driving emergence, release, transmission and persistence and spread of AMR bacteria and resistance genes, discussed in Section 3.1.2.(iii) of this advice, and the high importance to human and animal health of this antimicrobial class, it is recommended that its use in food-production aquaculture should be restricted [118].

(iv) Use of a human medicinal product

Human medicinal products are available for administration by injection and orally.

No further conditions proposed to those already mentioned above.

Rationale: See Section 3.1.2.(iv) of this advice.

(v) Use of a third country veterinary medicinal product

According to the Regulation, third country VMPs may only be used in the same species and for the same indication. No further conditions proposed to those mentioned above.

Rationale: See Section 3.1.2.(v) of this advice.

Step 3. Consideration of Criteria (a) and (e) in view of proposed conditions to be placed on use outside the terms of a marketing authorisation

Criterion (a) – risk to animal health or public health if the antimicrobial is used in accordance with Articles 112, 113 and 114

SPCs recommend that 3rd- and 4th-generation cephalosporins should not be used in small herbivores. Target animal safety warnings in the SPCs of authorised VMPs should be followed. Consumer safety is mitigated through the application of the statutory withdrawal period in accordance with Article 115.

Criterion (e) Impact on aquaculture and farming if the animal affected by the condition receives no treatment

Proposed condition	**Potential impact on aquaculture and farming if animal affected by the condition receives no treatment**
For those indications not included in the SPC of the concerned product, use must be based on target pathogen identification and antimicrobial susceptibility testing that demonstrates that 3rd- and 4th-generation cephalosporins are likely to be effective and that antimicrobials from a lower AMEG category would not be effective, unless it can be justified that this is not possible.	This condition does not preclude treatment. See Annex 1. of report for further discussion.
Use of 3rd- and 4th-generation cephalosporins under Article 113 to treat salmonellosis should be restricted to use of injectable products in individual animals with potentially life-threatening infections.	An EU baseline survey conducted by EFSA in 2008 [161] found *Salmonella Typhimurium* on approximately 6% of pig production and breeding holdings in the EU overall, with much lower prevalence of *S. Choleraesuis*. The findings of a systematic review of studies published between 2000 – 2017 estimated a prevalence of Salmonellae in healthy cattle in Europe of 2%. [158, 162, 163]. However, prevalence of Salmonellae on farm or in healthy animals at slaughter does not give a full picture of the prevalence of outbreaks of clinical disease, for which evidence is difficult to find for the EU. Salmonellae are often resistant to many first-line antibiotics used in food-producing animals (ASuT resistance pattern); and second-line treatment options may be limited e.g. aminoglycosides, florfenicol, fluoroquinolones. Abortions, septicaemia, meningitis, encephalitis and death are potential sequelae to infection. As there are no 3rd- and 4th-generation cephalosporins VMPs currently authorised for group administration, it is unlikely that they have previously been used by this route for treatment and metaphylaxis of salmonellosis.

	The proposed conditions do not prevent treatment of individual animals in order to protect animal welfare. Outbreaks can be minimised by use of attention to biosecurity, husbandry and use of vaccination where available; however, eradication is not always feasible [53, 158].
Not to be used in poultry.	According to Commission Decision (2012)182, SPCs have included a contraindication for use of 3rd- and 4th-generation cephalosporins in poultry since 2012; therefore, a legal restriction on such use is unlikely to have a significant further impact on poultry farming.
Not to be used in food-producing aquaculture	No evidence was found for the use of 3rd- and 4th-generation cephalosporins in food-production aquaculture in the EU; therefore, although impacts on aquaculture cannot be fully foreseen, they are not expected to be significant under current circumstances.
Administration to individual animals only	There are no 3rd- and 4th-generation cephalosporins VMPs currently authorised for group oral administration, and no evidence was found that they have previously been used by this route. Therefore, although the impact on farming of restriction to individual animal use cannot be foreseen, it is not expected to be significant.

Step 4. Final conclusion - recommendations made for conditions to be placed on use outside the terms of a marketing authorisation

Based on the discussion above, the following conditions are proposed for use under Articles 112, 113 and 114:

- For those indications not included in the SPC of the concerned product, use must be based on target pathogen identification and antimicrobial susceptibility testing that demonstrates that 3rd- and 4th-generation cephalosporins are likely to be effective and that antimicrobials from a lower AMEG category would not be effective, unless it can be justified that this is not possible.
- Use of 3rd- and 4th-generation cephalosporins under Article 113 to treat salmonellosis should be restricted to use of injectable products in individual animals with potentially life-threatening infections.
- Not to be used in poultry.
- Not to be used in food-producing aquaculture
- To be used in individual animals only. Exemption: Ornamental or conservation aquatic animals kept in closed water tanks.

4.8. Polymyxins

4.8.1. Background information

Examples of substances in the class that are authorised in veterinary and human medicine in the EU

Examples of substances authorised for veterinary use	Examples of ATCvet codes
Colistin (polymyxin E)	QJ01XB01 QA07AA10 QJ51XB01 QJ51XB02
Polymyxin B	QJ01XB02 QA07AA05 QS01AA18 QS02AA11 QS03AA03
Examples of substances authorised for human use	**Examples of ATC codes**
Colistin (polymyxin E)	J01XB01 A07AA10
Polymyxin B	J01XB02 A07AA05 S01AA18 S02AA11 S03AA03

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

Substance	Species	MRL tissues	MRL milk	MRL eggs
Colistin	All food-producing species	Yes	Yes	Yes

EU-authorised VMP formulations, based on sales reported ESVAC

Species		Route of administration						
		Group administration		Individual animal administration				
		In-feed	In-water	Injection	Oral Powder	Oral e.g. tablet, paste	Topical/local (incl. intrauterine)	Intra-mammary
Major	**Cattle**	CST	CST	CST	CST	CST		CST
	Sheep (meat)	CST	CST	CST				
	Pigs	CST	CST	CST	CST			
	Chickens	CST	CST		CST			
	Dogs			CST			PMB	
	Cats			CST			PMB	
Limited market	**Turkeys**	CST	CST	CST	CST			
	Poultry incl. geese, ducks, gamebirds	CST	CST	CST	CST			
	Guinea-pig						PMB	
	Goats	CST	CST	CST				
	Rabbits	CST	CST	CST				
	Buffalo		CST					
	Horses			CST				
	Pigeons		CST					

CST (colistin), PMB (polymyxin B)

Summary of main indications and contra-indications for EU-authorised VMPs, based on selected SPCs

Main indications	*Colistin* Group oral formulations and oral powders for cattle, sheep, pigs and poultry are indicated for: Treatment and metaphylaxis of enteric infections caused by non-invasive *E. coli* susceptible to colistin. Injectable formulations are authorised for IM administration in cattle (calves) sheep (meat) and pigs to treat colisepticaemia, urinary and gastrointestinal infections due to *Salmonella* spp. and *E. coli* including oedema disease in piglets and gynaecological infections caused by *E. coli* and *Pseudomonas* spp. Injectable formulations in combination with tiamulin, amoxicillin and ampicillin are authorised (in Spain) variously for treatment of food-producing and companion animals for a variety of diseases. *Polymyxin B* Topical treatments for pet animals: For the treatment of otitis externa and small localised superficial skin infections caused by Gram-negative organisms e.g. *Pseudomonas* spp., *E. coli*
Contraindications	*Colistin* Do not use in horses, particularly in foals, since colistin, due to a shift in the gastrointestinal microflora balance could lead to the development of antimicrobial associated colitis (Colitis X), typically associated with *Clostridium difficile*, which may be fatal. *Polymyxin B (topical)* Do not use in case of perforated tympanic membrane.

Examples of EU-authorised HMP formulations, from Article 57 database

Substance	Route of administration		
	Injection	Oral e.g. tablet, liquid	Topical/local
Colistin (polymyxin E)	x	x	
Polymyxin B			x

Existing recommendations

WOAH recommendations

Polymyxins are categorised VHIA by WOAH (formerly OIE). *Specific comments:* This class is used in the treatment of septicaemias, colibacillosis, salmonellosis, and urinary infections. Polymyxin E (colistin) is used against Gram-negative enteric infections.

Additional WOAH recommendations for polymyxins:

- Not to be used as preventive treatment applied by feed or water in the absence of clinical signs in the animal(s) to be treated;
- Not to be used as a first line treatment unless justified, when used as a second line treatment, it should ideally be based on the results of bacteriological tests;
- Extra-label/off-label use should be limited and reserved for instances where no alternatives are available. Such use should be in agreement with the national legislation in force; and
- Urgently prohibit their use as growth promotors.

WHO classifications

WHO: HPCIA

- (C1: Yes) Limited therapy for infections with MDR Enterobacteriaceae (e.g. *Klebsiella* spp., *E. coli*, *Acinetobacter*, *Pseudomonas* spp.).
- (C2: Yes) May result from transmission of Enterobacteriaceae from non-human sources.

- (P1: Yes) High numbers of people affected by diseases who are seriously ill in healthcare facilities in any countries for which the antimicrobial is the sole or one of few therapies available.
- (P2: Yes) In multiple countries there is high use in people in critical care settings or where multidrug resistant organisms are prevalent.
- (P3: Yes) Colistin resistant bacteria and the *mcr* family genes can be transmitted via the food chain.

WHO AWaRe: Polymyxin B and colistin are in the Reserve group

AMEG and CVMP recommendations

Polymyxins are included in the AMEG Category B, for which there is a higher AMR risk to public health. For these antimicrobials, the risk to public health resulting from veterinary use needs to be mitigated by specific restrictions. These restricted antimicrobials should only be used for the treatment of clinical conditions when there are no alternative antimicrobials in a lower category that could be effective. Especially for this category, use should be based on the results of antimicrobial susceptibility testing, whenever possible.

A referral procedure for veterinary medicinal formulations containing colistin at 2 000 000 IU/ml and intended for administration in drinking water to any food-producing species was conducted by the CVMP in 2010. It concluded that indications for the treatment of salmonella infections in calves, lambs, pigs and poultry should be deleted since supporting clinical data were inadequate and treatment of subclinical infections might interfere with national control programmes. Indications for the products for treatment of non-invasive *E. coli* infections in calves, lambs, pigs and poultry were found to be adequately supported.

In 2014 the CVMP undertook a further referral, for all products containing colistin as sole active substance for oral administration in food-producing animals. In addition to deletion of indications relating to salmonella infections, it was also recommended to restrict the indications for use to treatment of enteric infections caused by susceptible non-invasive *E. coli* only and that any indications for prophylactic use should be removed. Based on the recommended treatment durations for the proposed indication and the concentration-dependent activity of colistin, it was concluded that the treatment duration should be limited so as not to exceed 7 days in order to reduce the selection pressure for resistance.

In addition, with the withdrawal of the indication for salmonellosis, and owing to concerns regarding safety in horses, this animal species was removed from the SPC for orally administered products and a related contraindication for use in horses was added.

In 2016 the CVMP recommended the withdrawal of the marketing authorisations for all veterinary medicinal products for oral use containing colistin in combination with other antimicrobial substances since no benefit could be demonstrated for the combination products over the monotherapy product for the given indications, which included gastrointestinal and respiratory infections. CVMP noted that colistin combination products were intended to address needs where antimicrobial distribution would be required both in the gastrointestinal tract and beyond (e.g. gastrointestinal infection coupled with septicaemia and multi-organ disease) or where extended spectrum of antimicrobial cover was needed (e.g. polymicrobial infections). Acceptable clinical or other data were not provided to support these scenarios. Furthermore, the CVMP concluded that '*the benefit-risk balance for all veterinary medicinal products containing colistin in combination with other antimicrobial substances to be administered orally to food-producing species is negative, due to a lack of clinical relevance and in view of over-*

exposure of colistin that could pose a potential risk to animal and human health from an acceleration of the occurrence of colistin resistance'.

Further information on the justification for these measures is available [164-166].

Following the identification in 2015 of the *mcr-1* gene conferring resistance to colistin, and the increasing importance of colistin in human medicine, continued use of the substance in veterinary medicine has come under intense scrutiny. The CVMP/AMEG provided advice to the Commission on the use of colistin products in animals in the EU in 2016 [167]. This advice acknowledges that colistin is a last resort antibiotic in human medicine for treatment of severe nosocomial infections due to multidrug-resistant (MDR) Gram-negative bacteria that increasingly account for high morbidity and mortality. It was recognised that there is the potential for transfer from animals to humans of colistin-resistant pathogens and colistin resistance genes in commensal organisms through food and other routes of exposure. However, colistin remains of therapeutic importance in veterinary medicine, in particular for the treatment of serious enteric *E. coli* infections in poultry, weaning piglets and calves. Owing to high levels of resistance to other antibiotics in this pathogen, the only alternatives to colistin may be other CIAs. Recommendations were made to set targets to substantially reduce veterinary use of colistin in the EU, and to move the substance into the AMEG's higher risk category.

Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Disclaimer: The information in this section reflects reported use of antimicrobials outside the terms of a marketing authorisation. No evaluation is made in this section by the working group on the efficacy or safety of the reported uses, or on their potential impact on development and dissemination of AMR.

Information from published sources

Literature reports indicate that historically colistin was administered for prevention of pre- and post-weaning diarrhoea in piglets [168, 169]. Although injectable colistin products are authorised for treatment of *Salmonella* spp. infections, textbooks also refer to use of oral formulations for this indication [33, 96].

Polymyxin B is used for the treatment of endotoxemia in horses, due to its unique property of binding to non-specific endotoxins in the blood [170].

Information from the open call for data on use of antimicrobials in animals

The information below is summarised from the open call for data. Inclusion in the table does not endorse use or imply that it is consistent with use according to legislative provisions in Articles 112 to 114.

Colistin		Alternatives	Consequences of unavailability
Species	**Indication**		
Dairy cattle, small ruminants, pigs	Salmonellosis, to reduce levels of excretion	Neomycin, tetracyclines and apramycin, dependent on susceptibility Fluoroquinolones	
All food-producing species	Gram-negative infections including septicaemia and diarrhoea	Fluoroquinolones, 3rd- and 4th-generation cephalosporins, gentamicin Vaccination	Increased mortalities and welfare issues
Poultry	Respiratory/systemic colibacillosis	Neomycin, tetracyclines, fluoroquinolones, sulfonamides	
Avian species: geese, ducks	Colibacillosis	Wider spectrum antibiotics/ no alternatives	
Rabbits	Colibacillosis	Fluoroquinolones	Increased treatment duration

Horses	Colibacillosis, diarrhoea	Cephalosporins, fluoroquinolones or no alternatives	Unable to treat, welfare issues
Ornamental fish	Bacterial infections	Dependent on susceptibility	Deaths if untreated
Various spp.	Use of colistin + amoxicillin combinations for septicaemia, respiratory, gastrointestinal and genitourinary infections		Increased use of fluoroquinolones or no alternatives
Dogs	Injectable formulation used topically to treat otitis media due to MDR *Pseudomonas* spp.		Euthanasia
Polymyxin B		**Alternatives**	**Consequences of unavailability**
Species	**Indication**		
Horses	Endotoxaemia (Reg (EU) 122/2013)	Flunixin	Less effective treatment
Horses	Eye infections	None	Enucleation, septicaemia

4.8.2. Evaluation

Scope of permitted use according to the MRL Regulation

Colistin is included in Table 1 (allowed substances) of the Annex to Regulation (EU) 37/2010, having MRLs in all food-producing species, and hence can be used in accordance with Articles 113 and 114 of Regulation (EU) 2019/6. There are no 'Other provisions' mentioned in Table 1 that are of relevance to use outside the terms of the marketing authorisation.

Polymyxins can be used in non-food-producing species in accordance with Article 112.

Substances/indications in equines out of scope of evaluation for conditions due to listing in Regulation (EC) 1950/2006, as amended by Regulation (EU) 122/2013

Polymyxin B is listed for systemic treatment for endotoxaemia associated with severe colic and other gastrointestinal diseases in equines.

Examples of veterinary-authorised formulations/species

Colistin is authorised for administration in feed and drinking water for all major and some limited market food-producing species. It is also authorised for administration by injection in cattle, sheep, pigs, goats, horses, dogs and cats and for intramammary administration in cows.

No authorised VMPs were found for use in aquaculture in the EU.

Polymyxin B is authorised for topical administration (skin, eye, ear) in dogs, cats and rodents, for treatment of susceptible Gram-negative bacterial infections.

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Importance for human health

In human medicine, polymyxins (colistin) are one of few available therapies for serious systemic healthcare-associated infections due to MDR Enterobacterales, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, especially in seriously ill patients in ICUs. It is used as last resort in combination with meropenem, aminoglycosides or tigecycline for the treatment of infections caused by

carbapenemase-producing Gram-negative bacteria. Colistin should be used with care due to potential nephrotoxicity. Colistin is also administered by inhalation for the treatment of infections in cystic fibrosis patients and in patients with ventilator-associated pneumonia [171].

Infections caused by MDR Gram-negative bacteria are an increasing threat to healthcare delivery globally and colistin has increasingly been used in hospitals in the EU/EEA. Infections caused by carbapenem-resistant Gram-negative bacteria are associated with high levels of mortality and there were also an estimated 2,500 deaths due to colistin-resistant Gram-negative bacteria in the EU/EEA in 2015 [172].

Importance for animal health

Colistin is authorised in (group and individual) oral VMPs in the EU. In 2014 the CVMP recommended to restrict the indications for all VMPs containing colistin to be administered orally (in feed or water and tablets; calves, sheep, goats, pigs, poultry, rabbits) to "*Treatment and metaphylaxis of enteric infections caused by susceptible non-invasive E. coli*" only. Any indications for prophylactic, general indication or indication for any other pathogen were removed (Commission Decision (2015)1916 of 16 March 2015). According to the SPC, use should be based on susceptibility testing [173, 174]. Colistin is also authorised for parenteral and intramammary use. Colibacillosis (diseases due to *E. coli,* including enterotoxigenic strains) is a major cause of morbidity and mortality in neonatal and juvenile livestock of various species, especially swine [175-177]. Injectable formulations of colistin are authorised in a limited number of MSs for cattle (calves) sheep (meat) and pigs to treat colisepticaemia, urinary, gastrointestinal infections (*Salmonella* spp. and *E. coli* including oedema disease in piglets) and gynaecological infections caused by *E. coli* and *Pseudomonas*.

In dogs and cats, polymyxin B is among few alternatives for topical treatment of serious otitis due to Gram-negative infections and is included for this indication in the WSAVA list of essential medicines for cats and dogs [178]. It is also authorised for topical use in pets to treat small localised superficial skin infections caused by Gram-negative organisms e.g. *Pseudomonas* spp., *E coli.*

The 'open call for data' received reports of various uses of polymyxins in animals described as being outside of the terms of the marketing authorisation. Given the authorised formulations, indications etc., in many cases it is not possible to determine which aspect of use was not in line with the SPC. There were reports of use of colistin to treat limited market species, such as horses, goats, geese, ducks and ornamental fish, not included on the label of specific products, and use of oral formulations to treat broader Gram-negative infections including salmonellosis in pigs and ruminants. Injectable colistin combinations (+beta-lactam) were reported as used to treat peritonitis in calves and acute endotoxic mastitis in cattle. One expert also noted use of this combination to treat neonatal ruminants with diarrhoea caused by *E. coli* and clostridia. Polymyxin B was reported as used to treat eye infections in horses and otitis media due to MDR *Pseudomonas* spp. in dogs.

No evidence was found for use of polymyxins in aquaculture in the EU, although use has been reported in SE Asian countries [179].

Development and selection of resistance

Acquired resistance to polymyxins can be both chromosomal and plasmid-borne [167, 180]. Previously colistin resistance was thought to be entirely due to acquired mutations affecting the biosynthesis of lipopolysaccharide in the bacterial cell wall. Many of these mechanisms are recognised as being unstable. In 2015, the plasmid-borne *mcr-1* gene was identified. *Mcr*-1 encodes an enzyme (MCR-1) that modifies the lipid A, leading to resistance to polymyxins. Multiple *mcr* genes have now been described [181]. Resistance due to plasmid-mediated *mcr* genes has been detected in

Enterobacterales, *Acinetobacter* and *Pseudomonas* spp. and is reported globally from animals, food products, environment and in human clinical and non-clinical (screening) specimens [167, 180, 182-188].

mcr and ESBL genes have been identified on the same plasmid in salmonellae from food-producing animals, indicating the possibility for co-selection of resistance. [167, 188, 189]. Co-existence of *mcr*-genes and genes encoding for carbapenem resistance (NDM) have been found in *E. coli* isolates (on different plasmids) from food-producing animals and meat in China [190-192].

Although information about colistin resistance in bacteria derived from animals and food animal products is still limited, a widespread dispersion of *mcr* genes in livestock animals has been described [193]. Recent mandatory EU surveillance reported as overall (very) low i.e. <2% but variable prevalence of colistin resistance in *Salmonella* spp. (excluding intrinsically resistant strains) and indicator *E. coli* from different food-producing animal species and countries, but with moderate to high levels particularly in isolates from poultry in some countries [59].

EFSA does not monitor for antimicrobial resistance in aquaculture food production and information on colistin resistance in this sector is scare, particularly from the EU. However, *mcr*-1 has been detected in *E. coli* from farmed fish in Lebanon and China [24, 194]. Other references worldwide identify the presence of mcr genes in fish produce, but it is not always clear if this produce originates from farmed aquaculture [195].

Target pathogens

In a literature review performed by EFSA [108] of publications since 2010 and national AMR monitoring reports, the levels of resistance to colistin in pathogenic *E. coli* from pigs was 9.7% [range 0-76.9%], based on EU studies mostly from gastrointestinal infections, and in broilers it was 8.4% [range 1-13.4%].

A colistin-resistant gene (*mcr*-9) has recently been discovered in horses in Sweden [196] where it was associated with a *bla*SHV gene on a plasmid. The same colistin-resistant gene (*mcr*-9) has been found in extended spectrum beta-lactamase (ESBL)-producing *E. coli* at several equine hospitals in the UK [197]. In these isolates, the gene was associated with a different *bla*SHV gene and on a different plasmid, suggesting a different source than the Swedish horse isolates. Furthermore, another colistin-resistant gene (*mcr*-5.3) was identified in a horse with pneumonia in Brazil that died in 2012 [198].

The *mcr*-1 gene has incidentally been detected in *E. coli* from dogs and pet food in the EU, and globally [187, 199-201]. According to a literature review performed by EFSA (EFSA 2021), resistance to polymyxin in *Pseudomonas aeruginosa* from dogs and cats remains at very low levels.

Transmission of resistance

Epidemiology suggests that colistin resistance genes can be transferred from animals to humans and between animals via resistant bacteria or plasmids [167, 188]. *mcr* genes have been found in similar plasmids in the same bacterial species from food-producing animals and humans [167, 188, 202]. The more frequent isolation of *mcr* genes among animal isolates compared to human isolates, together with the higher use of colistin in livestock compared to human medicine in certain countries has been suggestive of transmission from animals to humans. The ban of the use of colistin in China as a growth promoter in agriculture has led to a decrease in colistin resistance in animals as well as humans [203].

In conclusion, although not quantifiable at present, there is evidence for the selection and potential transmission of resistance to polymyxins from animals to humans and other animals via pathogenic or commensal bacteria capable of transferring resistance to human and animal pathogens.

In conclusion,

- Polymyxins are essential antimicrobials in both veterinary and human medicine, often used as last resort to treat serious, life-threatening infections.
- Although prevalence of resistance to colistin in animal isolates of *E. coli* and *Salmonella* spp. from food-producing animals is generally low on mandatory surveillance, there is evidence to support the transfer of resistance to polymyxins from animals to humans and other animals.

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to Polymyxins.

Criterion (c) – availability of other treatments for animals

EFSA noted high levels of resistance to first line antimicrobials (e.g. aminopenicillins, potentiated sulfonamides, tetracyclines) in pathogenic *E coli* from swine, horses, sheep, goats and calves, suggesting their limited efficacy against these infections in many EU countries [55, 108, 154, 204]. Alternatives to colistin for resistant *E. coli* are limited to other AMEG category B substances i.e. fluoroquinolones (not poultry laying eggs for human consumption), 3rd- and 4th-generation cephalosporins (not poultry), or, depending on resistance profile and disease/patient characteristics, aminoglycosides or aminopenicillin-BLI combinations [167, 205]. In pigs, vaccinations (sows or piglets) can be an effective way to reduce the occurrence of neonatal and post-weaning diarrhoea caused by *E. coli*; however, it is necessary to use the appropriate vaccine for the most prevalent ETEC pathotype on the farm and to ensure that the vaccine is administered at the optimal time, consequently vaccination may not be consistently effective. Other measures can be introduced to reduce the need for antibiotics to treat infections such as ETEC (e.g. later weaning, improved genetics, changes in nutrition, improved housing and biosecurity) [206-208]; however these changes take time to implement and cannot replace antibiotics when treatment is needed for acutely sick animals.

In the open call for data, it was reported that colistin has been used outside the marketing authorisation to treat salmonellosis in pigs and ruminants. Due to frequent MDR to first-line antibiotics (ASSuT), treatment of salmonellosis should be based on AST; options may include aminoglycosides, 3rd-generation cephalosporins, fluoroquinolones, florfenicol, amoxicillin-clavulanate or TMPS according to susceptibility [53, 158, 160]. This use is discussed in more detail in Step 2.

Alternatives to polymyxin B for topical treatment of otitis externa due to Gram-negative infections in companion animals are limited, especially for *Pseudomonas aeruginosa* which is often MDR. Fluoroquinolones or aminoglycosides may be options, but aminoglycosides are inactivated by purulent discharges and should be used with care due to potential ototoxicity. There are no topical treatments authorised for treatment of otitis media in the dog. Fluoroquinolones and gentamicin, along with non-antibiotic alternatives have been recommended [209, 210].

Criterion (d) – availability of other antimicrobial treatments for humans

In human medicine, alternative antibiotics for the treatment of MDR Gram-negative infections are very limited but include combinations with beta-lactamase inhibitors such as ceftazidime-avibactam, ceftolozane-tazobactam, imipenem-relebactam, meropenem-vaborbactam and cefiderocol, as well as novel tetracyclines or fosfomycin, but these may also have limitations to their use. In the case of MDR-*A. baumannii*, the only alternative is cefiderocol and the novel tetracyclines [112, 114, 211].

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

- Polymyxins are essential antimicrobials in both veterinary and human medicine, often used as last resort to treat serious, life-threatening infections. In humans, colistin is essential to treat patients

in ICU who are seriously ill due to healthcare-associated infections caused by MDR carbapenem resistant Gram-negative bacteria. There are few alternatives available for these patients.

- In animals, colistin is important for the treatment of serious *E. coli* infections, in particular ETEC, which are associated with high morbidity and mortality in neonatal and juvenile food-producing species including limited market species. Colistin is also used outside the marketing authorisation for the treatment of salmonellosis in pigs and small ruminants. There are high levels of resistance to first-line antimicrobials in pathogenic *E. coli* and *Salmonella* spp. from food-producing animals and alternatives are limited to other AMEG category B substances in many cases.
- Although prevalence of resistance to colistin in animal isolates of *E. coli* and *Salmonella* spp. is generally low on mandatory surveillance in food-producing species, resistance is both chromosomal and plasmid-borne, and there is evidence to support the transfer of resistance to polymyxins from animals to humans and other animals.
- The extent of use of polymyxins outside the marketing authorisation is unknown. Most uses reported to the open call for data related to use in minor species or for potentially unauthorised indications (e.g. salmonellosis).

Therefore, considering the points above relevant to criteria (b), (c) and (d), it should be considered if conditions or a prohibition should be placed on the use of Polymyxins outside the terms of the marketing authorisation.

Step 2. Considerations of conditions to be placed on use outside the terms of a marketing authorisation

Please refer to Section 3.1.2. of the main report for the general rationale behind the proposed conditions.

(i) Use for unauthorised indications

Condition proposed: *For those indications not included in the SPC of the concerned product, use must be based on target pathogen identification and antimicrobial susceptibility testing that demonstrates that polymyxins are likely to be effective and that antimicrobials from a lower AMEG category would not be effective, unless it can be justified that this is not possible.*

Rationale: See Section 3.1.2 (i) of the advice.

Condition proposed: *Formulations intended for oral group administration must not be used for treatment or metaphylaxis of Salmonella spp.*

Rationale: The primary mechanisms for controlling Salmonella in pigs in the EU are through elimination or control and reduction programmes [156], including use of vaccination and husbandry measures outlined above [157]. Despite these measures, Salmonella can be re-introduced onto the farm through contaminated feed and water or wildlife such as rodents, birds and foxes. Clinical salmonellosis infection in pigs is usually due to host-adapted *S.* Choleraesuis (causing septicaemia) or non-host adapted *S*. Typhimurium (enterocolitis). Ubiquitous serotypes such as *S*. Typhimurium and *S*. Enteritidis generally cause human infections, but serious systemic illness in humans due to *S*. Choleraesuis is rare. Use of antibiotics has been justified to reduce severity of signs and prevent suffering in individual animals but does not reduce the prevalence or duration of shedding by sick or recovered animals, hence it has been concluded that use of antimicrobials for *Salmonella* control (metaphylaxis) in pigs should be discouraged due to the public health risk and use should be limited to individuals with life-threating salmonellosis (bacteriemia with high fever, depression and dyspnoea) [158, 159].

Salmonella infection in cattle can manifest as haemorrhagic enteritis, endotoxaemia, septicaemia, pneumonia and abortions. Host-adapted *S*. Dublin is the most common serotype in cattle and rarely causes infections in humans; *S*. Typhimurium is the second most common serotype. Control programmes are also implemented in some EU countries. Antimicrobial treatment is controversial due to the public health risk and possibility that cattle infected by *S.* Dublin may become chronic sub-clinical carriers that maintain infection in the herd. However, faecal shedding is a lesser problem in calves. Antimicrobial use in calves has been justified in case of enteritic salmonellosis to prevent development of bacteraemia and multiple organ disease, in which case systemic antimicrobial treatment is always needed [53, 160].

In the EU, the principal control strategy for salmonella in poultry[11] is elimination and exclusion by testing and culling of infected flocks. National control programmes concern salmonella serotypes with public health significance (Regulation EC 2160/2003) and are directed at *S.* Enteritidis, *S*. Typhimurium and monophasic *S.* Typhimurium. Commission Regulation EC 1177/2006 states that antimicrobials shall not be used as part of national control programmes for salmonella in poultry, except under exceptional circumstances to prevent undue suffering and for the salvage of valuable genetic material in breeding flocks in order to establish salmonella-free flocks.

Salmonella infections in poultry with serotypes of **animal health relevance** (*S.* Pullorum causing pullorum disease, *S*. Gallinarum causing fowl typhoid, and *S.* Arizonae) have been reviewed by EFSA in the context of the Animal Health Law (Regulation EU 2016/429). The zoonotic potential for these avian-adapted *Salmonella* serotypes is very low. S*.* Arizonae is associated with turkeys and appears to have been eradicated from EU production; whereas *S.* Pullorum and *S.* Gallinarum occur sporadically in the EU. Clinical signs relate to septicaemia and morbidity and mortality are highly variable depending on bird and management factors. High mortality may be recorded in young chicks. For *S.* Gallinarum, vaccine strategies may be employed. Clinical disease can be suppressed but not eliminated by antibiotic treatment and there may be asymptomatic long-term carriage by recovered birds. S. Pullorum infection may be transmitted vertically. *S.* Gallinarum, in particular, can survive in favourable environments for protracted periods and outbreaks may occur recurrently. Antibiotic treatments have been found to reduce mortality, but not to eliminate infection from a treated flock. In addition, disease has serious economic impact. In the EU and elsewhere elimination and exclusion (eradication) is the principal control strategy for these diseases [212, 213].

In terms of public health risk, most concern relates to serovars of Salmonella that have been associated with human foodborne diseases outbreaks. In the EU, data for 2020 show that most outbreaks were due to *S*. Enteritidis (57.9%), *S.* Typhimurium, Monophasic *S*. Typhimurium, *S.* Infantis and *S.* Derby. *S.* Enteritidis was primarily linked to broilers and layers/eggs, S. Typhimurium to broilers and pig sources, Monophasic S. Typhimurium to pigs and broilers, *S.* Infantis to broilers and *S.* Derby to pigs and turkeys [59]. EFSA/ECDC monitoring data from 2019-2020 show overall high resistance levels to ampicillin, sulfonamides and tetracyclines (ASuT) in *Salmonella* spp. from human cases and moderate-very high ASuT resistance in *Salmonella* isolates from food-producing species in most member states, limiting first-line treatment options. A generally (very) low (excluding intrinsically resistant strains) but variable prevalence of colistin resistance was reported in *Salmonella* spp. from different food-producing animal species and countries [59]. It should be borne in mind that invasive salmonella infections in humans are treated by preference with 3rd-generation cephalosporins, fluoroquinolones or, in children, azithromycin; colistin is not routinely used for this infection.

[11] According to Reg 2016/429, Article 4(9), 'poultry' means birds that are reared or kept in captivity for (a) the production of meat, eggs for consumption, other products; (b) restocking supplies of game birds; (c) the purpose of breeding birds used for the types of production referred to in points (a) and (b).

In conclusion, there may be justification for antibiotic use to reduce severity of signs of salmonellosis and prevent suffering in individual animals with potentially life-threatening infection, excepting poultry, also considering that many member states do not have 'stamping out' policies for salmonellosis other than in poultry. Certain injectable formulations of colistin authorised in the EU include salmonellosis within the scope of authorised indications. However, it is proposed that colistin should be prohibited for treatment or metaphylaxis of *Salmonella* spp. by use in formulations intended for oral group administration.

Condition proposed: *Must not be used for the treatment or metaphylaxis of Salmonella spp. in poultry.*

Rationale: According to Regulation EC 1177/2006, antimicrobials shall not be used as part of national control programmes for zoonotic salmonella in poultry. In regard to treatment of *S.* Pullorum and *S.* Gallinarum, eradication should be the principal control strategy.

(ii) Use for unauthorised target species

Colistin is authorised in VMPs for use in all major terrestrial food-producing species and for major companion animal species. It is also authorised for use in various limited market species, e.g. goats, horses, rabbits, buffalo, homing pigeons.

Polymyxin B is authorised for use in cats, dogs and small rodents.

No conditions proposed.

Rationale: See Section 3.1.2.(ii) of this advice.

(iii) Administration by an unauthorised route or use of extemporaneous formulation

Authorised VMPs containing colistin are available for administration orally including group medication, via injection, intramammary and topical use.

Condition proposed*: Not for use in food-producing aquaculture.*

Rationale: Aquatic systems represent a potentially important setting (a 'hotspot') for driving emergence, release, transmission and persistence and spread of AMR bacteria and resistance genes, as noted in Section 3.1.2.(iii) of this report. Although EFSA does not monitor for antimicrobial resistance in aquaculture food production and information on colistin resistance in this sector is still limited, serious concerns have been raised regarding the potential for aquaculture as a reservoir for *mcr*-genes that are of relevance to human and animal health [24, 195, 214]. Taking these factors into account, as there has been no formal public health risk assessment for use of colistin VMPs in food-production aquaculture and considering the importance of this class in human medicine, it is proposed that use outside the marketing authorisation in this sector should not be allowed.

Condition proposed:
When the intended route of administration is outside that included in the SPC, and for extemporaneous formulations, then the product should be administered to individual animals, only.

Rationale: See Section 3.1.2 (iii) of the advice.

(iv) Use of a human medicinal product

Human formulations of colistin are authorised for administration via oral, injection and inhalational routes. In humans, colistimethate sodium is administered by nebulisation to treat MDR Gram-negative pulmonary infections, e.g. *Pseudomonas aeruginosa*, and in cystic fibrosis patients [167]. Information

could not be found regarding the use of colistin via this route of administration for veterinary indications in the EU.

Authorised VMPs and HMPs containing polymyxin B are available in topical formulations only (auricular, ocular, cutaneous use).

Condition proposed*: Human medicinal products should be administered to individual animals only.*

Rationale: See Section 3.1.2 (iv) of the advice.

(v) Use of a third country veterinary medicinal product

According to the Regulation, third country VMPs may only be used in the same species and for the same indication. No additional conditions proposed to those above.

Rationale: See Section 3.1.2 (v) of the advice.

Step 3. Consideration of Criteria (a) and (e) in view of proposed conditions to be placed on use outside the terms of a marketing authorisation

Criterion (a) – risk to animal health or public health if the antimicrobial is used in accordance with Articles 112, 113 and 114

SPCs recommend that colistin should not be used in horses, particularly foals, as a shift in the gastrointestinal microflora balance could lead to the development of antimicrobial associated colitis (Colitis X), typically associated with *Clostridium difficile*, which may be fatal. Use of polymyxin B topical ear preparations is contraindicated in case of perforated tympanic membrane. Nephrotoxicity and neurotoxicity are reported as being associated with overdosage in animals and humans. Target animal safety warnings in the SPCs of authorised VMPs should be followed. Consumer safety relating to use under Articles 113 and 114 is mitigated through the application of the statutory withdrawal period in accordance with Article 115.

Criterion (e) Impact on aquaculture and farming if the animal affected by the condition receives no treatment

Proposed condition	**Potential impact on aquaculture and farming if animal affected by the condition receives no treatment**
For those indications not included in the SPC of the product, use must be based on target pathogen identification and antimicrobial susceptibility testing that demonstrates that polymyxins are likely to be effective and that antimicrobials from a lower AMEG category would not be effective, unless it can be justified that this is not possible.	This condition does not preclude treatment. See Annex 1 of report for further discussion.
Formulations intended for oral group administration must not be used for treatment or metaphylaxis of Salmonella spp.	An EU baseline survey conducted by EFSA in 2008 [161] found *Salmonella Typhimurium* on approximately 6% of pig production and breeding holdings in the EU overall, with much lower prevalence of *S. Choleraesuis*. The findings of a systematic review of studies published between 2000 –

	2017 estimated a prevalence of Salmonellae in healthy cattle in Europe of 2% [158, 162, 163]. However, prevalence of Salmonellae on farm or in healthy animals at slaughter does not give a full picture of the prevalence of outbreaks of clinical disease, for which evidence is difficult to find for the EU. Salmonellae are often resistant to many first-line antibiotics used in food-producing animals (ASuT resistance pattern); and second-line treatment options may be limited e.g. aminoglycosides, florfenicol, fluoroquinolones. Abortions, septicaemia, meningitis, encephalitis and death are potential sequelae to infection. Lack of effective antibiotics for group administration for treatment and metaphylaxis may result in more rapid spread of disease in the herd and hence higher morbidity and mortality. The proposed conditions do not prevent treatment of individual animals in order to protect animal welfare. In the longer term, outbreaks can be minimised by if attention to biosecurity, husbandry and use of vaccination where available; however, eradication is not always feasible. [53, 158]
Must not be used for the treatment or metaphylaxis of *Salmonella* spp. in poultry.	According to Regulation EC 1177/2006, antimicrobials shall not be used as part of national control programmes for zoonotic salmonella in poultry. In regard to *S.* Pullorum and *S.* Gallinarum, eradication should be the principal control strategy; therefore, a legal restriction on such use is unlikely to have a significant impact on poultry farming.
Not for use in food-producing aquaculture	No evidence was found for the use of polymyxins in food-production aquaculture in the EU; therefore, although impacts on aquaculture cannot be fully foreseen, they are not expected to be significant under current circumstances.
When the intended route of administration is not included in the SPC, or when using an extemporaneous formulation, then the product should be administered to individual animals, only.	There is little or no evidence supporting the effectiveness or need for alternative routes of administration in relation to use of colistin in food-producing animals. Therefore, although the impact on farming of restriction to individual animal use cannot be foreseen, it is not expected to be significant.

Step 4. Final conclusion - recommendations made for conditions to be placed on use outside the terms of a marketing authorisation

Conditions do not apply to use of polymyxin B for systemic treatment for endotoxaemia associated with severe colic and other gastrointestinal diseases in equines.

- For those indications not included in the SPC of the concerned product, use must be based on target pathogen identification and antimicrobial susceptibility testing that demonstrates that

polymyxins are likely to be effective and that antimicrobials from a lower AMEG category would not be effective, unless it can be justified that this is not possible.

- Formulations intended for oral group administration must not be used for treatment or metaphylaxis of *Salmonella* spp.
- Must not be used for the treatment or metaphylaxis of *Salmonella* spp. in poultry.
- Not for use in food-producing aquaculture.
- When the intended route of administration is outside that included in the SPC or when using an extemporaneous formulation, the product should be administered to individual animals, only.
- Human medicinal products should be administered to individual animals only.

4.9. Cyclic polypeptides

4.9.1. Background information

Examples of substances in the class that are authorised in veterinary and human medicine in the EU

Examples of substances authorised for veterinary use	Examples of ATCvet codes
Bacitracin	QD06AX05 QJ01XX10 QR02AB04 QA07AA93
Examples of substances authorised for human use	**Examples of ATC codes**
Bacitracin	D06AX05 J01XX10 R02AB04

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

Substance	Species	MRL tissues	MRL milk	MRL eggs	Other provisions
Bacitracin	bovine	No MRL required	Yes	-	For intramammary use in lactating cows only
	rabbit	Yes	-	-	-

EU-authorised VMP formulations, based on sales reported to ESVAC

Species		Route of administration						
		Group		Individual				
		In-feed	In-water	Injection	Oral e.g. tablet, paste, powder	Topical/local (incl. intrauterine)	Intra-mammary	Oral powder
Major	**Cattle**						BAC	
	Sheep (for meat)							
	Pigs							
	Chickens							
	Dogs					BAC		
	Cats					BAC		
Limited market species As listed in SPCs	**Rabbits**	BAC	BAC					

BAC (bacitracin)

Summary of main indications and contra-indications for EU-authorised VMPs, based on selected SPCs

Main indications	Bacitracin is available in antimicrobial combination products for intramammary use in lactating cows to treat clinical and subclinical mastitis due to *Staphylococcus aureus, Strep. dysgalactiae, S. uberis* and *E. coli.* It is authorised in antimicrobial combination products for topical treatment in dogs and cats of otitis externa caused by susceptible bacteria. Bacitracin is available in oral drinking water formulation and as a premix for meat-producing rabbits, for prophylaxis and reduction of symptoms and mortality due to epizootic enterocolitis associated with infections by *Clostridium perfringens.* In dogs, cats and cattle, bacitracin is available for cutaneous use to treat topical and superficial skin infections caused by Gram-positive and Gram-negative bacteria e.g. impetigo, paromychia, furunculosis, infected wounds and eczema, and for prophylaxis of wounds and burns.
Contraindications	Hypersensitivity to Bacitracin

Examples of EU-authorised HMP formulations, from Article 57 database

Substance	Route of administration

	Injection	Oral e.g. tablet, liquid	Topical/local
Bacitracin		x	x

Existing recommendations

WOAH recommendations

Polypeptides (including bacitracin) are categorised VHIA by WOAH (formerly OIE). *Specific comments:* Bacitracin is used in the treatment of necrotic enteritis in poultry.

WHO classifications

WHO: IA

- (C1: No)
- (C2: No)

WHO AWaRe: -

AMEG and CVMP recommendations

Cyclic polypeptides are included in the AMEG Category D. There are alternative treatments in human and veterinary medicine for their indications and that do not select for resistance to Category A substances through specific multiresistance genes.

These antibiotics are not devoid of negative impact on resistance development and spread. To keep the risk from use of these antibiotic classes as low as possible it is important that responsible use principles are complied with in everyday practice. Unnecessary use and unnecessarily long treatment periods should be avoided and group treatment restricted to situations where individual treatment is not feasible.

Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Disclaimer: The information in this section reflects reported use of antimicrobials outside the terms of a marketing authorisation. No evaluation is made in this section by the working group on the efficacy or safety of the reported uses, or on their potential impact on development and dissemination of AMR.

Information from published sources

It is reported that bacitracin has been used to treat ulcerative keratitis in horses; although poor susceptibility was reported in *Strep. equi* ssp. *zooepidemicus* [215].

In textbooks, it is mentioned that bacitracin has been used to treat clostridial diseases in poultry (e.g. necrotic enteritis); however, this may relate to use in third countries [36].

Information from the open call for data on use of antimicrobials in animals

Reported use of cyclic polypeptides outside the marketing authorisation			Alternatives	Consequences of unavailability
Substance	Species	Indication		
Bacitracin (in combination with other antibioitics)	Dogs, cats, horses	Eye infections only susceptible to these antibiotics Ulcerative keratitis in horses	-	Health and welfare Alternatives less effective

4.9.2. Evaluation

Scope of permitted use according to the MRL Regulation

Bacitracin is included in Table 1 (allowed substances) of the Annex to Regulation (EU) 37/2010 for rabbits and bovines and hence can be used in all food-producing species in accordance with Articles 113 and 114 of Regulation (EU) 2019/6. 'Other provisions' restrict the use in bovines to 'intramammary use in lactating cows only' but no provisions are given relating to use in rabbits.

Bacitracin can be used in non-food-producing species in accordance with Article 112.

Examples of veterinary-authorised formulations/species

Bacitracin is authorised in combination with other antimicrobials as an intramammary product and a product for cutaneous administration in bovines. It is also authorised as formulations for drinking water and in-feed administration in rabbits.

There are no veterinary medicines containing bacitracin that are authorised for use in aquaculture in the EU.

For dogs and cats, bacitracin is available as local formulations for aural and cutaneous use.

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Importance for human health

Bacitracin interferes with bacterial cell wall formation by inhibiting peptidoglycan synthesis, the major cell wall component in Gram-positive bacteria. Additionally, bacitracin has an ability to degrade nucleic acid and is particularly active against RNA [216].

Bacitracin is active against most Gram-positive bacteria, particularly *Staphylococcus aureus* and *Streptococcus pyogenes*, *Corynebacterium diphtheriae* and *Clostridioides difficile*, but susceptibility of *Enterococcus* species is variable. Among Gram-negative bacteria, bacitracin shows activity against *Neisseria* (meningococci and gonococci), *Treponema pallidum*, and *Haemophilus influenzae* [216].

Bacitracin is highly nephrotoxic when administered parenterally; hence it is mainly used topically and can be found as a compound in many over-the-counter products indicated for wound care. It is frequently used in combination with neomycin and polymyxin B or with corticosteroids [216].

Bacitracin is nationally approved in some EU Member States, often as a combination for topical use. Approved indications include primary infected dermatoses, such as impetigo, bacterial otitis externa, ecthyma, folliculitis and paronychia; in secondarily infected dermatoses, such as infected eczema, secondarily infected lesions of infestations (e.g., scabies) and secondary bacterial infection accompanying viral infections.

Importance for animal health

Bacitracin is authorised in combination antibiotic products for intramammary use in lactating cows to treat clinical and subclinical mastitis due to *Staphylococcus aureus*, *Streptococcus dysgalactiae* and *Streptococcus uberis*.

Bacitracin is available in oral drinking water formulation and as a premix for in-feed administration to meat-producing rabbits, for prophylaxis and reduction of symptoms and mortality due to epizootic enterocolitis associated with infections due to *Clostridium perfringens*.

In dogs and cats, bacitracin is available in antimicrobial combination products for topical treatment of otitis externa caused by 'susceptible bacteria' and for cutaneous administration to treat a variety of superficial skin infections, furunculosis, infected wounds and for prophylaxis of wounds and burns.

Published literature suggests that bacitracin has been used outside the terms of the marketing authorisation for treatment of ulcerative keratitis in horses and use was also reported in dogs and cats to the open call for data. Use of bacitracin for treatment of clostridial infections in poultry is noted in textbooks, although these reports may relate to use in third countries.

Development and selection of resistance

A number of mechanisms of bacitracin resistance have been reported in bacteria [217]. In the bacitracin-producing organism *B. licheniformis*, the *bcr*ABC genes encode a putative heterodimeric ATP-binding cassette (ABC) transporter that has been proposed to mediate the active efflux of bacitracin. Homologues of this transporter have been identified in *Bacillus subtilis* and *Streptococcus mutans*. A second recognized mechanism of bacitracin resistance is the overproduction of undecaprenol kinase. This enzyme converts undecaprenol to undecaprenyl-phosphate (UP), increasing the amount of lipid carrier present in the cell. It is proposed that up-regulation of this enzyme increases the levels of UP, thus overcoming the sequestration of UPP by bacitracin and increasing the resistance of the organism to bacitracin. Other reported mechanisms of bacitracin resistance are proposed to be mediated by a membrane-associated phospholipid phosphatase in *B. subtilis*. In *S. mutans*, it has been shown that inactivation of the *rgp*A gene, which is involved in glucose-rhamnose polysaccharide formation in the cell wall, results in increased bacitracin sensitivity [67].

MCR-1 confers cross-resistance to bacitracin, usage of bacitracin in food animals may serve as a non-colistin usage risk factor for the transmissible colistin resistance [218].

Bacitracin resistance in *Clostridium* spp. seems to be rare [219, 220].

Transmission of resistance

The *bcr*ABD operon located on plasmids in *C. perfringens* and *E. faecalis* is part of a MDR encoding conjugative plasmid associated with high-level resistance to bacitracin in *E. faecalis* in chickens. *E. faecalis* isolates in humans and chickens have shown to have homology and thus point to zoonotic potential.

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to Cyclic polypeptides.

Criterion (c) – availability of other treatments for animals

Cyclic polypeptides are in the AMEG's Category D and there are alternative antimicrobials available dependent on the specific disease, pathogen and target animal species under treatment. Alternative Category D antibiotics for treatment of Gram-positive bacteria include penicillins for the treatment of mastitis in cattle and fusidic acid for otitis externa and cutaneous infections in cats and dogs. For treatment of epizootic enterocolitis in rabbits, alternatives are Category C antibiotics (e.g. pleuromutilins).

Criterion (d) – availability of other antimicrobial treatments for humans

There are many alternative treatment options for treatment of all infections for which bacitracin is approved/used.

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

- Due to nephrotoxicity, bacitracin is mostly used topically, for otitis and superficial skin infections caused by Gram-positive bacteria in humans.
- In food-producing animals, bacitracin is used to treat intramammary infections in cattle and epizootic enterocolitis in rabbits. There are alternatives to bacitracin available for the given infections in both human and veterinary medicine.
- There is a potential pathway for transmission of resistance to bacitracin between animals in target pathogens and from animals to humans in commensal bacteria.
- Human formulations are reported as used outside the marketing authorisation for topical treatment of eye infections in companion animals; the extent of exposure to bacitracin related to this use is expected to be low.

Therefore, considering the points above relevant to criteria (b), (c) and (d), it is recommended that no conditions should be placed on the use of Cyclic polypeptides outside the terms of the marketing authorisation, although responsible antimicrobial use principles should be applied.

4.10. Pleuromutilins

4.10.1. Background information

Examples of substances in the class that are authorised in veterinary and human medicine in the EU

Examples of substances authorised for veterinary use	Examples of ATCvet codes
Tiamulin	QJ01XQ01
Valnemulin	QJ01XQ02
Examples of substances authorised for human use	**Examples of ATC codes**
Lefamulin	J01XX12

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

Substance	Species	MRL tissues	MRL milk	MRL eggs	Other provisions
Tiamulin	Porcine, chicken, turkey, rabbit	Yes	-	Yes (chicken only)	-
Valnemulin	Porcine, rabbit	Yes	-	-	-

EU-authorised VMP formulations, based on sales reported to ESVAC

Species		Route of administration						
		Group		Individual				
		In-feed	In-water	Injection	Oral e.g. tablet, paste, powder	Topical/local (incl. intrauterine)	Intra-mammary	Oral powder
Major	**Cattle**							
	Sheep (for meat)							
	Pigs	TIA VAL	TIA	TIA				TIA, VAL
	Chickens	TIA	TIA					TIA
	Dogs							
	Cats							
Limited market species As listed in SPCs	**Rabbits**	TIA, VAL	TIA					
	Turkeys	TIA	TIA					
	Racing pigeons		TIA		TIA			TIA

TIA (tiamulin), VAL (valnemulin)

Summary of main indications and contra-indications for EU-authorised VMPs, based on selected SPCs

Main indications	Pleuromutilins are authorised for oral administration and are indicated in pigs for enteric diseases: swine dysentery (*Brachyspira hyodysenteriae*), porcine colitis (*Brachyspira pilosicoli*), porcine proliferative enteropathy (ileitis) (*Lawsonia intracellularis*); for enzootic pneumonia (*Mycoplasma hyopneumoniae), Pasteurella multocida* and pleuropneumonia (*Actinobacillus pleuropneumoniae*). In rabbits, tiamulin and valnemulin are authorised for epizootic rabbit enterocolitis (ERE). In chickens tiamulin is authorised for Chronic Respiratory Disease caused by *Mycoplasma gallisepticum* and airsacculitis and infectious synovitis caused by *Mycoplasma synoviae*. In turkeys, tiamulin is authorised for infectious sinusitis and airsacculitis caused by *Mycoplasma gallisepticum, Mycoplasma synoviae* and *Mycoplasma meleagridis*. Tiamulin is also available for oral use in non-food pigeons to treat respiratory disease, hepatic infections, mycoplasmosis.

Contraindications	Pleuromutilins should not be administered in conjunction with ionophores.

Examples of EU-authorised HMP formulations, from Article 57 database

Substance	Route of administration		
	Injection	Oral e.g. tablet, liquid	Topical/local
Lefamulin	x	x	

Existing recommendations

WOAH recommendations

Pleuromutilins are categorised VHIA by WOAH (formerly OIE). *Specific comments:* The class of pleuromutilins is essential against respiratory infections in pigs and poultry. This class is also essential against swine dysentery (*Brachyspira hyodysenteriae*) however it is only available in a few countries, resulting in an overall classification of VHIA.

WHO classifications

WHO: IA

- (C1: No)
- (C2: No) To date pleuromutilins have only been used as topical therapy in people. There has to date been no transmission of resistance in *S. aureus*, including MRSA, from nonhuman sources.

WHO AWaRe: Lefamulin in the Reserve group

AMEG and CVMP recommendations

Pleuromutilins are included in the AMEG Category C: this category includes antibiotics for which there are alternatives in human medicine for their indications but which comply with one or both of the following criteria:

- For the veterinary indication under treatment, there are few or no alternatives belonging to Category D. Some examples of these indications are given in Table 4 of the AMEG advice [8], alongside the relevant (sub)class.
- The antibiotic selects for resistance to a substance in Category A through specific multiresistance genes.

Antibiotics placed in this category present a higher AMR risk for human and/or animal health than antibiotics placed in Category D. These antibiotics should only be used when there is no available substance in Category D that would be clinically effective.

Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Disclaimer: The information in this section reflects reported use of antimicrobials outside the terms of a marketing authorisation. No evaluation is made in this section by the working group on the efficacy or safety of the reported uses, or on their potential impact on development and dissemination of AMR.

Information from published sources

Pleuromutilins are not authorised for use in ruminants; however, considering the difficulty in controlling *M. bovis* in cattle and increasing resistance to authorised antibiotics, there are a few reports relating to use of pleuromutilins in calves to treat this disease [221, 222]. Tiamulin is also reported as used to treat joint infections in kids due to *Mycoplasma mycoides* [223].

Avian intestinal spirochaetosis (AIS) is a disease of poultry caused by various *Brachyspira* spp. occurring in breeder and layer flocks. There are no VMPs approved for this indication, although there is some published evidence to support effectiveness of pleuromutilins [36].

Information from the open call for data on use of antimicrobials in animals

The information below is summarised from the open call for data. Inclusion in the table does not endorse use or imply that it is consistent with use according to legislative provisions in Articles 112 to 114.

Substance	Species	Indication	Alternatives	Consequences of unavailability
Tiamulin	Pigs	*Mycoplasma suis, Mycoplasma hyorhinis*	Tetracyclines	
Tiamulin	Pheasants, partridges	Mycoplasma, Motile protozoal infections, Bacterial infections, Dysbacteriosis	Tylosin, doxycycline	
Valnemulin/tiamulin (in drinking water)	Rabbit	Epizootic rabbit enteropathy, colitis		

4.10.2. Evaluation

Scope of permitted use according to the MRL Regulation

Tiamulin and valnemulin are included in Table 1 (allowed substances) of the Annex to Regulation (EU) 37/2010 and hence can be used in all food-producing species in accordance with Articles 113 and 114 of Regulation (EU) 2019/6. There are no 'Other provisions' that would be important for use outside the terms of the marketing authorisation.

Pleuromutilins can be used in non-food-producing species in accordance with Article 112.

Examples of veterinary-authorised formulations/species

Tiamulin is available in in-water and in-feed formulations for group administration to pigs, rabbits and poultry. It is also available in injectable (pigs) and oral powder formulations for individual animal treatment. Tiamulin is authorised in formulations for use in racing pigeons.

Valnemulin is authorised in in-feed formulation for groups of pigs and rabbits, and as an oral powder for treatment of individual pigs.

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Importance for human health

Pleuromutilins are active against Gram-positive (*Staphylococcus* spp. – including MRSA and VRSA, and *Streptococcus* spp. including MDR strains) and fastidious Gram-negative bacteria (e.g., *Haemophilus spp., Moraxella catarrhalis, Neisseria* spp., *Legionella pneumophila*) as well as against *Mycoplasma* and *Chlamydia* spp. [224, 225].

Lefamulin is a relatively recently approved pleuromutilin for the treatment of community acquired pneumonia (CAP) in adults when it is considered inappropriate to use antibacterial agents that are commonly recommended for the initial treatment of CAP or when these have failed [226].

Retapamulin was approved by EMA in 2007 as a topical agent to treat impetigo and infected small lacerations, abrasion or sutured wounds caused by *Staphylococcus aureus* and *Streptococcus pyogenes*. The marketing authorisation in the EU has been withdrawn [227].

Pleuromutilins did not meet Criterion A (high human importance) for the Reserved List as there are other alternatives to treat CAP.

Importance for animal health

Pleuromutilins are authorised for individual and group oral administration in pigs. They are used to treat enteric diseases, porcine colitis (*Brachyspira pilosicoli*), porcine proliferative enteropathy (ileitis) (*Lawsonia intracellularis*), and are of particular importance for treatment of swine dysentery (*Brachyspira hyodysenteriae*) (see (c), below). Pleuromutilins are also used to treat respiratory infections: enzootic pneumonia (*Mycoplasma hyopneumoniae*), *Pasteurella multocida* and pleuropneumonia (*Actinobacillus pleuropneumoniae*).

In rabbits, tiamulin and valnemulin are authorised for group oral administration for epizootic rabbit enterocolitis (ERE).

In chickens tiamulin is authorised for group oral administration for Chronic Respiratory Disease caused by *Mycoplasma gallisepticum* and airsacculitis and infectious synovitis caused by *Mycoplasma synoviae*. In turkeys, tiamulin is authorised for infectious sinusitis and airsacculitis caused by *Mycoplasma gallisepticum, Mycoplasma synoviae* and *Mycoplasma meleagridis*.

There are no VMPs approved for treatment of avian intestinal spirochaetosis (AIS), a disease of poultry caused by various *Brachyspira* spp. occurring in breeder and layer flocks; however, there is some published evidence to support effectiveness of pleuromutilins [36].

Pleuromutilins are not authorised for use in ruminants but studies have been published investigating use in calves to treat *Mycoplasma bovis* [221, 222]. Tiamulin is also reported as used to treat joint infections in kids due to *Mycoplasma mycoides mycoides* [223].

According to the Open call for data, tiamulin may be used in pigs to treat *Mycoplasma* species that are not included in authorised indications (*M. hyorhinins, M. suis*). There were also reports of use to treat various infections in gamebirds, which are unauthorised species.

Tiamulin is authorised for administration to non-food-producing pigeons to treat respiratory disease, hepatic infections, mycoplasmosis.

Pleuromutilins have not been authorised for use in companion animals in the EU, and no evidence was found for use in these species.

Development and selection of resistance

Pleuromutilins have shown low potential for resistance development and little cross-resistance with other classes despite more than 30 years of use in veterinary medicine [224, 228]. The primary resistance mechanisms are related to chromosomal mutations in the 23S rRNA genes and in *rpl* genes encoding the large ribosomal proteins L3 and L4. Mutations in 23S rRNA confer resistance in staphylococci, *Brachyspira* and *Mycoplasma* spp. Mutations in *rpl* have been described in *Staphylococcus* spp. Multiple mutations are needed to achieve high-level resistance. A new resistance gene, *tva(A)*, has recently been identified in *B. hyodysenteriae* [229]. This gene reduces susceptibility and does not confer clinical resistance but facilitates higher level resistance via mutations in genes encoding ribosome associated functions.

Other mechanisms include acquisition of certain *vga* genes and *cfr* genes, located on plasmids or transposons. *Vga* encode ABC-F transporters also confer resistance to streptogramin A and lincosamides in *Staphylococcus* spp. (including LA-MRSA), *Enterococus faecium* and *Erysipelothrix rhusiopathae* and have been detected in isolates from pigs and other species. Similar plasmids carrying *vga* genes have been identified in porcine and human MRSA (Kadlec 2010). The *cfr* gene encodes for an rRNA methylase that confers the PhLOPSA resistance pattern (phenicols, lincosamides, oxazolidinones, pleuromutilins and streptogramin A). It is mainly located on plasmids in staphylococci and has been detected in (LA)MRSA from pigs in the EU and in a human hospital outbreak of MRSA in Spain [28, 59, 230, 231]. C*fr*-mediated resistance also been identifed in enterococci from humans and animals. *Vga, cfr* and *lsa* genes have also been detected in staphylococcal isolates from pet dogs and cats [232, 233]. Plasmids carrying *vga* and *cfr* genes have also been found to carry resistance genes to other antimicrobial classes (e.g. *erm, tet, aaD, dfr*). Hence co-selection for these MDR genes may also occur due to the more frequent use of other antibiotic classes in veterinary medicine e.g. macrolides, tetracyclines, trimethoprim [228].

Pleuromutilins are not included in the antimicrobial panel for AMR monitoring under mandatory EFSA/ECDC surveillance of resistance in zoonotic and indicator bacteria from food-producing animals. Monitoring of MRSA is voluntary and data are provided by few member states; as yet linezolid resistance has only been detected sporadically in isolates from pigs and further surveillance is needed to determine the true prevalence [28].

Decreased susceptibility of *Brachyspira hyodysenteriae* and to lesser extent *B. pilosicoli* to pleuromutilins has been reported in several EU countries but resistance develops slowly [228, 234-236]. No data are available regarding the occurrence of resistance to pleuromutilins in *L. intracellularis*. Due to difficulties associated with their culture, there is a lack of standardised testing and breakpoints for these pathogens. *Mycoplasma* spp. from pigs and poultry have largely retained high susceptibility to pleuromutilins in vitro; although there has been some reduction in the susceptibility of *M. hyopneumoniae* [34, 237].

Transmission of resistance

Transfer of plasmids carrying *vga* and *cfr* genes from animal bacteria e.g. enterococci, (LA)MRSA, to human commensals and pathogens is of concern, especially in regard to *cfr* since this gene also confers resistance to linezolid, a human antibiotic of last resort. The prevalence of *cfr* in animal isolates from the EU is not clear but appears to be at a low level at present. A recent risk assessment [238] estimated the risk to general public health due to use of pleuromutilins in pigs in Denmark as low in relation to both LA-MRSA and enterococci.

In conclusion,

Currently, lefamulin is the only pleuromutilin that is authorised for use in human medicine in the EU, for the treatment of CAP when more commonly recommended antibiotics are inappropriate or have failed (SPC Xenlata). Pleuromutilins are more important in veterinary medicine, in particular for treatment of swine dysentery and other enteric infections in pigs, and to treat diseases caused by mycoplasmas in pigs and poultry.

Decreased susceptibility to pleuromutilins in *B. hyodysenteriae* and *Mycoplasma* spp. is caused by chromosomal mutations and, where reported, has developed slowly. Of concern to both public and animal health is emergence of horizontally-transferable MDR-genes (*vga, cfr*) in staphylococci (including MRSA) and enterococci, which may be transmitted from animals to humans and other animals. Based on limited surveillance, *cfr* genes, which confer resistance to linezolid, appear to be at

low prevalence in the EU, although globally widespread, at present. Co-selection for pleuromutilin resistance may occur due to the use of various different antimicrobial classes.

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to Pleuromutilins.

Criterion (c) – availability of other treatments for animals

Effective alternatives are authorised for most of the indications for pleuromutilins, except for swine dysentery. High levels of resistance to alternative antibiotics in *B. hyodysenteriae* means that pleuromutilins are often the only remaining effective treatment; coupled with a lack of effective commercial vaccines to control disease outbreaks, this has consequences for animal welfare and production.

Criterion (d) – availability of other antimicrobial treatments for humans

There are numbers of alternative treatment options recommended for treatment of CAP such as beta-lactams, macrolides, fluoroquinolones [239].

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

- The pleuromutilin lefamulin is authorised in human medicine in the EU for the treatment of community-acquired pneumonia cause by Gram-positive bacteria (*Streptococcus pneumoniae*, *S. aureus*), certain Gram-negative bacteria (*Haemophilus influenzae*, *Legionella pneumophila*) and other bacteria (*Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*). There are a number of alternative antimicrobial classes that are effective for this indication.
- In veterinary medicine, pleuromutilins are used predominantly in pigs and rabbits for treatment of enteric diseases and in pigs and poultry for infections caused by mycoplasmas. Pleuromutilins are particularly important for the treatment of swine dysentery, a disease with major impact on pig health and welfare for which pleuromutilins in many cases may be the only effective antibiotic treatment.
- In important veterinary pathogens (e.g. *Brachyspira hyodysenteriae*, *Mycoplasma* spp.) resistance develops slowly and has remained at low levels. Of concern to both public and animal health is emergence of horizontally-transferable MDR-genes (*vga, cfr*) in staphylococci (including MRSA) and enterococci, which may be transmitted from animals to humans and other animals. These genes confer resistance to pleuromutilins, lincosamides and streptogramin A, and in the case of *cfr*, also to phenicols and importantly to linezolid, the latter being a human antibiotic of last resort. Co-selection for pleuromutilin resistance may occur due to the use of various different antimicrobial classes in veterinary medicine.
- Pleuromutilins are included in the AMEG Category C, primarily considering their importance for the treatment of swine dysentery and the potential to select for the MDR *cfr* gene in (LA)MRSA. They are available in in formulations for individual and group (in-feed/water) administration for pigs, poultry and rabbits. No evidence was found for their use in companion animals and limited evidence for their use outside the terms of the marketing authorisations to treat different indications or food-producing animal species. Hence it seems unlikely that use outside the terms of the marketing authorisation would contribute substantially to the AMR risk to public and animal health beyond the risk relating to authorised use.

Therefore, considering the points above relevant to criteria (b), (c) and (d), it is recommended that no conditions should be placed on the use of Pleuromutilins outside the

terms of the marketing authorisation, although responsible antimicrobial use principles should be applied.

4.11. Macrolides

4.11.1. Background information

Examples of substances in the class that are authorised in veterinary and human medicine in the EU

Examples of substances authorised for veterinary use	Examples of ATCvet codes
Azithromycin	QJ01FA10 QS01AA26
Erythromycin	QJ01FA01
Gamithromycin	QJ01FA95
Spiramycin	QJ01FA02
Tildipirosin	QJ01FA96
Tilmicosin	QJ01FA91
Tulathromycin	QJ01FA94
Tylosin	QJ01FA90
Examples of substances authorised for human use	**Examples of ATC codes**
Azithromycin	J01FA10 S01AA26
Clarithromycin	J01FA09
Erythromycin	J01FA01
Josamycin	J01FA07
Midecamycin	J01FA03
Oleandomycin	J01FA05
Roxithromycin	J01FA06
Spiramycin	J01FA02

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

Substance	Species	MRL tissues	MRL milk	MRL eggs	Other provisions
Erythromycin,	All food-producing species	Yes	Yes	-	-
Gamithromycin	All ruminants, Porcine	Yes	-	-	Not for use in animals from which milk is produced for human consumption
Spiramycin	Bovine, porcine, chicken	Yes	Yes	-	Not for use in animals from which eggs are produced for human consumption.
Tildipirosin	Bovine, caprine, porcine	Yes	-	-	Not for use in animals from which milk is produced for human consumption.
Tilmicosin	All food-producing species	Yes	Yes	-	Not for use in animals from which eggs are produced for human consumption.
Tulathromycin	Bovine, ovine, caprine, porcine	Yes	-	-	Not for use in animals from which milk is produced for

					human consumption
Tylosin	All food-producing species	Yes	Yes	Yes	-
Tylvaosin	Porcine, poultry	Yes	-	Yes	-

EU-authorised VMP formulations, based on sales reported to ESVAC

Species		Route of administration						
		Group		Individual				
		In-feed	In-water	Injection	Oral powder	Oral e.g. tablet, paste	Topical/local (incl. intrauterine)	Intra-mammary
Major	**Cattle**	SPI, TYL	ERY, SPI, TLM, TYL	ERY, GAM, SPI, TLD, TLM, TUL, TYL				ERY, SPI, TYL
	Sheep (for meat)		TYL	ERY, GAM, TLM, TUL, TYL				
	Pigs	SPI, TLM, TYL, TYV	SPI, TLM, TYL, TYV	ERY, GAM, SPI, TLD, TUL, TYL	TLM, TYL, TYV			
	Chickens	ERY, SPI, TYL	ERY, SPI, TLM, TYL, TYV	ERY, TYL	TYL	ERY, TYL		
	Dogs			ERY, TYL		SPI		
	Cats			TYL		SPI		
Limited market species	**Turkeys**	TYL	ERY, TLM, TYL, TYV	ERY	TYL	ERY, TYL		
	Poultry	TYL	ERY, SPI, TLM, TYL, TYV			ERY, TYL		
	Pheasants		TYV			TYL		
	Goats		TYL	ERY, TYL				
	Rabbits	TLM			TLM			
	Pigeons		ERY, SPI, TYL		ERY, SPI, TYL	ERY, SPI, TYL		
	Rodents		TYL					
	Ornamental birds		ERY, SPI, TYL		SPI	TYL		

ERY (erythromycin), GAM (gamithromycin), SPI (spiramycin), TLD (tildipirosin), TLM (tilmicosin), TUL (tulathromycin), TYL (tylosin), TYV (tylvalosin).

Summary of main indications and contra-indications for EU-authorised VMPs, based on selected SPCs

Main indications	*Cattle* Treatment and metaphylaxis of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni* and *Mycoplasma bovis.* Treatment of infectious bovine keratoconjunctivitis (IBK) associated with *Moraxella bovis*. Treatment of mastitis and metritis caused by Gram-positive organisms.

	Treatment of interdigital necrobacillosis due to *Fusobacterium necrophorum*. *Pigs* Treatment and metaphylaxis of swine respiratory disease (SRD) associated with *Actinobacillus pleuropneumoniae, Pasteurella multocida, Mycoplasma hyopneumoniae, M. hyorhinis, Haemophilus parasuis* and *Bordetella bronchiseptica*. Treatment of porcine proliferative enteropathy (ileitis) caused by *Lawsonia intracellularis*, swine dysentery, caused by *Brachyspira hyodysenteriae.* Treatment of erysipelas and metritis and arthritis due to *Mycoplasma* and *Staphylococcus* spp. *Sheep* Treatment of respiratory disease due to *Mannheimia haemolytica* and *Pasteurella multocida*. Treatment of infectious pododermatitis (foot rot) associated with virulent *Dichelobacter nodosus* and *Fusobacterium necrophorum* requiring systemic treatment. Treatment of acute ovine mastitis caused by *Staphylococcus aureus* and *Mycoplasma agalactiae* *Goats* Treatment of respiratory infections, metritis and mastitis caused by Gram-positive bacteria and *Mycoplasma* spp. *Chickens* Treatment and metaphylaxis of chronic respiratory disease due to *Mycoplasma gallisepticum* and *M. synoviae*. Treatment and metaphylaxis of necrotic enteritis caused by *Clostridium perfringens*. *Turkeys* Treatment and metaphylaxis of respiratory disease caused by *Mycoplasma gallisepticum*, *M. synoviae, Ornithobacterium rhinotracheale*. *Rabbits* Treatment and metaphylaxis of respiratory disease caused by *Pasteurella multocida* and *Bordetella bronchiseptica* *Pheasants* Treatment of respiratory disease associated with *Mycoplasma gallisepticum*. *Dogs and cats* For treatment of respiratory, enteritis and skin infections due to *Staphylococcus* and *Streptococcus* spp.
Contraindications	Macrolides may cause hepatoxicity and gastrointestinal irritation when administered orally. Tylosin and tilmicosin should not be used in horses due to risk of inflammation of the caecum. Tilmicosin displays variable cardiovascular toxicity according to animal species, but this risk is particularly important in human users.

Examples of EU-authorised HMP formulations, from Article 57 database

Substance	Route of administration		
	Injection	Oral e.g. tablet, liquid	Topical/local
Azithromycin	x	x	x
Clarithromycin	x	x	
Erythromycin	x	x	x
Josamycin		x	x
Midecamycin		x	
Oleandomycin			x
Roxithromycin		x	
Spiramycin	x	x	

Existing recommendations

WOAH recommendations

Macrolides are categorised VCIA by WOAH (formerly OIE). *Specific comments:* The wide range of applications and the nature of the diseases treated make macrolides extremely important for veterinary medicine. Macrolides are used to treat mycoplasma infections in pigs and poultry, haemorrhagic digestive disease in pigs (*Lawsonia intracellularis*) and liver abscesses (*Fusobacterium*

necrophorum) in cattle, where they have very few alternatives. This class is also used for respiratory infections in cattle.

WHO classifications

WHO: HPCIA (Macrolides and ketolides)

- (C1: Yes) Limited therapy for *Legionella*, *Campylobacter*, and MDR *Salmonella* spp. and *Shigella* infections.
- (C2: Yes) May result from transmission of *Campylobacter* spp. and *Salmonella* spp. from non-human sources.
- (P1: Yes) High absolute number of people affected by diseases for which the antimicrobial is the sole or one of few therapies available.
- (P2: Yes) High frequency of use in human medicine.
- (P3: Yes) Transmission of resistant *Campylobacter* spp. from non-human sources.

WHO AWaRe: Watch: e.g. Azithromycin, Clarithromycin, Erythromycin, Spiramycin

AMEG and CVMP recommendations

Macrolides are included in the AMEG Category C: this category includes antibiotics for which there are alternatives in human medicine for their indications but which comply with one or both of the following criteria:

- For the veterinary indication under treatment, there are few or no alternatives belonging to Category D. Some examples of these indications are given in Table 4 of the AMEG advice [8], alongside the relevant (sub)class.
- The antibiotic selects for resistance to a substance in Category A through specific multiresistance genes.

Antibiotics placed in this category present a higher AMR risk for human and/or animal health than antibiotics placed in Category D. These antibiotics should only be used when there is no available substance in Category D that would be clinically effective.

The CVMP review of the use of macrolides, lincosamides and streptogramins identified that of most concern to public health is emergence of resistance in *Campylobacter* spp. in poultry and pigs, although the outcome of public health risk assessments due to veterinary use is equivocal [240]. The reflection paper proposed that the duration of treatment with such products should be limited to the minimum time needed for cure of the disease. This was followed up by a referral for tylosin products administered orally to pigs, which restricted the treatment duration to three weeks and deleted the indication for swine dysentery (*Brachyspira hyodysenteriae*) due to concerns about high levels of resistance [241].

In 2019 a referral for VMPs containing tylosin administered parenterally for the treatment of mastitis in cows caused by *Mycoplasma* spp. considered that this indication had not been supported by (pre)clinical data and that ineffective treatment of *M. bovis* could lead to spread of the pathogen in the cattle herd, risking animal health and welfare. The indication was therefore removed from SPCs and a warning added in SPC 4.5 that efficacy data do not support the use of tylosin for the treatment of bovine mastitis caused by *Mycoplasma* spp.

Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Disclaimer: The information in this section reflects reported use of antimicrobials outside the terms of a marketing authorisation. No evaluation is made in this section by the working group on the efficacy or safety of the reported uses, or on their potential impact on development and dissemination of AMR.

Information from published sources

Evidence was found in published sources for use of macrolides to treat *Rhodococcus equi* infections in foals. Drugs of first choice for the treatment of *Rhodococcus equi* infection are the combination of macrolides (e.g. erythromycin and human-authorised azithromycin and clarithromycin) and rifampicin [242, 243], for a minimum of four weeks.

Macrolides are also part of recommended treatment (in combination with e.g. rifampicin and a fluoroquinolone) in cats and dogs for rare but serious life-threatening infections due to *Mycobacteria* spp. [244]. Erythromycin has been used in dogs for treatment of enteritis caused by *Campylobacter jejuni* [245].

According to textbooks, human-authorised macrolides e.g. azithromycin and clarithromycin have been used in dogs and cats to treat respiratory infections and atypical bacterial infections e.g. *Borrelia burgdorferi, Helicobacter spp., Chlamydophila felis*, although with variable efficacy. Azithromycin has also been used to treat the protozoan infection, cryptosporidiosis in, cats. Azithromycin has been used to treat *Lawsonia intracellularis* in horses. It is also used in treatment of exotic animals and birds. In dogs, tylosin has been used to treat 'antibiotic-responsive diarrhoea' [33, 46, 119, 125, 246].

Erythromycin and tylosin are included in the WSAVA List of Essential medicines for Cats and Dogs for treatment of severe *Campylobacter* infections and chronic enteric infections responsive to antimicrobial therapy [178].

There are reports of the use of erythromycin to treat bacterial kidney disease (*Remibactium salmoninarum*, BKD) in Atlantic salmon outside the EU (see below).

Information from the open call for data on use of antimicrobials in animals

The information below is summarised from the open call for data. Inclusion in the table does not endorse use or imply that it is consistent with use according to legislative provisions in Articles 112 to 114.

Substance	Species	Indication	Alternatives	Consequences of unavailability
Azithromycin	Rabbits	Pasteurellosis	marbofloxacine	
Azithromycin	Equine (foals)	*Rhodococcus equi* infection	plasma therapy in prevention and treatment, off-label gamithromycin, doxycyline	Alternatives less efficacious
Azithromycin, Clarithromycin	Equine (foals)	*Lawsonia intracellularis*		
Clarithromycin	Cat	Tuberculosis (cutaneous)	often used in combination with other human antibiotics	
Azithromycin	Cat	Tuberculosis (cutaneous)	often used in combination with other human antibiotics	
Azithromycin	Cat	Cryptosporidiosis	Tylosin	
Azithromycin	Dog	Babesiosis		
Tylosin	Mink	enteritis	no	
Azithromycin	Dogs, cats	Respiratory diseases, Babesiosis,	other macrolides	
Clarithromycin	Cetaceans	Mycobacteriosis	Depending on sensitivity	

4.11.2. Evaluation

Scope of permitted use according to the MRL Regulation

Various macrolides are included in Table 1 (allowed substances) of the Annex to Regulation (EU) 37/2010 and hence can be used in all food-producing species in accordance with Articles 113, 114 and 115 of Regulation (EU) 2019/6. 'Other provisions' restrict the use of certain macrolides either for animals producing eggs or milk for human consumption.

Macrolides can be used in non-food-producing species in accordance with Article 112.

Substances/indications in equines out of scope due to listing in Regulation (EC) 1950/2006, as amended by Regulation (EU) 122/2013

Use of azithromycin is listed for the treatment of *Rhodococcus equi* infections in equines.

Examples of veterinary-authorised formulations/species

Various macrolides are available for group administration in-water and/or in-feed to all major food-producing animals and some limited market species e.g. pheasants, goats, rabbits, pigeons, ornamental birds. They are also available for administration by injection for treatment of individual food-producing species and for intramammary administration to cattle.

There are no veterinary medicines containing macrolides that are authorised for use in aquaculture in the EU.

For dogs and cats, macrolides are available as injectable and oral formulations.

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Importance for human health

Macrolides are active against most Gram-positive bacteria (e.g., *Staphylococcus* spp., including beta-lactamase-producing strains, *Streptococcus* spp., *Enterococcus* spp., *Clostridium* spp.) but only selected Gram-negative organisms (e.g. *Neisseria gonorrhoeae*, *Helicobacter pylori* and *Campylobacter* spp., *Shigella* and *Salmonella* spp.) as well as several species responsible for intracellular infections, such as *Mycobacterium* spp., *Chlamydia* spp., *Mycoplasma pneumoniae*, and *Legionella* spp. [247-251].

Macrolides are among the most used classes of antibiotics in humans. They are used in the management of RTIs, acute bacterial sinusitis, acute bacterial otitis media, pharyngitis, tonsillitis, acute exacerbation of chronic bronchitis, mild to moderately severe CAP, uncomplicated chlamydia infections, urethritis, cervicitis, acute exacerbation of chronic bronchitis (adequately diagnosed), SSTIs, campylobacteriosis and *H. pylori* infections. Macrolides are an important treatment alternative for patients allergic to penicillin and cephalosporins.

Although there is a comparatively high prevalence of food-borne zoonotic *Campylobacter* spp. infections in humans, only serious cases need treatment and the proportion of fatalities is low. The increasing incidence of fluoroquinolone resistance in *Campylobacter* spp. has rendered macrolides such as erythromycin and azithromycin the antibiotics of choice for human campylobacteriosis and *H. pylori* infections. Resistance to macrolides in human *Campylobacter* spp. infections remains low in the EU, and they are first choice for oral treatment of 'at-risk' patients, e.g. children.

Importance for animal health

Food-producing species

Macrolides are authorised in VMPs in the EU, predominantly for use in food-producing animals for gastrointestinal and respiratory infections. They are used to treat *Mycoplasma* spp., primarily a cause of respiratory and synovial infections with high morbidity leading to important impacts on health and welfare in ruminants (*M. bovis*), pigs (*M. hyopneumoniae, M. hyorhinis*) and occurring sporadically in poultry (*M. gallispeticum, M. synoviae)* in the EU [252-254].

Macrolides are also used to treat other pathogens causing respiratory disease in cattle and sheep (*Mannheimia haemolytica, Pasteurella multocida, Histophilus somni*), swine (*Actinobacillus pleuropneumoniae, Pasteurella multocida, Haemophilus parasuis* and *Bordetella bronchiseptica*) and turkeys (*Ornithobacterium rhinotracheale*).

In pigs they are used to treat enteric diseases e.g. swine dysentery (*Brachyspira hyodysenteriae*) and porcine proliferative enteropathy (PPE) (*Lawsonia intracellularis*). PPE is of major importance in the swine industry and may manifest as proliferative haemorrhagic enteropathy with high mortality in acute outbreaks. The indication for swine dysentery has been removed from orally administered tylosin products due to concerns relating to resistance. Macrolides are also used for treatment of swine erysipelas (*Erysipelothrix rhusiopathae*).

In cattle and other ruminants, macrolides are used for a variety of infections e.g. metritis, mastitis, interdigital necrobacillosis (*Dichelobacter nodosus*, *Fusobacterium necrophorum*). They are also approved for treatment of infectious bovine keratoconjunctivitis (IBK) associated with *Moraxella bovis*.

In poultry, in addition to the indications for mycoplasma infections, they may be used for staphylococcal infections fowl cholera (*Pasteurella multocida*) and necrotic enteritis (*Clostridium perfringens*).

In rabbits, macrolides are used for respiratory disease (*Pasteurella multocida* and *Bordetella bronchiseptica)* and epizootic rabbit enteropathy (ERE).

Companion animals

In dogs, spiramycin is authorised, often in combination with metronidazole, for Gram-positive and anaerobic infections of the oral cavity and sinuses. Macrolides have few authorised uses in companion animals.

No macrolide VMPs authorised for use in horses or in aquaculture in the EU were identified.

Outside the terms of a marketing authorisation, macrolides (e.g. erythromycin, clarithromycin) are used in combination with rifampicin to treat cases of pneumonia in foals due to *Rhodococcus equi* when this is severe and life-threatening.

Macrolides are also part of recommended treatment (in combination with e.g. rifampicin and a fluoroquinolone) in cats and dogs for rare but serious life-threatening infections due to *Mycobacteria* spp. [244, 255].

Erythromycin may be used for treatment of enteritis due to *Campylobacter jejuni* in dogs, when treatment is warranted [256, 257].

Erythromycin has been reported to be used for treatment of bacterial kidney diseases (BKD) (*Renibacterium salmoninarum*) in Atlantic salmon and is used for this indication in Chile [258]. Due to the intracellular location and limited susceptibility of this pathogen, prolonged treatment is required

(28 to 30 days) and development of resistance has been reported [259]. Hence in the EU control is more often through eradication programmes; although this may also be challenging [259-261]. It is also reported that erythromycin has been used to treat various other aquaculture diseases in several regions outside the EU e.g. China, Philippines, Japan [262].

According to the Open call for data, macrolides are used outside the terms of the marketing for limited market animal species not included in the SPC e.g. mink, cetaceans. In addition, macrolides authorised only in human medicines e.g. azithromycin and clarithromycin are used to treat *Rhodococcus equi, Lawsonia intracellularis* and mycobacterial infections amongst other infections in companion animals, as noted above.

Development and selection of resistance

Mechanisms of resistance to macrolides include modification of the target site, drug inactivation and drug efflux. Resistance is conferred by chromosomal mutations as well as horizontal transfer of resistance genes. The most common mechanism is target site modification mediated by different rRNA methylases (*erm* genes), which confers resistance to macrolides, lincosamides and streptogramin B. *erm* genes have been identified on plasmids and transposons and are widely distributed in Gram-positive, Gram-negative and anaerobic bacteria from human and animal sources. Many efflux genes have been identified in Gram-positive and Gram-negative bacteria (e.g. *mef*A*, mef*E, *msr*), but not all confer resistance to 16-member ring macrolides. Enzymatic inactivation is a less common resistance mechanism (*mph, ere* genes) [263-265].

Monitoring under mandatory EFSA/ECDC surveillance shows that resistance to macrolides in *C. jejuni* from food-producing animals and humans remains low in Europe overall; but it is at moderate levels in *C. coli* and is higher in certain EU countries. Combined resistance to both ciprofloxacin and erythromycin in *C. jejuni/C.coli* from humans is very low/low. In *Salmonella* spp. and *E. coli* resistance to azithromycin is generally low [59]. Monitoring of MRSA is voluntary and data are provided by few member states. Most isolates are LA-MRSA. The prevalence ranges from 0% to 100% depending on animal production type and country. High levels of macrolide resistance have been reported in MRSA from pigs in Belgium, Portugal and calves from Belgium and Switzerland [59].

In the EU, *erm*(B) has been reported on plasmids and multidrug resistant islands in *C. coli* from poultry [266-268].

There is evidence for selection and spread of resistance to macrolides due to the use of these antimicrobials in food-producing animals. Long term, in particular low-dose use of macrolides selects for emergence of erythromycin resistant campylobacter in animal reservoirs [240, 263].

Recent EFSA Opinions indicate that there are generally low levels of resistance to macrolides in common respiratory pathogens from pigs and cattle in Europe [55, 108] (See Annex 3. EFSA Animal Health Law Scientific opinions). However, in regard to *Mycoplasma* spp., strains of *M. hyopneumoniae* and *M. hyosynoviae* with reduced susceptibility to macrolides have been detected in pigs, although this may not be reflected in loss of clinical efficacy, and high levels of resistance have been detected in *M. bovis* from cattle and *M. gallisepticum* from poultry [237, 269, 270].

In pigs, EFSA reported that high levels of resistance (based on ECOFF) to tylosin and tylvalosin, from 32 to 80%, have been observed in *Brachyspira hyodysenteriae* isolates [108]. CVMP has deleted the indication for treatment of swine dysentery from tylosin products administered orally to pigs.

EFSA does not monitor for antimicrobial resistance in aquaculture food production. Macrolide resistance has been reported in foodborne pathogens from aquatic food animals in Asia [116].

Transmission of resistance

Resistance to macrolides can be transferred from food-producing and companion animals to humans via zoonotic pathogens (*Campylobacter, Salmonella* spp., LA-MRSA, *Rhodococcus equi* and commensals [267, 271-274]. Most concern relates to poultry, which are the primary source of *C. jejuni* and campylobacter infection in humans [275]. A significant association has been shown between macrolide-resistance in *C. jejuni* isolates from poultry and from humans [89].

There is evidence for the selection and transmission of resistance to macrolides from animals to humans and other animals via zoonotic and target pathogens or commensal bacteria capable of transferring resistance to pathogens.

In conclusion,

- Macrolides are a highly important antibiotic class in human medicine, commonly used for various infections including upper and lower respiratory tract, STIs and soft tissue infections. They are first-choice for serious *Campylobacter* infections, especially in children. Macrolides are also authorised for a wide variety of infections in food-producing species, being very important for treatment of respiratory tract and mycoplasma infections in livestock and poultry species and for gastrointestinal diseases in poultry and pigs.
- Macrolides are used outside the terms of the marketing authorisation for limited market species and human-only authorised macrolides are used for unauthorised indications e.g. *Rhodococcus equi* in horses and mycobacterial infections in companion animals.
- EU surveillance shows generally low levels of resistance to macrolides in zoonotic pathogens (*Campylobacter* and *Salmonella* spp.) and *E. coli* from food-producing species. Resistance to macrolides is low in target respiratory pathogens from cattle and pigs, but increasing levels are detected in *Mycoplasma* spp. from pigs, poultry and cattle. Macrolide resistance can be transmitted from animals to humans and other animals via zoonotic and target pathogens or commensal bacteria capable of transferring resistance to pathogens.

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to Macrolides.

Criterion (c) – availability of other treatments for animals

Alternatives to macrolides for treatment of swine respiratory pathogens include amoxicillin(±clavulanate), amphenicols, pleuromutilins. Where mycoplasma are involved, tetracyclines or potentially fluoroquinolones may be used, although that latter are in AMEG Category B.

Alternatives to macrolides for *L. intracellularis* in swine are limited mostly to tetracyclines and pleuromutilins [33, 276, 277].

In cattle, alternatives for treatment of respiratory pathogens include amoxicillin(±clavulanate), amphenicols, TMPS and, according to susceptibility, tetracyclines. Alternative first-line antibiotics for mycoplasmas include tetracyclines; however, despite increasing resistance to both tetracyclines and macrolides [237], macrolides remain important for bovine respiratory disease and enzootic pneumonia in calves due to *Mycoplasma bovis* and complicated by secondary pathogens as amphenicols or fluoroquinolones may be the only alternatives [55, 278].

For interdigital necrobacillosis in cattle, amoxicillin, amphenicols, TMPS or tetracyclines may be alternatives from the same of lower AMEG category. In small ruminants, long-acting tetracyclines may be used to treat contagious footrot caused by *Dichelobacter nodosus*.

In poultry, alternatives for treatment of mycoplasma infections include tetracyclines, pleuromutilins, lincosamides or (AMEG Category B) fluoroquinolones. For necrotic enteritis, penicillins, aminoglycosides or spectinomycin are options.

For *Rhodococcus equi*, alternatives such as doxycycline have only been investigated for treatment of less severe cases [243, 272, 279, 280]. Based on either clinical trial results or published AST, alternative antibiotics for *Rhodococcus equi* include doxycycline, fluoroquinolones, and aminoglycosides.

Criterion (d) – availability of other antimicrobial treatments for humans

Fluoroquinolones are an alternative treatment option for RTIs (e.g. moxifloxacin for the treatment of moderately severe CAP) [281, 282]. Fluoroquinolones and tetracyclines are an alternative for treatment of campylobacteriosis. For severe cases or invasive infections, parenteral treatment is more likely: fluoroquinolones (if susceptible), aminoglycosides, carbapenems or TMPS [283, 284].

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

- Macrolides are a highly important antibiotic class in human medicine, commonly used for various infections including, respiratory tract and soft tissue infections and infections caused by intracellular pathogens. They are also very important for oral treatment of campylobacteriosis, especially in children; although alternative antibiotics, administered parenterally, are more likely to be used for severe infections. Although macrolides are one of limited alternatives for certain infections, they are not regarded as an antibiotic of 'last resort' for human use and were placed in the AMEG's Category C.
- Macrolides are authorised in all major food-producing species to treat a wide range of diseases, in particular respiratory and gastrointestinal infections and infections caused by mycoplasmas. Many of the indications are serious life-threatening infections with significant morbidity or mortality. For some indications, e.g. PPE (*Lawsonia intracellularis*) in pigs and complicated mycoplasma pneumonias, there are limited alternatives that would be equally effective; however, for most of the common indications alternatives from AMEG Categories C or D are available.
- There is evidence for the selection and transmission of resistance to macrolides from treated animals to humans and other animals. In relation to public health, most concern relates to the potential for transmission of macrolide-resistant *C. jejuni* isolates from poultry to humans; however, resistance generally remains at low levels. Regarding animal health, there are concerns about increasing resistance to macrolides in *Brachyspira* spp. in pigs and in mycoplasma in food-producing species.
- Macrolides are used widely in both human and veterinary medicine [89]. The uses identified outside the terms of a marketing authorisation relate to minor species or a few specific minor indications. Considering that macrolides are authorised for use in all major animal species and many limited market species, for administration by various routes including to groups of animals, and taking account of the broad indications authorised, it is considered unlikely that use in compliance with Articles 112 to 114 would contribute substantially to the AMR risk to public and animal health beyond the risk relating to authorised use.

Therefore, considering the points above relevant to criteria (b), (c) and (d), it is recommended that no conditions should be placed on the use of Macrolides outside the terms of the marketing authorisation, although responsible antimicrobial use principles should be applied.

4.12. Ketolides

4.12.1. Background information

Examples of substances in the class that are authorised in human medicine in the EU

Examples of substances authorised for human use	Examples of ATC codes
Telithromycin	J01FA15
Solithromycin	J01FA16

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

Ketolides are not included in Table 1 (allowed substances) of the Annex to the MRL Regulation (EU) 37/2010 and cannot be used in food-producing animals in the EU.

Examples of EU-authorised HMP formulations, from Article 57 database

No ketolides are authorised in HMPs in the EU at present.

Existing recommendations

WOAH recommendations

Ketolides are not classified by WOAH (formerly OIE).

WHO classifications

WHO: HPCIA (Macrolides and ketolides)

- (C1: Yes) Limited therapy for *Legionella*, *Campylobacter*, and MDR *Salmonella* spp. and *Shigella* infections.
- (C2: Yes) May result from transmission of *Campylobacter* spp. and *Salmonella* spp. from non-human sources.
- (P1: Yes) High absolute number of people affected by diseases for which the antimicrobial is the sole or one of few therapies available.
- (P2: Yes) High frequency of use in human medicine.
- (P3: Yes) Transmission of resistant *Campylobacter* spp. from nonhuman sources.

WHO AWaRe: Watch: Telithromycin, Solithromycin

AMEG recommendations

Ketolides are included in the AMEG Category A: these classes are not authorised in veterinary medicine but are authorised in human medicine in the EU. These antibiotic classes may only be used exceptionally in individual companion animals in compliance with the prescribing "cascade". Substances in these classes cannot be used for food-producing animals in the absence of established maximum residue limits.

AMEG noted that ketolides are important for treatment of *Streptococcus pneumoniae* infection in humans [8].

Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Disclaimer: The information in this section reflects reported use of antimicrobials outside the terms of a marketing authorisation. No evaluation is made in this section by the working group on the efficacy or safety of the reported uses, or on their potential impact on development and dissemination of AMR.

Information from published sources

Evidence supporting the use of, or specific need for, ketolides in animals is lacking.

Information from the open call for data on use of antimicrobials in animals

No information on use outside the terms of a marketing authorisation was provided in the open call for data.

4.12.2. Evaluation

Scope of permitted use according to the MRL Regulation

Ketolides are not included in the Annex to the MRL Regulation (EU) 37/2010 and cannot be used in food-producing animals in the EU.

Ketolides can be used in non-food-producing species in accordance with Article 112.

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Ketolides have been developed for the treatment of respiratory tract infections due to Gram-positive and Gram-negative bacteria causing CAP (*Streptococcus pneumoniae, Haemophilus spp., Moraxella* spp.), particularly those resistant to beta-lactams and macrolide antimicrobials. Telithromycin is active against atypical organisms such as *Chlamydia* spp., *Mycoplasma* spp. and *Legionella* spp. Ketolides are not active against Enterobacterales and *Pseudomonas aeruginosa* [250].

Safety and efficacy of telithromycin has been extensively studied in numerous trials involving several respiratory tract infections, including CAP, pharyngitis, sinusitis, acute exacerbations of chronic bronchitis and asthma [250]. Major adverse effects related to hepatotoxicity, gastrointestinal upsets, blurred/loss of vision and cardiac abnormalities (long QT syndrome).

Despite initial clinical promise, no ketolides are currently on the EU market due to the identified safety concerns. Telithromycin has been authorised for use in the EU but was withdrawn by the marketing authorisation holder in 2018. Considering the safety aspects, telithromycin is not needed for treatment of serious infections in humans at present; however, future development of this class cannot be excluded.

Importance for animal health

Ketolides are not authorised in VMPs in the EU and no evidence was found documenting their use in animals.

Development and selection of resistance

Similar mechanisms of resistance to macrolides have been described for ketolides. These include modification of the target, drug inactivation and drug efflux. The most common mechanisms are target site modification mediated by different rRNA methylases (*erm* genes), which also confers resistance to macrolides, lincosamides and streptogramin B, and drug efflux conferred via the *mef*A gene. Inducible resistance to telithromycin has been documented through the expression of *erm*(B) and *mef*A genes in

some *Staphylococcus* and *Streptococcus* spp. [285]. Whilst several studies have underpinned the importance of *erm*(B) and *mef*(A) genes in mediating telithromycin resistance, it is acknowledged that other mechanisms may additionally exist.

No large data sets specific to the prevalence of ketolide resistance in the EU were identified in the public domain. One published report documented that amongst a worldwide collection of 13,874 *S. pneumoniae* isolates from humans (isolated between 1999 and 2003), only 10 were resistant to telithromycin with MICs ≥ 4 µg/mL; all such isolates contained the *erm(B)* gene [286].

The *erm*(B) gene has been detected on plasmids in *Staphylococcus, Streptococcus* and *Enterococcus* spp. and multiple drug resistance islands in various *Campylobacter* spp., including at low prevalence in isolates from food-producing animals in the EU [230, 266, 287, 288].

Transmission of resistance

Although ketolides are not authorised in VMPs, there is evidence for the potential for selection and transfer of resistance between animals and from animals to humans through target and zoonotic pathogens and commensal bacteria if use in animals became established.

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to Ketolides.

Criterion (c) – availability of other treatments for animals

No use of ketolides in animals was identified either in publications or in the open call for data.

Criterion (d) – availability of other antimicrobial treatments for humans

Ketolides are not currently marketed in HMPs in the EU – alternatives are available in human medicine.

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

- Currently, there are no ketolides marketed in human medicines in the EU and, due to safety concerns and the availability of alternatives, they cannot be regarded as highly important to human health.
- No VMPs containing ketolides have been authorised in the EU and no authorised ketolide VMPs were found in third countries.
- Inducible resistance to telithromycin has been documented through the expression of *erm*(B) and *mef*A genes in some *Staphylococcus* and *Streptococcus* spp., similar to those expressed in certain erythromycin-resistant isolates. The *erm*(B) gene has been detected on plasmids in *Staphylococcus, Streptococcus* and *Enterococcus* spp. and multiple drug resistance islands in various *Campylobacter* spp., including at low prevalence in isolates from food-producing animals in the EU. Hence there is evidence for the potential for selection and transfer of resistance between animals and from animals to humans through zoonotic and commensal bacteria if use in animals became established.
- No evidence was found for use of ketolides in animals in the EU or third countries, and in the absence of MRL status and authorised medicines, ketolides could only be used as extemporaneous preparations in non-food-producing animals.

Therefore, considering the points above relevant to criteria (b), (c) and (d), it is recommended that no conditions should be placed on the use of Ketolides outside the terms of the marketing authorisation, although responsible antimicrobial use principles should be applied.

4.13. Lincosamides

4.13.1. Background information

Examples of substances in the class that are authorised in veterinary and human medicine in the EU

Examples of substances authorised for veterinary use	Examples of ATCvet codes
Clindamycin	QD10AF01 QG01AA10 QJ01FF01
Lincomycin	QJ01FF02 QJ51FF02
Pirlimycin	QJ51FF90
Lincomycin combinations with other antibacterial	QJ01FF52 QJ51RF03
Examples of substances authorised for human use	**Examples of ATC codes**
Clindamycin	D10AF01 G01AA10 J01FF01
Lincomycin	J01FF02

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

Substance	Species	MRL tissues	MRL milk	MRL eggs	Other provisions
lincomycin	All food-producing species	Yes	Yes	Yes	-
pirlimycin	bovine	Yes	Yes	-	-

EU-authorised VMP formulations, based on sales reported to ESVAC

Species		Route of administration						
		Group		Individual				
		In-feed	In-water	Injection	Oral Powder	Oral e.g. tablet, paste	Topical/local (incl. intrauterine)	Intra-mammary
Major	**Cattle**		LIN	LIN				LIN, PIR
	Sheep (for meat)			LIN				
	Pigs	LIN	LIN	LIN	LIN			
	Chickens	LIN	LIN	LIN				
	Dogs		CLI	LIN		CLI	CLI	
	Cats		CLI	LIN		CLI		
Limited market species As listed in SPCs	**Turkeys**	LIN	LIN	LIN				
	Poultry		LIN	LIN	LIN			
	Ducks	LIN	LIN					
	Geese	LIN	LIN					
	Pheasants		LIN					
	Guinea-fowls		LIN					
	Goats			LIN				
	Pigeons		LIN					

LIN (lincomycin), CLI (clindamycin), PIR (pirlimycin)

Summary of main indications and contra-indications for EU-authorised VMPs, based on selected SPCs

Main indications	Lincomycin is authorised for infections due to Gram-positive organisms (e.g. *Staphylococcus* spp. and *Streptococcus* spp.) and certain Gram-negative anaerobes (e.g. *Bacteroides*, *Fusobacterium* spp.) In cats and dogs lincomycin is authorised for respiratory infections, septicaemia, skin infections and abscesses.

	In pigs, it is authorised for treatment of swine dysentery (*Brachyspira hyodysenteriae*), PPE (*Lawsonia intracellularis*), enzootic pneumonia (*Mycoplasma hyopneumoniae*) and septic arthritis (including *M. hyosynoviae*). In chickens lincomycin is authorised for treatment of necrotic enteritis (*Clostridium perfringens*) and, in combination with spectinomycin, for treatment of chronic respiratory disease due to *Mycoplasma gallispeticum* and *E coli*. In cattle, intramammary preparations containing lincomycin or pirlimycin are authorised for mastitis due to Gram-positive cocci including *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae* and *Streptococcus uberis*. Clindamycin In dogs and cats, clindamycin is authorised for oral treatment of infected wounds, abscesses, oral and dental infections due to *Staphylococcus*, *Streptococcus*, *Bacteroides* and *Fusobacterium* spp., and *Clostridium perfringens*. In dogs it is also indicated for superficial pyoderma due to *Staph. pseudintermedius* and osteomyelitis due to *Staph. aureus*. A topical formulation of clindamycin is available to treat superficial wounds and superficial interdigital pyoderma in dogs.
Contraindications	Lincosamides may cause fatal enterocolitis in horses, ruminants, rabbits and rodents, usually due to an overgrowth *of C. difficile*. There are no products authorised for systemic use in ruminants.

Examples of EU-authorised HMP formulations, from Article 57 database

Substance	Route of administration		
	Injection	Oral e.g. tablet, liquid	Topical/local
Clindamycin	x	x	x
Lincomycin	x	x	

Existing recommendations

WOAH recommendations

Lincosamides are categorised VHIA by WOAH (formerly OIE). *Specific comments:* Lincosamides are essential in the treatment of *Mycoplasma pneumonia*, infectious arthritis and haemorrhagic enteritis of pigs.

WHO classifications

WHO: HIA

- (C1: No)
- (C2: Yes) May result from transmission of *Enterococcus* spp. and *Staphylococcus aureus*, including MRSA, from nonhuman sources.

WHO AWaRe: Access: Clindamycin. Watch: Lincomycin

AMEG and CVMP recommendations

Lincosamides are included in the AMEG Category C: this category includes antibiotics for which there are alternatives in human medicine for their indications but which comply with one or both of the following criteria:

- For the veterinary indication under treatment, there are few or no alternatives belonging to Category D. Some examples of these indications are given in Table 4 of the AMEG advice [8], alongside the relevant (sub)class.
- The antibiotic selects for resistance to a substance in Category A through specific multiresistance genes.

Antibiotics placed in this category present a higher AMR risk for human and/or animal health than antibiotics placed in Category D. These antibiotics should only be used when there is no available substance in Category D that would be clinically effective.

Lincosamides are important in humans for treatment of staphylococcal infections, but alternatives exist. In veterinary medicine, there are few or no alternatives of lesser risk for treatment of deep infections e.g. osteomyelitis and serious skin infections in companion animals.

Lincosamides select for *erm* genes that mediate cross-resistance between macrolides, lincosamides and streptogramins and for the *cfr* gene that imparts resistance to oxazolidinones.

The CVMP review of the use of macrolides, lincosamides and streptogramins identified that of most concern was emergence of resistance in *Campylobacter* spp. in poultry and pigs, although the outcome of public health risk assessments due to veterinary use is equivocal [240]. The reflection paper proposed that the duration of treatment with such products should be limited to the minimum time needed for cure of the disease. In subsequent referrals, the indication for swine dysentery has been removed from certain products containing lincomycin [289]. For Linco-Spectin 100 products, a warning is included in SPC 4.5 advising of resistance to lincomycin in *B. hyodysenteriae* and warning that products may not be efficacious against this disease.

Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Disclaimer: The information in this section reflects reported use of antimicrobials outside the terms of a marketing authorisation. No evaluation is made in this section by the working group on the efficacy or safety of the reported uses, or on their potential impact on development and dissemination of AMR.

Information from published sources

The WSAVA List of essential medicines for cats and dogs includes clindamycin. Several of the proposed uses would be outside the terms of EU marketing authorisations:

- Anaerobic infections *in general*
- As part of treatment for life-threatening infections such as sepsis and acute pneumonia (in association with a fluoroquinolone or other antimicrobial to provide Gram-negative coverage).
- CNS infections
- Mycoplasma, Neospora, Toxoplasmosis

Information from the open call for data on use of antimicrobials in animals

The information below is summarised from the open call for data. Inclusion in the table does not endorse use or imply that it is consistent with use according to legislative provisions in Articles 112 to 114.

Substance	Species	Indication	Alternatives	Consequences of unavailability
Lincomycin	Bovine	Severe joint ill	Clav-amox, Penicillin/streptomycin	Last-line treatment
Clindamycin	Dogs and cats	Osteomyelitis, endocarditis, toxoplasmosis	None	Animal cannot be treated with impacts on animal welfare
Clindamycin	Ornamental birds, reptiles	Infections susceptible to this antibiotic only e.g. osteomyelitis		Animal cannot be treated with impacts on animal welfare
Clindamycin	Cetaceans, pinnipeds,	Susceptible anaerobic infections		Severe disease or death

	penguins, pelicans, turtles			
Clindamycin (human IV formulation)	Dogs, cats	Sepsis	Metronidazole IV	Inability to treat anaerobic sepsis by IV route
Clindamycin (human IV formulation)	Ornamental birds and reptiles	Osteomyelitis		Inadequate treatment, animal welfare
Lincomycin + spectinomycin combi	Mink	Enteritis, mastitis, metritis, pneumonia, *E coli* infections	Probiotics/none	Increased mortality, chronic illness, welfare issues
Lincomycin + spectinomycin combi	Fish	Bacterial disease	None	Mortality
Lincomycin + spectinomycin combi	Pheasant, partridge	Bacterial infection	Amoxicillin	Mortality
Lincomycin + spectinomycin combi	Calves	Encephalitis	Not according to susceptibility testing	High level of sickness and disease, welfare issues
Lincomycin + spectinomycin combi	Cattle	Peritonitis	Penicillin-streptomycin	Suffering, mortality, economic losses

4.13.2. Evaluation

Scope of permitted use according to the MRL Regulation

Lincomycin and pirlimycin are included in Table 1 (allowed substances) of the Annex to Regulation (EU) 37/2010 and hence can be used in all food-producing species in accordance with Articles 113 and 114 of Regulation (EU) 2019/6. There are no 'Other provisions' that would be specifically important for use outside the terms of the marketing authorisation.

Lincosamides can be used in non-food-producing species in accordance with Article 112.

Examples of veterinary-authorised formulations/species

Lincomycin is authorised in formulations for group administration to pigs and poultry (in-feed, in-water) and cattle (in-water). Lincomycin is also available in injectable formulation for administration to pigs, poultry, dogs, cats and, usually in combination with spectinomycin, to cattle and sheep.

Lincomycin and pirlimycin are authorised in intramammary preparations for cattle.

Clindamycin is authorised in oral formulations for administration to dogs and cats and in a topical formulation for dogs.

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Importance for human health

Due to superior microbiological activity and bioavailability of clindamycin, lincomycin is infrequently used clinically today. The antibacterial spectrum of activity of clindamycin is similar to that of the macrolides, streptogramins, and chloramphenicol [290]. Clindamycin is active against Gram-positive bacteria e.g. *Staphylococci* (including many beta-lactamase-producing strains), *Streptococci*, including penicillin-resistant *Streptococcus pneumoniae*, but it is not typically active against *Enterococcus* spp. or Gram-negative bacteria [291]. It also demonstrates a potent activity against anaerobic bacteria such

as *Bacteroides fragilis*, *Clostridium perfringens, Fusobacterium* spp., *Prevotella melaninogenica* and *Peptostreptococcus* spp. [290].

Clindamycin is used in combination for the treatment of inhalational anthrax, however the burden of this disease is low [292]. Currently, clindamycin is regarded as the first-choice medicine for bacterial vaginosis. Other important indications are for the treatment of staphylococcal anaerobic infections, including mixed infections (for which they must be combined with an antibiotic with activity against aerobic Gram-negative bacilli) [133]. Clindamycin is also used for treatment of *Toxoplasma gondii*.

Clindamycin is nationally approved in the EU and is indicated for the treatment of serious infections caused by anaerobic bacteria, including intra-abdominal infections, SSTIs; tonsillitis and dental infection.

The high prevalence of clindamycin-resistant staphylococci, streptococci, and anaerobes in some geographic locations limits the clinical usefulness of this agent. Also, as a bacteriostatic antibiotic, clindamycin is not considered to be suitable to treat severe infections as monotherapy, especially in immunocompromised hosts [291].

Importance for animal health

Lincosamides are mainly authorised in veterinary medicine for treatment of infections due to Gram-positive organisms (e.g. *Staphylococcus* spp. and *Streptococcus* spp.) and certain Gram-negative anaerobes (e.g. *Bacteroides, Fusobacterium* spp.)

In pigs, lincomycin is authorised for oral group treatment of swine dysentery (*Brachyspira hyodysenteriae*), porcine proliferative enteropathy - PPE (*Lawsonia intracellularis*) and enzootic pneumonia (*Mycoplasma hyopneumoniae*) and by injection for septic arthritis (including *M. hyosynoviae*) and for other infections caused by Gram-positive bacteria. Pleuromutilins are considered to be more effective than lincomycin in control of swine dysentery and mycoplasma infections in pigs [33].

In chickens lincomycin is authorised for treatment of necrotic enteritis (*Clostridium perfringens*) and in combination with spectinomycin, for treatment of chronic respiratory disease due to *Mycoplasma gallispeticum* and *E. coli*.

In cattle, the major use of lincosamides is for local treatment of mastitis and intramammary preparations containing lincomycin or pirlimycin are authorised for IMI due to Gram-positive cocci including *Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae* and *Streptococcus uberis.*

Lincomcycin is available for administration by injection to dogs and cats, but more often clindamycin is used for oral treatment of infected wounds, abscesses, oral and dental infections due to *Staphylococcus, Streptococcus,* and anaerobes - *Bacteroides* and *Fusobacterium* spp., and *Clostridium perfringens*. In dogs, clindamycin is also indicated for superficial pyoderma due to *Staphylococcus pseudintermedius* and osteomyelitis due to *Staph. aureus*.

No lincosamides were identified with a marketing authorisation for use in fish in the EU.

According to published literature, clindamycin is used in dogs and cats for indications outside the terms of the marketing authorisation e.g. osteomyelitis, prostatitis, as combination therapy for sepsis and acute pneumonia and for protozoal infections (toxoplasmosis, neosporosis, babesiosis) [33, 125, 178]. According to the open call for data, lincosamides are also used for unauthorised limited market species (e.g. ornamental birds, reptiles, zoo species) and human formulations of clindamycin may be used for the intravenous administration route.

Lincosamides are not used for systemic treatment in horses, ruminants and rabbits due to the potential to cause overgrowth of *Clostridium difficile* and serious fatal diarrhoea.

Development and selection of resistance

Resistance to lincosamides occurs mostly alongside cross-resistance to macrolides and streptogramin B (MLSB). The most common mechanism is target site modification mediated by different rRNA methylases (e.g. *erm* genes). *erm* genes have been identified on plasmids and transposons and are widely distributed in Gram-positive, Gram-negative and anaerobic bacteria from human and animal sources. The *cfr* gene also encodes for an rRNA methylase that confers the PhLOPSA resistance pattern (phenicols, lincosamides, oxazolidinones, pleuromutilins and streptogramin A). It is mainly located on plasmids in staphylococci but occurs with low frequency and has been detected sporadically in in LA-MRSA from pigs [59, 230].

Resistance to lincosamides can also occur by active efflux. In staphylococci, efflux pumps encoded by *vga* genes also confer cross-resistance to streptogramin A and pleuromutilins.

Specific resistance to lincosamides is due to enzymatic inactivation and occurs rarely e.g. *lnu* genes have been observed on plasmids in Gram-positive organisms and *C. perfringens* from broilers [293, 294].

Lincosamides are not included in the antimicrobial panel for AMR monitoring under mandatory EFSA/ECDC surveillance of resistance in zoonotic and indicator bacteria from animals; however, considering that resistance to lincosamides mostly occurs with cross-resistance to macrolides, the data reported for macrolides can be considered relevant (see Macrolides in Section 4.11.). In addition, *lnu* genes have been identified in staphylococci from cattle, pigs and from MRSP in a dog [294].

In target animal pathogens e.g. *Brachyspira hyodysenteriae, B. pilosicoli* and *M. hyopneumoniae*, mutational MLS resistance remains important. Very high levels of resistance to lincosamides have been detected in *Brachyspira* spp. from pigs and *Enterococcus* spp. from broilers in the EU [108, 295]. EFSA identified high levels of resistance to lincosamides in *S. pseudintermedius* isolates from cats and dogs in the EU [136]. Reduced susceptibility to lincosamides has been shown in recent field isolates of mycoplasma from poultry and ruminants [237]. Information on prevalence of resistance in anaerobes is limited, but high levels of resistance to lincomycin were detected in *C. perfringens* from Belgian broilers [293] and in *Lawsonia* spp. from pigs [289, 294].

Transmission of resistance

There is evidence for the selection and transmission of resistance to lincosamides from animals to humans and other animals via zoonotic and target pathogens or commensal bacteria capable of transferring resistance to pathogens.

In conclusion,

- Lincosamides are important in human and veterinary medicine for treatment of infections due to Gram-positive organisms, in particular *Staphylococcus* spp. and *Streptococcus* spp., and anaerobes (e.g. *Bacteroides, Fusobacterium* spp.).
- In addition, in veterinary medicine they may be used for swine dysentery and PPE infections in pigs and mycoplasma infections in pigs and poultry. They are often used in combination with spectinomycin.
- Mechanisms of resistance to lincosamides mostly also confer resistance to macrolides and are widely distributed in Gram-positive, Gram-negative and anaerobic bacteria from human and food-

producing and companion animal sources. Resistance to lincosamides can be transmitted from animals to humans and other animals via zoonotic and target pathogens or commensal bacteria capable of transferring resistance to pathogens.

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to Lincosamides.

Criterion (c) – availability of other treatments for animals

There are few alternatives available for treatment of anaerobic infections in animals, in particular for food-producing species. Amoxicillin-clavulanate is an option, including for *Bacteroides* and *Prevotella* spp., producing beta-lactamases. Otherwise, 3rd-generation cephalosporins (AMEG Category B) are a last resort. In companion animals, metronidazole may also be used.

In pigs, *Brachyspira* spp. may also be resistant to macrolides in the presence of lincomycin resistance. There are few alternatives, but pleuromutilins are considered to be more effective than lincomycin in control of swine dysentery. Pleuromutilins or macrolides also have higher activity for treatment of PPE [296]. For mycoplasma infections in pigs, tetracyclines, macrolides (depending on susceptibility) or florfenicol are alternatives [33, 34].

In poultry, alternatives for treatment of mycoplasmas include pleuromutilins, tetracyclines and, as last resort, fluoroquinolones (AMEG Category B) [36]. Penicillins are an alternative for treatment of necrotic enteritis.

In dogs and cats, guidelines recommend use of 1st-generation cephalosporins, amoxiclav or (according to susceptibility) TMPS as first-tier alternatives for treatment of superficial pyoderma due to *Staph. pseudintermedius* [103]. For osteomyelitis, treatment should be based on CAST, but alternatives are available for beta-lactamase-producing staphylococci e.g. cephalosporins, fluoroquinolones, and anaerobes (metronidazole) [33].

In companion animals, alternatives are available for treatment of toxoplasmosis (TMPS, azithromycin, pyrimethamine), neosporosis (totrazuril, pyrimethamine) and babesiosis (imidocarb, tetracyclines), although these uses are mostly outside the terms of a marketing authorisation and may not be the preferred option.

Criterion (d) – availability of other antimicrobial treatments for humans

To treat above-mentioned infections in humans including staphylococcal infections, alternative antibiotic agents (e.g. penicillin–beta-lactamase inhibitor combinations tetracycline, cephalosporins and metronidazole,) are available [291]. For anthrax, there are alternatives to clindamycin, e.g. vancomycin or linezolid, that can be included as part of combination therapy [297]. For treatment of toxoplasmosis, alternatives include pyrimethamine + sulfadiazine, atovaquone and dapsone [298].

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

- Lincosamides are important in human and veterinary medicine for treatment of infections due to Gram-positive organisms, in particular *Staphylococcus* spp. and *Streptococcus* spp., and anaerobes.
- In addition, in veterinary medicine they are used as a first-line option for swine dysentery (according to susceptibility) and PPE infections in pigs and mycoplasma infections in pigs and poultry.
- Mechanisms of resistance to lincosamides mostly also confer resistance to macrolides and are widely distributed in Gram-positive, Gram-negative and anaerobic bacteria from human and food-

producing and companion animal sources. Resistance to lincosamides can be transmitted from animals to humans and other animals via zoonotic and target pathogens or commensal bacteria capable of transferring resistance to pathogens.

- There is a risk for animal and public health due to development of resistance to lincosamides. The lincosamides are included in the AMEG's Category C primarily due to their capacity to cross-select for resistance to oxazolidinones (Category A) via selection of the MDR *cfr* gene. This gene occurs with low prevalence in animal isolates in the EU and may also be selected other veterinary antimicrobials.
- There is in general a sufficient range of alternatives to lincosamides for treatment of the main indications in human and veterinary medicine.
- The uses outside the terms of the marketing authorisation identified for lincosamides relate to use in minor unauthorised species, use to treat minor indications including protozoal infections occurring in companion animals and use of a human intravenous formulation of clindamycin.
- Considering that lincosamides are authorised for use in all major animal species and many limited market species, for administration by various routes including to groups of animals, and taking account of the adequacy of alternative antimicrobials, it is considered unlikely that use outside the terms of the marketing authorisation would contribute substantially to the AMR risk to public and animal health beyond the risk relating to authorised use.

Therefore, considering the points above relevant to criteria (b), (c) and (d), it is recommended that no conditions should be placed on the use of Lincosamides outside the terms of the marketing authorisation, although responsible antimicrobial use principles should be applied.

4.14. Streptogramins

Streptogramins are authorised in human medicinal products in the EU. At present they are not authorised in veterinary medicinal products in the EU; although MRLs are available for one substance in the class.

4.14.1. Background information

Examples of substances included in the class that are authorised in human medicine in the EU

Examples of substances authorised for human use	Examples of ATC codes
Pristinamycin	J01FG01

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

Substance	Species	MRL tissues	MRL milk	MRL eggs	Other provisions
Virginiamycin	Poultry	Yes	-	-	Not for use in animals from which eggs are produced for human consumption

Examples of EU-authorised HMP formulations, from Article 57 database

Substance	Route of administration		
	Injection	Oral e.g. tablet, liquid	Topical/local
Pristinamycin		x	

Existing recommendations

WOAH recommendations

Streptogramins are categorised VIA by WOAH (formerly OIE). *Specific comments:* Virginiamycin is an important antimicrobial in the prevention of necrotic enteritis (*Clostridium perfringens*).

WHO classifications

WHO: HIA

- (C1: No)
- (C2: Yes) May result from transmission of *Enterococcus* spp. and MRSA from non-human sources.

WHO AWaRe: quinupristin-dalfopristin is included in the Reserve group; pristinamycin is in the Watch group

AMEG recommendations

Streptogramins are included in the AMEG Category A: these classes are not authorised in veterinary medicine but are authorised in human medicine in the EU. These antibiotic classes may only be used exceptionally in animals in compliance with the prescribing "cascade".

However, the AMEG considers streptogramins to be obsolete in the EU for human use [8].

Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Disclaimer: The information in this section reflects reported use of antimicrobials outside the terms of a marketing authorisation. No evaluation is made in this section by the working group on the efficacy or safety of the reported uses, or on their potential impact on development and dissemination of AMR.

Information from published sources

Virginiamycin is approved with veterinary medicinal claims in various countries outside the EU, including USA, Canada, South America, South Africa, Australia and New Zealand.

According to the country, virginiamycin may be indicated for treatment of swine dysentery and prevention of necrotic enteritis in broiler chickens caused by *Clostridium perfringens*. Both diseases have potential for serious economic and animal welfare impacts and may be associated with mortality, which can be >10% in the case of necrotic enteritis in poultry flocks. In some countries, virginiamycin is approved for reduction of the incidence of liver abscesses in cattle and reduction of the risk of laminitis in non-food horses.

No other significant evidence could be found for the use of streptogramins in non-food-producing animals.

Information from the open call for data on use of antimicrobials in animals

The information below is summarised from the open call for data. Inclusion in the table does not endorse use or imply that it is consistent with use according to legislative provisions in Articles 112 to 114.

Substance	Species	Indication	Alternatives	Consequences of unavailability
Virginiamycin	Horse	laminitis	various palliative therapies: anti-inflammatory agent	laminitis related morbidity
	Broilers	necrotic enteritis	reliance on beta-lactams, and potentially macrolides under the cascade	

4.14.2. Evaluation

Scope of permitted use according to the MRL Regulation

Streptogramins are not authorised for use in VMPs in the EU. However, virginiamycin is included in Table 1 (allowed substances) of the Annex to the MRL Regulation (EU) 37/2010 and hence can be used in food-producing species in accordance with Articles 113 and 114 of Regulation (EU) 2019/6. 'Other provisions' state that it is not for use in animals from which eggs are produced for human consumption.

Streptogramins can be used in non-food-producing species in accordance with Article 112.

Examples of veterinary-authorised formulations/species

There is currently no veterinary-authorised formulation of streptogramins in the EU.

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Importance for human health

Quinupristin-dalfopristin was previously regarded as one of few available treatments for VRE and MDR *E. faecium* and MDR *Staphylococcus aureus* infections in humans [299]. Quinupristin-dalfopristin no longer appears to be marketed in the EU and is not used to treat either MRSA or VRE as there are more effective alternatives with better safety [300, 301].

Pristinamycin is nationally authorised in some EU member states for oral administration to treat acute maxillary sinusitis, acute exacerbations of chronic bronchitis, mild to moderate community-acquired pneumonia (CAP) and skin and soft tissue infections (SSTI).

Streptogramins have now largely been replaced in human medicine and have limited availability in the EU/EEA.

Importance for animal health

In accordance with Article 113(2), virginiamycin could be used in veterinary medicine for the same species and indications for which it is authorised in third countries: treatment of swine dysentery, prevention of necrotic enteritis in broilers, reduction of the incidence of liver abscesses in cattle and reduction of the risk of laminitis in non-food horses (provided that these uses/indications are also compliant with requirements of Article 107(3) in regard to prophylactic use of antibiotics). The 'open call for data' received reports of some uses of virginiamycin in animals described as being cascade use. There were reports of use of virginiamycin for laminitis in horses and for necrotic enteritis in fattening chickens.

No significant evidence could be found for use of streptogramins in non-food-producing animals (excluding non-food horses) in the EU.

Selection and development of resistance

Since group A and B streptogramins are chemically unrelated and have different binding sites, the mechanisms of resistance differ.

The most common mechanism of resistance to streptogramin B found in Gram-positive cocci is modification of 23S rRNA target binding site by rRNA methylases encoded by erythromycin-resistant methylase (*erm*) genes. This modification confers resistance to all or most macrolides, lincosamides, and streptogramins B (MLS_B) antibiotics. Different plasmids and transposons carrying *erm* genes conferring MLS_B resistance have been recovered widely in enterococci, staphylococci and streptococci from poultry and pig farms worldwide [240, 302, 303]. In a study of glycopeptide-resistant *E. faecium* from Danish Pigs, the *ermB* gene was linked on the same transferable element, probably a plasmid, to the *vanA* gene which confers resistance to vancomycin in enterococci, suggesting the possibility of co-selection [304].

Another mechanism of resistance to streptogramin B compounds found in staphylococci and rarely in *E. faecium* is hydrolysis of the lactone ring brought about by lyase enzymes which are encoded by plasmid-borne *vgb* genes. There is little information on the occurrence of *vgb* genes in staphylococci from animals.

Streptogramin A can be inactivated by acetyltransferases, encoded by *vat* (virginiamycin acetyl transferase) genes. *VatA, vatB* and *vatC* are found in staphylococci and are carried on plasmids but rarely identified in staphylococci from animals. *Vat*(D) and *vat*(E) genes are globally widespread in enterococci from poultry and pigs [305-311]. The *vat* genes are often co-transferred with *vga, vgb* or *erm* genes [303].

In staphylococci and enterococci, the multiresistance *cfr* gene encoding Cfr methyltransferase mediates resistance to streptogramin A in addition to phenicols, lincosamides, pleuromutilins and oxazolidinones (PhLOPSA) [312]. The *cfr* gene may be located chromosomally or on one of several plasmids [313] . Horizontal transmission of the *cfr* gene mediated by plasmids and transposons was demonstrated in MRSA isolates from humans and animals in China, and may play an important role in co-dissemination of *cfr* with *exA*f and *ermC* [314]. It is mainly located on plasmids in staphylococci but occurs with low frequency and has been detected sporadically in in LA-MRSA from pigs in Europe [59, 230].

Both streptogramin A and B compounds are subject to multidrug efflux pumps although these do not necessarily increase MICs to levels required for clinical resistance. In staphylococci, streptococci and enterococci, ABC transporters confer resistance to variably lincosamides, pleuromutilins and streptogramin A (PLSA) and are encoded by, for example, *vga* and *lsa* genes which may be located on transposons or plasmids.

There is a lack of comprehensive and recent surveillance data on streptogramin-resistance in MRSA and in indicator *Enterococcus* spp. in food-animals in the EU. Data reported to EFSA in 2017-18 identified that 100% of MRSA from Finnish pig meat were resistant to both quinupristin-dalfopristin (Q/D) and lincosamides, and 30.8% were resistant to macrolides [230]. MRSA isolates from Belgian calves showed Q/D resistance in 15.5% isolates in 2015 [315]. 2013 is the last year for which routine surveillance data for indicator *Enterococcus* spp. are available under EFSA/ECDC surveillance, and samples were submitted by few member states [316]. Q/D resistance was found to be very high in isolates of *E. faecium* from broiler meat (54.5 – 73.3%, 3 countries) and Danish pig meat (72.7%); in bovine meat from Denmark and Netherlands Q/D-resistance was 41.7% and 60%, respectively. Data from broilers were available from 4 EU countries and showed overall resistance to Q/D in *E faecium* of 73.7%; in *E faecium* from fattening pigs from Spain, resistance was 94.7%. In *E. faecium* from Belgian broilers (n=73), 65.8% were multi-resistant, of which 57.5% were co-resistant to erythromycin, Q/D and tetracyclines. In the EFSA/ECDC report [316], it was suggested that the high level of Q/D resistance could be related to cross-selection due to the therapeutic use of macrolides in animals.

Little information is available in relation to streptogramin-resistance in bacterial isolates from companion animals. Q/D resistance mediated through *ermB* and *vatD* genes has been demonstrated in *E. faecium* from horses; prevalence in samples from horses treated with virginiamycin to prevent laminitis was high (70%) and not different to that in non-exposed animals [317]. In a study that investigated 632 staphylococcal isolates from companion animals in Portugal obtained between 1999 to 2014, Couto, Monchique [233] reported the presence of the *cf*r gene in an *S. pseudintermedius* isolate taken from a dog that had been treated with florfenicol as last resort. The *vg*a(A) and *ls*a(E) genes associated with PLSA were identified in the staphylococcal isolates from pets in China [232].

Transmission of resistance

Identical *erm*(B) gene sequences have been found in unrelated *E. faecium* isolates from humans, pigs and poultry, suggesting exchange between human and animal strains [271]. Similarity has been shown between *vat*(E)-carrying plasmids from poultry *E faecium* and the *vat*(E) plasmid from a human *E faecium* isolate [318]. The close structural relatedness of the plasmid carrying *vga(A)* from porcine MRSA to a plasmid from human clinical *S. aureus* suggested that the plasmid type had been exchanged between humans and pigs [319]. The *cfr* gene, conferring the PhLOPSA resistance phenotype, has been reported in enterococci, staphylococci and other bacterial genera from humans, pigs, cattle and poultry worldwide [320] including from pigs in Germany in LA-MRSA isolates which are also capable of human colonization and infection [321]. In conclusion, *erm, cfr* and various efflux genes have been identified in enterococci and staphylococci from livestock species worldwide. There is evidence for potential

exchange of streptogramin-resistance genes between human and animal strains of *E. faecium* and *S. aureus*.

Quantitative risk assessments performed for geographic regions outside the EU have suggested very low risk in relation to use of virginiamycin in food-producing animals and impacts on human health outcomes, but may not be applicable to the EU situation [322-325].

Although not quantifiable at present, there is evidence for the for selection and transfer of resistance to streptogramins (that might also confer resistance to human CIAs) from animals to humans and other animals through zoonotic and target pathogens or commensal bacteria capable of transferring resistance to pathogens.

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to Streptogramins.

Criterion (c) – availability of other treatments for animals

In the European Union virginiamycin was previously used as a feed additive but such use intended for growth promotion was banned in 1998 by Council Regulation (EC) No 2821/98. Although virginiamycin is included in the Annex to the MRL Regulation (EU)37/2010 as a substance allowed for use in poultry, there are no authorised VMPs in the EU containing streptogramins.

Virginiamycin is approved for veterinary medicinal use in several third countries, most importantly for prevention of necrotic enteritis in poultry and treatment of swine dysentery in pigs, both diseases having important economic and animal welfare consequences. There is no fully effective vaccine available and limited alternative antibiotics for treatment of swine dysentery. Mostly pleuromutilins (tiamulin and valnemulin) or, according to susceptibility, tylosin (injection) and lincomycin may be used. Doxycycline has also been used across Europe for the treatment of swine dysentery [229].

Improved management of diet, husbandry and control of coccidial infections are important for prevention of necrotic enteritis in broilers [326]. There have been difficulties associated with development of a fully effective vaccine [327]. In the EU, alternative antibiotics for the treatment of necrotic enteritis include penicillin, lincomycin and macrolides.

Measures to reduce the risk of laminitis include control of predisposing factors e.g. underlying metabolic/endocrine disease, carbohydrate overload, altered weight [328].

Criterion (d) – availability of other antimicrobial treatments for humans

Pristinamycin is nationally authorised in some EU member states for oral administration to treat acute maxillary sinusitis, acute exacerbations of chronic bronchitis, mild to moderate community-acquired pneumonia (CAP) and skin and soft tissue infections (SSTI).

Quinupristin-dalfopristin was previously regarded as one of few available treatments for VR and MDR *E. faecium* and MDR *Staphylococcus aureus* infections in humans. Quinupristin-dalfopristin no longer appears to be marketed in the EU and is not used to treat either MRSA or VRE as there are alternatives with better activity that are also less toxic.

For the treatment of MRSA, in addition to vancomycin, last resort options include also new lipoglycopeptides (oritavancin, dalbavancin, telavancin), glycylcyclines, 5th-generation cephalosporins (ceftaroline, ceftobiprole) and daptomycin. In regard to VRE, alternatives include glycylcyclines and daptomycin. Nevertheless, resistance to those last resort antibiotics options has already been reported.

Streptogramins have now been replaced in human medicine and have limited availability in the EU/EEA due to other existing treatment alternatives.

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

- Quinupristin-dalfopristin was previously regarded as one of few available treatments for vancomycin-resistant and multidrug-resistant *E. faecium* and multidrug-resistant *S. aureus* infections in humans. Although pristinamycin still has limited availability, use of streptogramins for these critical indications has now been replaced by more effective alternatives with better safety and streptogramins are almost obsolete in human medicine in the EU.
- In accordance with Article 113(2), veterinary medicines containing virginiamycin from third countries could be used for the species and indications for which they are authorised, namely treatment of swine dysentery, prevention of necrotic enteritis in broilers, reduction of the incidence of liver abscesses in cattle and reduction of the risk of laminitis in horses. However, prophylactic use must be in compliance with the restrictions in Article 107(3), which are likely to minimise these uses.
- Although there is a lack of contemporaneous surveillance data on the prevalence of resistance to streptogramins in animal isolates from the EU, data collected within the last 10 years showed high levels of resistance in MRSA and indicator Enterococcus spp. from different livestock species in some countries. There is evidence for the selection and potential transmission of streptogramin-resistance genes (e.g. *erm*, *vat*, *vga* and *cfr*) from animals to humans and other animals through zoonotic and target pathogens or commensal bacteria e.g. enterococci and staphylococci. These multi-resistance genes may cross or co-select for important antibiotic classes including oxazolidinones, macrolides and vancomycin. However, the limited use of streptogramins outside the terms of the marketing authorisation is unlikely to significantly increase the selection pressure compared with the alternative antimicrobials that select the same resistance genes and are more frequently used in veterinary practice e.g. macrolides.
- According to restrictions on the use of streptogramins under Article 113(2) and the responsible use measures in Article 107(3), the use of streptogramins outside the terms of the marketing authorisation is likely to be very infrequent.

Therefore, considering the points above relevant to criteria (b), (c) and (d), it is recommended that no conditions should be placed on the use of Streptogramins outside the terms of the marketing authorisation, although responsible antimicrobial use principles should be applied.

4.15. Aminoglycosides and Aminocyclitols

4.15.1. Background information

Examples of substances included in the class that are authorised in veterinary and human medicine in the EU

Examples of substances authorised for veterinary use	Examples of ATCvet codes
Amikacin	QD06AX12 QJ01GB06 QS01AA21
Apramycin	QJ01GB90 QA07AA92 QJ51GB90
Dihydrostreptomycin	QJ01GA90 QS01AA15 QA07AA90 QJ51GA90
Framycetin	QJ01GB91 QS01AA07
Gentamicin	QD06AX07 QJ01GB03 QS01AA11 QA07AA91 QJ51GB03
Kanamycin	QJ01GB04 QS01AA24 QA07AA08
Neomycin	QD06AX04 QJ01GB05 QS01AA03 QA07AA01
Paromomycin	QJ01GB92 QA07AA06
Streptomycin	QJ01GA01 QA07AA04
Spectinomycin	QJ01XX04
Examples of substances authorised for human use	**Examples of ATC codes**
Amikacin	D06AX12 J01GB06 S01AA21
Bekanamycin	J01GB13
Capreomycin	J04AB30
Dihydrostreptomycin	S01AA15
Framycetin	S01AA07
Gentamicin	D06AX07 J01GB03 S01AA11
Kanamycin	J01GB04 S01AA24 A07AA08
Neomycin	D06AX04 J01GB05 S01AA03 A07AA01
Netilmicin	J01GB07 S01AA23
Paromomycin	A07AA06
Streptomycin	J01GA01 A07AA04
Tobramycin	J01GB01 S01AA12
Spectinomycin	J01XX04

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

Substance	Species	MRL tissues	MRL milk	MRL eggs	Other provisions
Apramycin	Bovine	Yes			Not for use in animals from which milk is produced for human consumption.
Apramycin	Ovine, porcine, chicken, rabbit	No MRL required			For oral use only. Not for use in animals from which milk or eggs are produced for human consumption.
(Dihydro)/ streptomycin	All ruminants, porcine, rabbit	Yes	Yes (ruminants)		
Neomycin/ framycetin	All food-producing species	Yes	Yes	Yes	
Gentamicin	All mammalian food-producing species and fin fish	Yes	Yes		
Kanamycin	All food-producing species except fin fish	Yes	Yes		Not for use in animals from which eggs are produced for human consumption.
Paromomycin	All food-producing species	Yes		Yes	Not for use in animals from which milk is produced for human consumption.
Spectinomycin	All Food-producing species	Yes	Yes		Not for use in animals from which eggs are produced for human consumption.

EU-authorised VMP formulations, based on sales reported to ESVAC

Species		Route of administration						
		Group		Individual				
		In-feed	In-water	Injection	Oral e.g. tablet, paste, powder	Topical/local (incl. intrauterine)	Intra-mammary	Oral powder
Major	**Cattle**	NEO	APR, DHS, GEN, NEO, PM, STR, SPT	APR, DHS, FRM, GEN, KAN, NEO, PM, STR, SPT	DHS	DHS, GEN, NEO	DHS, FRM, GEN, KAN, NEO, STR	DHS, GEN, NEO, STR
	Sheep (for meat)	NEO	APR, DHS, NEO, PM, STR	DHS, GEN, KAN, NEO, STR, SPT	DHS, SPT	NEO	DHS, FRM	NEO
	Pigs	APR, GEN, NEO, PM, SPT	APR, DHS, GEN, NEO, PM, STR, SPT	DHS, GEN, KAN, NEO, PM, STR, SPT	DHS, SPT	DHS, NEO		APR, GEN, NEO, STR
	Chickens	NEO, PM,	APR, NEO, PM, SPT	KAN, SPT	NEO			
	Dogs		NEO, STR	DHS, GEN, KAN, NEO, STR, SPT	DHS, GEN, KAN, NEO,	NEO, GEN		STR
	Cats		NEO	DHS, GEN, KAN, NEO, STR, SPT	DHS, GEN, KAN, NEO	GEN		
Limited market species	**Turkeys**	NEO, PM	NEO, PM, SPT	SPT	NEO			
	Ducks	NEO	NEO					
	Geese	NEO	NEO					

As listed in SPCs	**Horses**		NEO, STR	AMK, DHS, KAN, NEO, STR	DHS	GEN, NEO		STR
	Goats	NEO	DHS, NEO, PM, SPT	DHS, KAN, NEO, SPT		NEO	DHS, NEO	
	Rabbits	APR, NEO, PM	APR, DHS, NEO, PM					
	Minks		NEO					
	Fur animals	NEO			DHS			
	Guinea fowls	NEO						
	Quails	NEO	NEO					
	Pheasants	NEO						
	Racing pigeons		SPT		NEO			NEO
	Partridges	NEO	NEO					
	Ornamental birds		GEN	GEN				
	Rodents			GEN				
	Reptiles			GEN				
	Buffaloes			GEN				

AMK (amikacin), APR (apramycin), DHS (dihydrostreptomycin), FRM (framycetin), GEN (gentamicin), KAN (kanamycin), NEO (neomycin), PM (paromomycin), STR (streptomycin), SPT (spectinomycin)

Summary of main indications and contra-indications for EU-authorised VMPs, based on selected SPCs

Main indications	Various aminoglycosides are authorised for treatment of enteric infections, such as colibacillosis and salmonellosis, and respiratory infections in various food-producing animals. In companion animals, injections of gentamicin or amikacin are authorised for the treatment of septicaemia and respiratory infections. In cattle, neomycin, streptomycin, kanamycin and framycetin, in combination with other antimicrobial agents, are used in preparations for intramammary administrations to cows with mastitis. Dihydrostreptomycin and neomycin are authorised in combination with penicillin for treatment of a broad range of indications in food-producing animals and companion animals. Aminoglycosides are also used for topical treatment of infections of the eye and ear. Paromomycin is authorised for oral administration to calves, lambs and goat kids for reduction of *Cryptosporidium parvum*. Spectinomycin is authorised as an oral dose for lambs and piglets for the treatment of neonatal enteritis due to *E. coli* infections. It is also available as an injection for cattle, pigs and horses. Spectinomycin is often used in formulations in combination with lincomycin, for administration in drinking water to pigs for treatment of *E. coli* and *Lawsonia intracellularis* infections and poultry for respiratory disease due to *Mycoplasma gallisepticum* and *E. coli* (chronic respiratory disease). Injectable formulations are also available for all major food-producing and companion animal species.
Contraindications	Aminoglycosides can induce ototoxicity and nephrotoxicity. Not to be used in patients with (severe) kidney damage. In patients with renal insufficiency or dehydration, the dosage should be evaluated carefully. Ear applications: do not use in case of damaged tympanic membrane. Aminoglycosides should be used with care outside the marketing authorisation in food-producing species due to the prolonged accumulation of residues in the kidneys [125].

Examples of EU-authorised HMP formulations, from Article 57 database

Substance	Route of administration		
	Injection	**Oral e.g. tablet, liquid**	**Topical/local**
Amikacin	x		x
Bekanamycin			x
Capreomycin	x		
Dihydrostreptomycin		x	
Framycetin			x
Gentamicin	x		x
Kanamycin	x		x
Neomycin		x	x
Netilmicin	x		x

Paromomycin		x	
Streptomycin	x		
Tobramycin	x		x
Spectinomycin	x		

Existing recommendations

WOAH recommendations

Aminoglycosides are categorised as VCIA by WOAH (formerly OIE). *Specific comments:* The wide range of applications and the nature of the diseases treated make aminoglycosides extremely important for veterinary medicine. Aminoglycosides are of importance in septicaemias; digestive, respiratory and urinary diseases. Gentamicin is indicated for *Pseudomonas aeruginosa* infections with few alternatives. Apramycin and Fortimycin are currently only used in animals. Few economic alternatives are available.

Aminocyclitols are categorised VCIA by OIE. *Specific comments:* Used for respiratory infections in cattle and enteric infections in multiple species.

WHO classifications

Aminoglycosides

WHO: CIA

- (C1: Yes) Sole or limited therapy as part of treatment of enterococcal endocarditis and multidrug-resistant (MDR) tuberculosis and MDR Enterobacteriaceae.
- (C2: Yes) May result from transmission of *Enterococcus* spp., Enterobacterales (including *E. coli*), and *Mycobacterium* spp. from non-human sources.
- (P1: No) In some countries there is a high proportion of use in patients in health care settings with serious infections for which, because of resistance, it is one of few alternatives.
- (P2: Yes) High frequency of use in human medicine.
- (P3: Yes) Transmission of *Enterococcus* spp., Enterobacteriaceae (including *E. coli*), and *Mycobacterium* spp. from non-human sources.

WHO AWaRe: Reserve: Plazomicin; Watch: e.g. Kanamycin, Neomycin, Netilmicin, Sisomicin, Streptomycin, Tobramycin; Access: Amikacin, Gentamicin

Aminocyclitols

WHO: IA

- (C1: No) In some areas spectinomycin may be one of limited antimicrobials still active against *Neisseria gonorrhoeae*.
- (C2: No) May result from transmission of Enterobacterales, including *E. coli*, from non-human sources, but there is no demonstrated transmission from *E. coli* to *N. gonorrhoeae.*

WHO AWaRe: Access: Spectinomycin

AMEG and CVMP recommendations

Aminoglycosides (except spectinomycin) are included in the AMEG Category C: this category includes antibiotics for which there are alternatives in human medicine for their indications but which comply with one or both of the following criteria:

- For the veterinary indication under treatment, there are few or no alternatives belonging to Category D. Some examples of these indications are given in Table 4 of the AMEG advice [8], alongside the relevant (sub)class. For aminoglycosides, they were identified as Pseudomonas infections in companion animals and horses and weaning diarrhoea due to Enterobacterales in pigs.
- The antibiotic selects for resistance to a substance in Category A through specific multiresistance genes.

Antibiotics placed in this category present a higher AMR risk for human and/or animal health than antibiotics placed in Category D. These antibiotics should only be used when there is no available substance in Category D that would be clinically effective.

Spectinomycin is included in AMEG's Category D. There are alternative treatments in human and veterinary medicine for their indications and that do not select for resistance to Category A substances through specific multiresistance genes.

These antibiotics are not devoid of negative impact on resistance development and spread. To keep the risk from use of these antibiotic classes as low as possible it is important that responsible use principles are complied with in everyday practice. Unnecessary use and unnecessarily long treatment periods should be avoided and group treatment restricted to situations where individual treatment is not feasible.

The CVMP published a reflection paper on the use of aminoglycosides in animals in the EU in 2018 [329], acknowledging an increasing importance of this class for treatment of MDR Gram-negative infections in humans. It was concluded that there is a high risk of transfer aminoglycoside-resistance between animals and humans via zoonotic and commensal foodborne bacteria. The high levels of resistance in veterinary pathogens, including *E. coli*, to streptomycin and spectinomycin led to a recommendation that antimicrobial susceptibility testing should be conducted prior to use of these substances in animals; although noting that veterinary breakpoints are only available for a limited number of organisms.

A referral was conducted by CVMP in 2014 to review the indications and dosing regimen for VMPs containing gentamicin for use in horses [330]. It was concluded that there were insufficient data available to support various broad claims, including gastrointestinal and genitourinary infections cause by various pathogens; consequently, the indications were limited to treatment of lower respiratory tract infections due to aerobic Gram-negative bacteria susceptible to gentamicin, using a single daily dosing regimen.

The CVMP has made a recommendation for the suspension of VMPs containing paromomycin to be administered parenterally to pigs [331]. The indications for such products were broad and inadequately supported by (pre)clinical data and residues data. As data were not provided to support the dosing regimen, this led to concerns about ineffective treatment and risk of resistance development. Hence a referral concluded that the benefit-risk for the products was negative.

Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Disclaimer: The information in this section reflects reported use of antimicrobials outside the terms of a marketing authorisation. No evaluation is made in this section by the working group on the efficacy or safety of the reported uses, or on their potential impact on development and dissemination of AMR.

Information from published sources

Considering that aminoglycosides are authorised for a wide range of indications and species, it is difficult to determine if some published uses are outside a marketing authorisation.

Clearer examples include the intra-articular administration of amikacin for the treatment of septic arthritis in foals and dogs [9, 332] or the administration of gentamicin as aerosol in dogs [333].

Amikacin is often reserved to treat MDR infections in companion animals, e.g. septicaemia and pneumonia in neonatal foals and serious Gram-negative infections in dogs and cats due to MDR Enterobacterales or *Pseudomonas aeruginosa* [33, 125].

In cases where topical treatment is unsuitable for MRSP infections in companion animals, amikacin is often one of few systemic antimicrobials to which isolates may remain susceptible [334].

Aminoglycosides are applied in apiculture, ornamental aquaculture and in other minor species such as rabbits, reptiles and birds, although safety and efficacy have not been established in all cases, with use often being off-label [335].

Information from the open call for data on use of antimicrobials in animals

The information below is summarised from the open call for data. Inclusion in the table does not endorse use or imply that it is consistent with use according to legislative provisions in Articles 112 to 114.

Substance	Species	Indication	Alternatives	Consequences of unavailability
Amikacin (injectable human product)	Equines	Neonatal septicaemia Irrigation of tendon sheaths, intraperitoneal use	Gentamicin No alternatives using the same formulation. Metronidazole could be alternative for anaerobic intra-abdominal infections.	Deaths Welfare problems and deaths
Amikacin (human solution for infusion)	Equines	Septic arthritis	Gentamicin	Ineffective treatment and welfare problems
Amikacin (human solution for infusion)	Dogs, cats	MDR infections e.g. Acinetobacter		Inadequate treatment and welfare problems
Amikacin, neomycin (human formulations)	Teleosts, elasmobranchs, cetaceans	Susceptible bacterial diseases	Ceftazidime, tetraccycline	Severe disease, deaths
Apramycin, neomycin	mink	Enteritis	None	Welfare problems, deaths
Gentamicin (human product for infusions)	Horses	Joint infections, septic tenosynovitis, osteomyelitis, perioperative use, surgical colic, septic peritonitis, pneumonia in foals (Bordetella spp.)	Gentamicin (VMP, but different formulation)	Unable to treat life-threatening infections
Gentamicin (human product for infusions)	Dogs	Septic shock	Gentamicin VMPs are a different formulation	
Gentamicin	Equines	(pleuro)pneumonia, wound infections, septic arthritis, tendonitis	Streptomycin combinations (Fluoroquinolones)	Uncontrolled infections and death

		Severe systemic infections and septicaemia		
Gentamicin	Bovine, ovine, caprine	Septicaemia	None	Deaths
Gentamicin – (human formulation for intra-vitreal injection)	Horses	After vitrectomy		Inadequate treatment and welfare problems
Gentamicin (human formulations for implant)	Horses	Infected bone or soft tissue cavities, infected wounds		
Gentamicin injection	rabbits	Colibacillosis		
Gentamicin	Reptiles	Bacterial infections		
Gentamicin, neomycin	Ornamental birds			
Gentamicin and neomycin	Ornamental fish	Bacterial infections, gastrointestinal infections	Fluoroquinolones, amikacin, depending on susceptibility	Deaths
Neomycin	Fur animals			
Neomycin/streptomycin oral solutions	Pigs (unauthorised species)	Enteritis due to Enterobacterales		
Paromomycin	Ornamental birds, reptiles	Bacterial infections and Cryptosporidiosis.	None	Severe
Tobramycin (eye drops, human)	Horses, cats, dogs	Bacterial keratitis and corneal ulcers involving *Pseudomonas* spp.	None	May lead to enucleation or euthanasia
Kanamycin (eye drops, human formulation)	Dogs and cats	Eye infections	Other aminoglycosides	
Tobramycin (injectable human formulation)	Horses	Septic arthritis		Alternatives less effective
Spectinomycin	rabbits	colibacillosis	Quinolones	

4.15.2. Evaluation

Scope of permitted use according to the MRL Regulation

Various aminoglycosides are included in Table 1 (allowed substances) of the Annex to Regulation (EU) 37/2010 and hence they can be used in all food-producing species in accordance with Articles 113 and 114 of Regulation (EU) 2019/6. There are 'Other provisions' that restrict use of certain substances, e.g. apramycin and paromomycin cannot be used in animals from which milk is produced for human consumption and apramycin, kanamycin and paromomycin cannot be used in animals producing eggs for human consumption. Aminoglycosides can be used in non-food-producing species in accordance with Article 112.

Substances/indications in equines out of scope of evaluation for conditions due to listing in Regulation (EC) 1950/2006, as amended by Regulation (EU) 122/2013

Amikacin is listed for the treatment of septic arthritis in equines.

Examples of veterinary-authorised formulations/species

Aminoglycosides are available in formulations intended for group administration (in-feed, in-drinking water) for all major food-producing species and for many limited market species e.g. various poultry, goats, rabbits. They are available in injectable formulations for all major food-producing and companion animal species and for horses and goats. They are also available as intramammary

formulations for cattle, sheep and goats, and in topical formulations (e.g. eye and ear preparations) for various species.

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Importance for human health

Aminoglycosides are particularly active against Gram-negative, aerobic bacteria (e.g. *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii*) and Gram-positive bacteria (including MRSA and (vancomycin-resistant) VR-*Staphylococcus aureus* and VR-*Enterococci*) as well as *Mycobacterium* spp. [336-338].

Aminoglycosides have been used in clinical practice since 1940. They are primarily used in combination with other antibiotics. Aminoglycosides are used to treat severe infections such as septicaemia, endocarditis, complicated UTIs, severe pelvic inflammatory disease, peritonitis and other severe intra-abdominal infections [339]. In paediatrics, gentamicin is used for septicaemia, meningitis, biliary tract infections, acute pyelonephritis and endocarditis [340]. Aminoglycosides are used to treat serious infections caused by MDR Gram-negative bacteria when other alternatives are lacking, and endocarditis caused by difficult-to-treat pathogens when monotherapy with beta-lactam antibiotics is not sufficient. Beta-lactam antibiotics are often combined with an aminoglycoside for severe sepsis/septic shock to broaden the antibacterial spectrum and achieve rapid bactericidal, and possibly synergistic effects [341].

Aminoglycosides are nationally authorised in the EU for indications that include the treatment of bacteraemia, UTIs, chest infections, severe neonatal infections and other serious systemic infections due to susceptible organisms, in adults and children including neonates. Paromomycin is authorised for treatment of amoebiasis [342].

Spectinomycin now has limited use in human medicine, to treat uncomplicated gonococcal infections in patients allergic to penicillins or with infections that are resistant to penicillins [3].

Importance for animal health

Aminoglycosides are authorised in VMPs in the EU, for use in companion and food-producing animals (cattle, pigs, poultry, sheep, goats, horses, dogs and cats) for treatment of septicaemias, gastrointestinal, urinary and respiratory tract infections.

Food-producing species

In 2021, the sales of aminoglycosides (including spectinomycin) made up 5.9% of the total sales of antimicrobials for food-producing species, in mg/population correction unit (PCU) [15]. The most frequently used substances are neomycin, dihydrostreptomycin and spectinomycin. Other substances from the group that are used in food-producing species are apramycin, gentamicin, kanamycin and paromomycin. Aminoglycosides are among few alternatives for treatment of weaning diarrhoea in piglets and other infections due to MDR Enterobacterales (including salmonellosis) in various animal species. *E. coli* infections (e.g. septicaemia, meningitis, severe enteritis) are a major cause of morbidity and mortality in neonatal livestock and horses [175-177, 205]. Recent EFSA opinions noted high levels of resistance to first line antimicrobials (e.g. aminopenicillins, potentiated sulfonamides, tetracyclines), often involving multidrug resistance, in pathogenic *E. coli* from swine, poultry, calves, lambs and horses. This suggests the limited efficacy of first-line antibiotics against these infections in

many EU countries [55, 108, 154, 204, 295]. Aminoglycosides are a treatment option where Enterobacterales spp. remain susceptible [329].

Aminoglycosides are often used in combination with other antimicrobials, such as beta-lactams, in order to achieve a synergistic effect or to broaden the spectrum of activity. Streptomycin and neomycin are authorised in the EU in combination with penicillins for treatment of a broad range of non-specific indications, including as intramammary preparations for mastitis. Spectinomycin is authorised in combination with lincomycin and administered in drinking water to pigs for treatment of *E. coli* and *Lawsonia intracellularis* infections and to poultry for respiratory disease due to *Mycoplasma gallisepticum* and *E. coli* (chronic respiratory disease).

Paromomycin is authorised for reduction of the severity and the duration of diarrhoea associated with *Cryptosporidium parvum* in pre-ruminants.

There are no aminoglycosides authorised as VMPs in aquaculture in the EU and no evidence was found for their use or need in food-producing aquatic species in the EU.

Companion animals

In companion animals, injections of gentamicin or amikacin are authorised for the treatment of septicaemia and respiratory infections. Aminoglycosides are one of few treatment options in companion animals for MDR Gram-negative bacteria including *Pseudomonas* spp. causing a variety of serious, potentially life-threatening, infections (septicaemia, urinary and respiratory tract infections, otitis). They are used with caution due to potential nephrotoxicity and ototoxicity [104, 107, 178, 329, 343].

Aminoglycosides are also commonly used for topical treatment of infections of the eye and ear in companion animals.

In regard to use outside the terms of a marketing authorisation, aminoglycosides are applied in apiculture, ornamental aquaculture and in other minor species such as mink, rabbits, reptiles, zoo species and birds [335]. In horses and other companion animals, human formulations are used for alternative administration routes e.g. by infusion, intra-articular injection and topical use for ocular infections.

In MRSP cases, amikacin is often one of few systemic antimicrobials to which isolates remain susceptible. Prevalence of MRSP in companion animals varies across the EU. It is most commonly implicated in canine recurrent pyoderma, and may be involved in life-threatening surgical wound, urinary and respiratory tract infections.

Development and selection of resistance

The three main mechanisms of bacterial resistance to aminoglycosides are the reduction of the intracellular concentration of the antimicrobial, the enzymatic modification of the antibiotic and the modification of the molecular target. Resistance mechanisms are complex and differ between the aminoglycoside molecules and between bacterial species, and generally there is less cross-resistance when compared with other classes of antimicrobials. Enzymatic inactivation of aminoglycosides is the most common resistance mechanism [329, 335, 344, 345]. Aminoglycosides are differently affected by these enzymes. Among these enzymes, AAC(6')-Ib-cr confers resistance to gentamicin and fluoroquinolones such as ciprofloxacin [329]. The methylation of the ribosomal target responsible for high-level resistance against most aminoglycosides is an emerging mechanism of great concern in clinically relevant Gram-negative bacteria, but this mechanism is still uncommon in Europe. Methyltransfereases (*armA, rmtA*, *rmtB*, *rmtC*, *rmtD*, *rmtD2*, *rmtE*, *rmtF*, *rmtG*, *rmtH* and *npmA)* have been found on mobile genetic elements and have been associated with genes encoding resistance to

extended spectrum beta-lactams, quinolones and carbapenems. Except in mycobacteria, resistance genes are often located on mobile genetic elements, facilitating their spread between different bacterial species and between animals and humans [335, 346-349]. In *M. tuberculosis*, mutations in the genes *rps*L and *rrs* encoding the ribosomal protein S12 and the 16S rRNA, respectively, are responsible for most of the high-level streptomycin resistance [350].

EFSA/ECDC mandatory surveillance shows that the EU prevalence of resistance to gentamicin in *Campylobacter* spp. and *Salmonella* spp. is generally low in food-producing animals. Resistance to streptomycin was observed at low levels in *C. jejuni* isolates from carcasses and fresh meat, and at a moderate level in meat preparations. The median levels of resistance to gentamicin in *Salmonella* spp. and indicator *E. coli* from all animal species was low, with exceptions for individual countries [28, 59].

Resistance to aminoglycosides has been detected in isolates from companion animals including *Pseudomonas* spp., staphylococci including MRSP and Enterobacterales also producing ESBLs [335]. Based on a literature review performed by EFSA, moderate levels of resistance to gentamicin were demonstrated in *Pseudomonas* spp. from cats and dogs [136].

Veterinary pathogens from food-producing animals generally retain good susceptibility to gentamicin, but levels of resistance to streptomycin and spectinomycin are higher. There is evidence that the usage of aminoglycosides in veterinary medicine is associated with the increased prevalence of resistance to aminoglycosides and other antimicrobial classes in bacteria in animals [335, 351, 352].

No reports were found of clinically relevant resistance to paromomycin in protozoal species.

Transmission of resistance

Aminoglycoside-resistance has been found in many different bacterial species, including those with zoonotic potential such as *Salmonella* spp., *Campylobacter* spp. and (LA)MRSA. The same resistance genes have been found in isolates from humans and animals [353-356]. Evaluation of risk factors indicates that the probability of transmission of aminoglycoside-resistance from animals to humans through transfer of zoonotic pathogens or commensal foodborne bacteria and/or their mobile genetic elements can be regarded as high. The highest risk is anticipated from transfer of resistant enterococci or coliforms (*E. coli*) since infections with these pathogens in humans would potentially be treated with aminoglycosides [329, 335]. Aminoglycosides are not part of the recommended first-line treatment regimen for mycobacterial infections in companion animals.

Resistance to aminoglycosides can also be transferred between animals via commensal organisms or target pathogens.

In conclusion, there is evidence for the selection and transmission of resistance to aminoglycosides from animals to humans via zoonotic pathogens or commensal bacteria capable of transferring resistance to human pathogens. In addition, the usage of aminoglycosides in veterinary medicine is associated with the increased prevalence of resistance to aminoglycosides and other antimicrobial classes in bacteria in animals, which can be transmitted via target pathogens and commensal bacteria.

In conclusion,

- Aminoglycosides are important in human medicine, where they are used in combination with other antibiotics to treat serious infections such as septicaemia, cUTI and intra-abdominal infections. Importance is increasing for infections due to MDR Gram-negative bacteria, including *Pseudomonas* and *Acinetobacter* spp. Aminoglycosides are also important for treatment of staphylococcal infections, enterococcal endocarditis and mycobacterial infections.

- In veterinary medicine, aminoglycosides are authorised for use in companion and food-producing animals (e.g. cattle, pigs, poultry, sheep, goats, horses, dogs and cats) for treatment of septicaemias, gastrointestinal, urinary and respiratory tract infections. They are among few effective treatments for MDR Gram-negative infections associated with e.g. post-weaning diarrhoea in pigs and septicaemias in companion animals and are also used for control of cryptosporidiosis.
- The extent of use outside the terms of a marketing authorisation is unknown but should be considered in the context of the wide range of authorised species, indications and formulations. Nevertheless, there is evidence for use in a diverse range of minor species and for administration by unauthorised routes to treat specific infections in individual animals.
- The prevalence of resistance to gentamicin in indicator *E. coli* and veterinary pathogens from food-producing animals is generally low. However, resistance genes are located on MGEs and there is evidence for the selection and transmission of resistance to aminoglycosides between animals and from animals to humans via zoonotic pathogens or commensal bacteria capable of transferring resistance to pathogenic bacteria.

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to Aminoglycosides and Aminocyclitols.

Criterion (c) – availability of other treatments for animals

Aminoglycosides are used in food-producing and companion animal species to treat serious life-threatening infections with significant morbidity or mortality. In horses, gentamicin is one of the few available antimicrobials for treating Gram-negative infections.

Alternatives for resistant *E. coli* are available, but limited to substances from the higher AMEG Category B i.e. colistin (not foals), 3rd- and 4th-generation cephalosporins (not poultry) or fluoroquinolones. TMPS is also an alternative for food-producing animals and horses, but resistance is very common in Gram-negative bacteria.

In companion animals, for systemic treatment of MDR Gram-negative bacteria and pseudomonas infections, fluoroquinolones (AMEG Category B) are the only veterinary-authorised alternative, although susceptibility is variable [357], whilst polymyxins might be used for localised infections amenable to topical treatment (e.g. eye or ear infections). Other alternatives for MDR Gram-negative bacteria and MRS such as carbapenems, oxazolidinones and glycopeptides must not be used in animals in the EU [2].

Criterion (d) – availability of other antimicrobial treatments for humans

Alternatives to treat severe MDR infections are limited and include beta-lactam antibiotics, fluoroquinolones. For endocarditis caused by enterococci, alternative treatment options include high-dose daptomycin and the combination of ampicillin with ceftriaxone.

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

- Aminoglycosides are becoming increasingly important in human medicine, where they are used in combination with other antibiotics to treat serious infections due to MDR Gram-negative bacteria, including *Pseudomonas* and *Acinetobacter* spp., and are also important for treatment of staphylococcal infections, enterococcal endocarditis and mycobacterial infections. Alternatives are available but are limited.
- In veterinary medicine, aminoglycosides and aminocyclitols are authorised for use in all major food-producing and companion animals, and some limited market species, for treatment of various

infectious diseases including septicaemias, gastrointestinal, urinary and respiratory tract infections. In general, there are alternatives; although, aminoglycosides are among few effective treatments for MDR Gram-negative infections in animals, for which alternatives are likely to be AMEG Category B substances whose use is of greater concern to human and animal health.

- The prevalence of resistance to gentamicin in indicator *E. coli* and veterinary pathogens from food-producing animals is generally low. However, resistance genes are located on MGEs and there is evidence for the selection and transmission of resistance to aminoglycosides between animals and from animals to humans via zoonotic pathogens or commensal bacteria, in particular *E. coli* and enterococci, capable of transferring resistance to pathogenic bacteria.
- Aminoglycosides are used outside the terms of a marketing authorisation in a diverse range of minor species and for administration by unauthorised routes to treat specific infections in individual animals. Although the extent of this use is unknown, it is likely to be relatively low considering the wide range of authorised species, indications and formulations (including for group administration) available.

Therefore, considering the points above relevant to criteria (b), (c) and (d), it is recommended that no conditions should be placed on the use of Aminoglycosides and Aminocyclitols outside the terms of the marketing authorisation, although responsible antimicrobial use principles should be applied.

4.16. Tetracyclines

This section does not include the novel tetracyclines eravacycline and omadacycline, which are included in the Article 37(5) list of substances reserved for use in humans, only.

Minocycline is evaluated separately.

4.16.1. Background information

Examples of substances included in the class that are authorised in veterinary and human medicine in the EU

Examples of substances authorised for veterinary use	**Examples of ATCvet codes**
Chlortetracycline	QJ01AA03
Doxycycline	QJ01AA02
Oxytetracycline	QJ01AA06
Tetracycline	QJ01AA07
Examples of substances authorised for human use	**Examples of ATC codes**
Chlortetracycline	J01AA03
Demeclocycline	J01AA01
Doxycycline	J01AA02
Lymecycline	J01AA04
Metacycline	J01AA05
Oxytetracycline	J01AA06
Rolitetracycline	J01AA09
Tetracycline	J01AA07

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

Substance	**Species**	**MRL tissues**	**MRL milk**	**MRL eggs**
Chlortetracycline	All food-producing species	Yes	Yes	Yes
Doxycycline	Bovine Porcine Poultry	Yes	-	-
Oxytetracycline	All food-producing species	Yes	Yes	Yes
Tetracycline	All food-producing species	Yes	Yes	Yes

EU-authorised VMP formulations, based on sales reported to ESVAC

Species		**Route of administration**						
		Group		**Individual**				
		In-feed	**In-water**	**Injection**	**Oral e.g. tablet, paste, powder**	**Topical/local (incl. intrauterine)**	**Intra-mammary**	**Oral powder**
Major	**Cattle**	C, D, O, T	C, D, O, T	D, O, T		C, O, T	C, T	C, D, O, T
	Sheep (for meat)	O, T	C, D, O, T	O		O, T		O
	Pigs	C, D, O, T	C, D, O, T	D, O		O, T		C, D, O, T
	Chickens	C, D, O, T	C, D, O, T	O	O			C, O, T
	Dogs	O	D, O, T	O	C, D, O			O
	Cats	O	D, O, T	O	C, D, O			O
Limited market species As listed in SPCs	**Turkeys**	C, O, T	C, D, O, T	O	O			C, O
	Ducks	C	O					C, O
	Geese	C, O	D, O					
	Horses		O	O		C, O, T		O
	Goats	C, O, T	C, D, O, T	O		O, T		O
	Fish	C, O	O					O
	Salmon	O						
	Carp	O						O
	Trout							O

	Sea bream, Sea bass	O						
	Rabbits	C, O	C, D, O	O				
	Minks	C, O	O		O			
	Fur animals	O	O					
	Guinea fowls	C, O	O					
	Quails	C, O	O					
	Pheasants	C, O	O		O			
	Racing pigeons	O	C, D, O, T		D, O			D, O
	Partridges	C, O						
	Ornamental birds	O	D, O, T		O, T			O
	Rodents		D					
	Pet rabbits		D					
	Snakes		D					
	Ferrets	O			O			
	Buffaloes			O				
	Deer			O				
	Donkeys					O		

C – chlortetracycline (CTC), D – doxycycline, O – oxytetracycline (OTC), T - tetracycline

Summary of main indications and contra-indications for EU-authorised VMPs, based on selected SPCs

Main indications	Tetracyclines are authorised in many different formulations as indicated above, for use in all major food-producing and companion animal species, and many minor species, including horses. It should be noted that indications for some products refer only to 'treatment of organisms susceptible to [*tetracycline X*]' meaning that a wide range of indications are encompassed within authorised use. Injectable formulations are available for a wide range of indications as might be needed for treatment of individual animals, e.g. respiratory infections (e.g. pasteurellosis), mastitis (*Trueperella pyogenes*, *E. coli*, *Staphylococcus aureus*, *Streptococci*), metritis (*E. coli* or *Streptococcus pyogenes*), navel/joint ill, septicaemia (*Salmonella Dublin* and *Streptococcus pyogenes*), enzootic abortion in sheep (*Chlamydophila abortus*), hoof infections (*Dichelobacter nodosus*, *Fusobacterium necrophorum*), etc; and for certain specific infections e.g. Ehrlichiosis, leptospirosis, listeriosis, erysipelas; keratoconjunctivitis (*Moraxella bovis*). Tetracyclines are available as in-feed and drinking water formulations for administration to pigs, calves, lambs and poultry. The indications for terrestrial animals mostly relate to a range of respiratory diseases and enteritic diseases, e.g. in pigs, atrophic rhinitis caused by *Pasteurella multocida* and *Bordetella bronchiseptica*; bronchopneumonia caused by *Pasteurella multocida*, *Streptococcus suis* and *Mycoplasma hyorhinis*; pleuropneumonia caused by *Actinobacillus pleuropneumoniae*; streptococcal meningitis. In poultry, respiratory infections caused by *Mycoplasma* spp., *Escherichia coli*, *Haemophilus paragallinarum*, *Salmonella* spp., *Ornithobacterium rhinotracheale* and *Bordetella avium*; enteritis caused by *Clostridium perfringens*. In (pre-ruminant) calves, bronchopneumonia and pleuropneumonia caused by *Pasteurella* spp., *Streptococcus* spp., *Trueperella pyogenes*, *Histophilus somni* and *Mycoplasma* spp. In racing pigeons and ornamental birds, doxycycline is authorised for treatment of infections caused by *Chlamydophila psittaci*, *Pasteurella multocida*, *Mycoplasma* spp. In food-producing fish, premix formulations are also available for treatment and control of furunculosis due to *Aeromonas salmonicida* and columnaris disease (*Flavobacterium columnare*) in Atlantic salmon, and furunculosis and enteric redmouth disease (*Yersinia ruckeri*) in Rainbow trout. Oxytetracycline and doxycycline tablets are available for treatment of respiratory and soft tissue infections in cats and dogs. Named target pathogens include staphylococci, streptococci, *Bordetella bronchiseptica*, *Pasteurella* spp., *Leptospira* spp., *Chlamydophila felis*, *Ehrlichia canis*. Topical treatments containing tetracyclines are also available for treatment of eye infections, wounds and interdigital dermatitis.
Contraindications	Do not use in animals with impaired liver or kidney function. Should not be used in early pregnancy. Oxytetracycline is not to be administered to horses concomitantly with corticosteroids. For doxycycline, there are contraindications for use in animals with known photosensitivity and diseases associated with vomiting or dysphagia.

Examples of EU-authorised HMP formulations, from Article 57 database

Substance	Route of administration		
	Injection	Oral e.g. tablet, liquid	Topical/local
Chlortetracycline			x
Demeclocycline		x	x
Doxycycline	x	x	x
Lymecycline		x	
Metacycline		x	
Oxytetracycline			x
Rolitetracycline			x
Tetracycline		x	x

Existing recommendations

WOAH recommendations

Tetracyclines are categorised VCIA by WOAH (formerly OIE). *Specific comments:* The wide range of applications and the nature of the diseases treated make tetracyclines extremely important for veterinary medicine. This class is critically important in the treatment of many bacterial and chlamydial diseases in a wide range of animal species. This class is also critically important in the treatment of animals against heartwater (*Ehrlichia ruminantium*) and anaplasmosis (*Anaplasma marginale*) due to the lack of antimicrobial alternatives.

WHO classifications

WHO: HIA

- (C1: Yes) Limited therapy for infections due to *Brucella* spp., *Chlamydia* spp., and *Rickettsia* spp.
- (C2: No) Countries where transmission of brucellosis from non-human sources to humans is common should consider making tetracycline a critical antibiotic, as there is considerable concern regarding the availability of effective products where *Brucella* spp. are endemic.

  There are differences in activity and resistance mechanisms in tetracyclines (e.g. minocycline, doxycycline compared to chlortetracycline) against some bacteria such as *Acinetobacter* spp. In future editions, the tetracycline class may need to be separated into different groups.

WHO AWaRe: Watch: e.g. chlortetracycline, metacycline, oxytetracycline; Access: doxycycline, tetracycline

AMEG and CVMP recommendations

Tetracyclines are included in the AMEG Category D. There are alternative treatments in human and veterinary medicine for their indications and that do not select for resistance to Category A substances through specific multiresistance genes.

These antibiotics are not devoid of negative impact on resistance development and spread. To keep the risk from use of these antibiotic classes as low as possible it is important that responsible use principles are complied with in everyday practice. Unnecessary use and unnecessarily long treatment periods should be avoided and group treatment restricted to situations where individual treatment is not feasible.

AMEG Category D. Tetracyclines were recognized as important for treatment of *Brucella* spp. in humans. In animals: No alternatives for treatment of heartwater (*Ehrlichia ruminantium*) and anaplasmosis, although these are diseases with low incidence. Fewer alternatives for vector-borne diseases in dogs and cats.

The CVMP has conducted five referral procedures for doxycycline products. These relate to harmonisation of SPCs, indications and dosing regimens [358].

Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Disclaimer: The information in this section reflects reported use of antimicrobials outside the terms of a marketing authorisation. No evaluation is made in this section by the working group on the efficacy or safety of the reported uses, or on their potential impact on development and dissemination of AMR.

Information from published sources

Considering the broad range and often non-specific indications stated in many SPCs and the wide range of animal species for which this class is authorised, uses outside the SPC mostly relate to exotic or limited market species or use of human authorised formulations. In textbooks, it is mentioned that tetracyclines are also used for non-antibiotic effects e.g. anti-inflammatory and immunomodulatory effects. Examples include immune-mediated skin diseases e.g. pemphigus foliaceous and discoid lupus erythematosus in dogs and angular limb deformities (tendon contracture) in foals [53, 125, 359, 360].

Information from the open call for data on use of antimicrobials in animals

The information below is summarised from the open call for data. Inclusion in the table does not endorse use or imply that it is consistent with use according to legislative provisions in Articles 112 to 114.

Substance	Species	Indication	Alternatives	Consequences of unavailability
Doxycycline	Cats	Pyoderma resistant to alternatives, Lymphoplasmacytic pododermatitis	None	
Tetracyclines	Dogs	Immune-mediated skin disease		Possible euthanasia
Doxycycline	Equines	Lyme disease, anaplasmosis, theileriosis, bone and dental infections		
Oxytetracycline spray	Equines	'Thrush' – Fusobacterium infections		
Doxycycline (human tablet product)	Equines	Neonatal infections, intracellular infections, osteomyelitis, Lawsonia spp., borreliosis	Oxytetracycline, but need for IV use is impractical	For Lawsonia - Use of more modern macrolides, such as gamithromycin, azithromycin, clarithromycin (and rifampicin)
Tetracycline	Equines	Bacterial infections		
Tetracycline	Mink	Enteritis (E.coli), pneumonia, mastitis, wounds	None authorised	Increased mortality, chronic illness
Doxycycline	Fish	Various bacterial diseases	None. Use in re-circulating aquaculture systems.	Other TCs, but these may have higher environmental impact
Doxycycline	Rabbits	Pneumonia, enteritis, skin disease		
Doxycycline	Rats	URT		
Oxytetracycline	Goats	Chlamydia abortion		
Tetracycline	Goats	Mycoplasma spp.		
Oxytetracycline	Macaca fascicularis	Chronic diarrhoea due to Balantidium		Mortality
Oxytetracycline	Teleosts, penguins,	Bacterial and protozoal infections		

	pelicans, shorebirds			
Oxytetracycline	Guinea pigs	Peri-operative period for telemetry device implantation		Mortality
Oxytetracycline	Rabbits	Tularaemia (Francisella tularensis)		
Oxytetracycline	Sea bream, Seabass	Pasteurellosis Pasteurellosis + vibriosis		Florfenicol
Oxytetracycline	Ornamental fish			
Doxycycline	Ornamental birds	Chlamydiosis, ornithosis/ psittacosis, respiratory diseases		Animal welfare
Doxycycline	Reptiles	Mycoplasmas	None	
Chlortetracycline	Pheasant, partridge	Mycoplasma, Motile Protozoal infections, Bacterial infections, Dysbacteriosis	Tiamulin, doxycycline	Mortalities
Chortetracycline eye ointment (human product)	Horses	bacterial eye infections, immunologic keratitis		Possible enucleation or euthanasia
Chortetracycline eye ointment (human product)	Cat, ferret			
Doxycycline (human oral suspension product)	Cat	Protozoal infections, bacterial infections (mycoplasma, chlamydophila)	Tablets, but difficulty with administration	

4.16.2. Evaluation

Scope of permitted use according to the MRL Regulation

Various tetracyclines are included in Table 1 (allowed substances) of the Annex to Regulation (EU) 37/2010 and individual substances in the class can be used in all food-producing species in accordance with Articles 113 and 114 of Regulation (EU) 2019/6. MRLs are established for tissues, milk and eggs. There are no 'other provisions' that might be relevant for use outside the terms of the marketing authorisation.

Tetracyclines can be used in non-food-producing species in accordance with Article 112.

Glycylcyclines are sometimes included as a sub-class of tetracyclines; however, along with omadacycline and eravacycline, they are included in the substances reserved for human use (Annex to Regulation (EU) 2022/1255) and are outside scope of this advice [2].

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Importance for human health

In human medicine, tetracyclines are used to treat infections caused by many aerobic Gram-positive and Gram-negative bacteria (e.g., Enterobacterales, *Brucella* spp.) as well as atypical pathogens, such as *Rickettsia* spp. and *Chlamydia* spp. In the EU, approved indications include: RTIs (pneumonia and other lower RTIs due to susceptible strains of *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Klebsiella pneumoniae* and other organisms); *Mycoplasma pneumoniae* pneumonia; chronic bronchitis and whooping cough; UTIs caused by susceptible strains of *Klebsiella* spp., *Enterobacter* spp.,

Escherichia coli, Enterococcus faecalis and other organisms; sexually transmitted diseases (infections due to *Chlamydia trachomatis)* [361, 362]. Tetracycline remains an important agent in the therapy of severe diarrhoea due to *Vibrio cholerae* and in salvage eradication regimens for *Helicobacter pylori* [363]. Although these indications are important, newer alternatives are available. Doxycycline is included as part of protocols for treatment and prevention of malaria [364].

Importance for animal health

In veterinary medicine, tetracyclines are first-line antibiotics for a wide variety of infections in all major and several 'limited market' species (e.g. turkeys, goats, horses). Tetracyclines are the second most used antibiotic class in the EU, comprising 25.8% of the sales in mg/PCU [15]. In food-producing animals they are used to treat respiratory infections (due e.g. to mycoplasmas, *Pasteurella multocida, Bordetella bronchiseptica, Mannheimia haemolytica*), enteritis, clostridial infections, listeriosis, interdigital and digital dermatitis, metritis, enzootic abortion and mastitis. In fish, tetracyclines can be used to treat furunculosis due to *Aeromonas salmonicida,* columnaris disease and enteric redmouth disease in Rainbow trout. In dogs and cats, they are used for respiratory infections (e.g. *B. bronchispetica, P. multocida*) and soft tissue infections (mainly doxycycline). AMEG recognised that in animals tetracyclines are one of few alternative treatments for vector-borne diseases caused by intracellular bacteria, and in particular that there are no alternatives for treatment of heartwater (*Ehrlichia ruminantium*) and anaplasmosis; although these diseases occur with low incidence in the EU and resistance is rare [33]. Tetracyclines are included in the AMEG category D.

Indications for some veterinary medicinal products refer only to 'treatment of organisms susceptible to [*tetracycline X*]' meaning that a wide range of non-specified indications are encompassed in authorised use. Hence, according to the 'open call for data' use outside the marketing authorisation mostly related to use of a particular product in an unauthorised (minor or exotic) species, or to use of human formulations e.g. eye ointments, that have more limited availability in veterinary medicine. There are also reports of use of tetracyclines for non-antimicrobial effects, e.g. for treatment of immune-mediated skin diseases in companion animals and tendon contracture in foals.

Development and selection of resistance

Resistance to tetracyclines occurs in Gram-negative and Gram-positive bacteria due to several mechanisms, encoded by numerous *tet* genes borne on MGEs. The most common mechanisms are efflux systems and ribosomal protection. Resistance may also be due to enzymatic inactivation. The *tet*(B) gene is present in a wide range of Gram-negative bacteria and encodes an efflux pump conferring resistance to tetracyclines including doxycycline and minocycline. *Tet* genes may be chromosomal but several *tet* genes are also frequently found on multiresistance plasmids or other MGE. Widespread distribution of specific *tet* genes, such as *tet*(B) or *tet*(M), supports exchange between different ecosystems including humans and animals [365-367]. LA-MRSA is typically resistant to tetracyclines [64].

Owing to their long term and extensive use, acquired resistance to tetracyclines is widespread in Enterobacterales and other important pathogens. According to EFSA mandatory surveillance of AMR in zoonotic and indicator bacteria [28], prevalence of resistance to tetracyclines is generally high in *Salmonella* spp. from humans and pigs and moderate-high in isolates from poultry (except laying hens). In indicator *E. coli*, levels of resistance to tetracyclines are high in pigs and poultry and moderate in calves, although there is geographical variation and a decreasing trend in some countries. The occurrence of resistance to tetracyclines in *Campylobacter* spp. from humans and food-producing animals is generally high-extremely high in the EU. Under the voluntary monitoring of MRSA from food-producing animals 2019/20, tetracycline resistance was 100% in MRSA from most reported

animal populations. Likewise, levels of resistance to tetracyclines in key target animal pathogens are moderate to high e.g. in *Streptococcus suis* and respiratory pathogens (APP, *P. multocida*) from pigs; *E. coli* from gastrointestinal infections in pigs, calves and poultry and *Staph. pseudintermedius* from dogs [28]. Resistance in obligate intracellular pathogens e.g. *Anaplasma, Chlamydia* and *Ehrlichia* remains low [33, 294].

Transmission of resistance

Resistance to tetracyclines can be transferred from food-producing and companion animals to humans via zoonotic pathogens (e.g. *Campylobacter, Salmonella* spp., MRSA) or commensal bacteria capable of transferring resistance to pathogens. According to the JIACRA report, tetracycline resistance in *C. jejuni* from humans was related to tetracycline resistance in *C. jejuni* from poultry [89].

In conclusion,

- Tetracyclines are important as **first-line** antibiotics to treat a wide range of infections in humans and animals (food-producing and non-food animal species).
- There is evidence for the selection and transmission of resistance to tetracyclines from animals to humans and between animals via zoonotic and target pathogens or commensal bacteria capable of transferring resistance to pathogens.
- Widespread high prevalence of resistance to tetracyclines in common pathogens has limited their usefulness in both human and veterinary medicine.

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to Tetracyclines.

Criterion (c) – availability of other treatments for animals

Tetracyclines are in the AMEG's category D and in general there are alternative antimicrobials (e.g. aminopenicillins, sulfonamides) dependent on the specific disease, pathogen and target animal species under treatment. In some instances, the only alternatives may be from a higher AMEG category e.g. for infections due to mycoplasmas. For anaplasmosis, babesiosis and *Ehrlichia* infections in ruminants, oxytetracycline is the recommended treatment, although disease prognosis is poor and disease management largely reliant on vector control. For *Ehrlichia* and *Anaplasma* spp. infections in dogs, doxycycline is preferred but alternatives include chloramphenicol, imidocarb or fluoroquinolones [368]. These diseases occur with low incidence in Europe.

Non-antimicrobial uses:

One of the most common congenital deformities of newborn foals is contracted digital flexor tendons. There is a range of presentations from straightening of a forelimb through to a severe contracture where the knees or the fetlock and/or corono-pedal joint cannot be held straight and the foal 'knuckles over'. Since the early 1990's, it has become traditional in equine medicine to treat foals with 'contracted tendons' with super-high doses of oxytetracycline [369-371]. Oxytetracycline chelates calcium, *in vivo*, leading to temporary tendon relaxation. The SPC specifies a dose for horses as 5-10 mg/kg, which is within the range for toxic effects. In young foals, the principle of treatment for contracted tendons is to keep the foal's leg sufficiently straight to allow walking and stretching which will correct the issue. However, in severely contracted tendons there is no alternative treatment other than super-high dose/s of oxytetracycline.

For treatment of autoimmune pemphigus diseases in dogs the mainstay of treatment includes use of immunosuppressive drugs such as glucocorticoids or azathioprine, which are associated with well-

known adverse effects. Tetracycline alongside niacinamide is sometimes used for its anti-inflammatory properties, as an adjunctive therapy or alone to treat milder cases. Other alternatives e.g. cyclosporine, tacrolimus, may also be used but have potential for toxicity especially when administered orally [359, 372].

Criterion (d) – availability of other antimicrobial treatments for humans

For most of the approved indications, treatment alternatives are available: penicillins, cephalosporins, respiratory fluoroquinolones for pneumonia; beta-lactam-BLI, macrolides and cephalosporins for acute exacerbations of COPD; fosfomycin , pivmecillinam, cephalosporins and TMP-SMX for uncomplicated UTI and a number of classes (fluoroquinolones, cefepime, ceftazidime, piperacillin-tazobactam, carbapenems) for complicated UTI - here combinations with other antibacterials are usually needed; azithromycin for *Chlamydia*, *Mycoplasma* and *Ureaplasma* STIs; macrolides in acne [3].

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

- Tetracyclines are important as first-line antibiotics to treat a wide range of infections in humans and animals (food-producing and non-food animal species).
- There is evidence for the selection and transmission of resistance to tetracyclines from animals to humans and between animals via commensal and pathogenic bacteria. Widespread high prevalence of resistance to tetracyclines in common pathogens has limited their usefulness in both human and veterinary medicine.
- In general, there are alternatives to tetracyclines available for the given bacterial indications in both human and veterinary medicine. There are few alternatives in veterinary medicine for treatment of certain intracellular organisms (e.g. *Ehrlichia*), but levels of resistance to tetracyclines in these species are low.
- However, tetracyclines are in AMEG Category D and the alternative classes may be in a higher AMEG category, associated with higher AMR risk to public and animal health.
- Tetracyclines are authorised for use in all major food and non-food species, and some limited market species, to treat a broad range of infections, which are not always specified in the SPC. They are also available in formulations for oral group and individual medication and for parenteral and topical use. Therefore, it is unlikely that use outside the terms of the marketing authorisation would contribute to the AMR risk substantially beyond authorised use.

Therefore, considering the points above relevant to criteria (b), (c) and (d), it is recommended that no conditions should be placed on the use of Tetracyclines outside the terms of the marketing authorisation, although responsible antimicrobial use principles should be applied.

4.17. Minocycline

4.17.1. Background information

Minocycline has not been authorised in VMPs in the EU.

Examples of substances in the class that are authorised in human medicine only in the EU

Examples of substances authorised for human use	Examples of ATC codes
Minocycline	J01AA08 A01AB23

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

Minocycline is not included in Table 1 (allowed substances) of the Annex to the MRL Regulation (EU) 37/2010 and cannot be used in food-producing animals in the EU.

Summary of main indications and contra-indications for EU-authorised HMPs, based on selected SPCs

Main indications	Minocycline is notably indicated for the treatment of the following infections: • Gonorrhoea. • Non-gonococcal urethritis. • Prostatitis. • Moderate to severe acne; use in moderate acne only if topical treatment is ineffective, if acne is extensive or hard to reach and if there is a high risk of scarring. • Acute and chronic bronchitis. • Bronchiectasis. • Lung abscess. • Pneumonia. • Ear, nose and throat infections. • Urinary tract infections. • Pelvic inflammatory disease (e.g. salpingitis, oophoritis). • Skin and soft tissue infections caused by minocycline sensitive organisms. • Ophthalmic infections. • Nocardiosis. • Prophylactic treatment of asymptomatic meningococcal carriers. • Pre and post-operative prophylaxis of infection.
Contraindications	Severe liver impairment. Pregnancy and lactation. In France minocycline led to a restriction of the indication due to a risk of severe hypersensitivity syndromes and autoimmune disorders. Microbiologically documented infections of bacterial strains resistant to other cyclins and sensitive to minocycline (see Section 5.1 of the SPC) and for which no oral antibiotic seems appropriate".

Examples of EU-authorised HMP formulations, from Article 57 database

Substance	Route of administration		
	Injection	Oral e.g. tablet, liquid	Topical/local
Minocycline		x	x

Existing recommendations

<u>WOAH recommendations</u>

Minocycline is not classified by WOAH (formerly OIE).

<u>WHO classifications</u>

WHO: HIA (as tetracyclines)

- (C1: Yes) Limited therapy for infections due to *Brucella* spp., *Chlamydia* spp., and *Rickettsia* spp.
- (C2: No) Countries where transmission of brucellosis from non-human sources to humans is common should consider making tetracycline a critical antibiotic, as there is considerable concern regarding the availability of effective products where *Brucella* spp. are endemic.

  There are differences in activity and resistance mechanisms in tetracyclines (e.g. minocycline, doxycycline compared to chlortetracycline) against some bacteria such as *Acinetobacter* spp. In future editions, the tetracycline class may need to be separated into different groups.

WHO AWaRe: IV route in Reserve group; Oral route in Watch group.

AMEG recommendations

Tetracyclines are included in the AMEG Category D, minocycline was not assessed specifically. There are alternative treatments in human and veterinary medicine for their indications and that do not select for resistance to Category A substances through specific multiresistance genes.

These antibiotics are not devoid of negative impact on resistance development and spread. To keep the risk from use of these antibiotic classes as low as possible it is important that responsible use principles are complied with in everyday practice. Unnecessary use and unnecessarily long treatment periods should be avoided and group treatment restricted to situations where individual treatment is not feasible.

Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Disclaimer: The information in this section reflects reported use of antimicrobials outside the terms of a marketing authorisation. No evaluation is made in this section by the working group on the efficacy or safety of the reported uses, or on their potential impact on development and dissemination of AMR.

Information from published sources

Dogs and cats

According to textbooks, minocycline is used outside the marketing authorisation for susceptible skin and soft tissues, respiratory tract and joint infections. It may also be effective for infections due to *Rickettsia* spp., *Ehrlichia canis, Borrelia burgdorferi* and Brucellosis [46, 119].

According to the CVMP's Reflection paper on off-label use, minocycline has been used in the treatment of canine hemangiosarcoma [373].

Some references indicate potential efficacy for the treatment of MRSP in dogs [374, 375].

Horses

Minocycline is used in horses to treat susceptible bacterial infections and tick-borne diseases such as *Ehrlichia*, *Anaplasma*, and *Borrelia burgdorferi* (Lyme disease). There is some research that suggests that oral minocycline may have superior bioavailability and reach higher tissue-concentrations in horses when compared to oral doxycycline.

Information from the open call for data on use of antimicrobials in animals

No information on use outside the terms of a marketing authorisation was provided in the open call for data.

4.17.2. Evaluation

Scope of permitted use according to the MRL Regulation

Minocycline is not included in the Annex to the MRL Regulation (EU) 37/2010 and cannot be used in food-producing animals in the EU.

Minocycline can be used in non-food-producing animals in accordance with Article 112.

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Importance for human health

Minocycline has a spectrum of activity that is largely similar to that of the tetracyclines. It is reported to be effective in vitro against some tetracycline-resistant *Staphylococcus* spp., *Streptococcus* spp., and certain strains of tetracycline-resistant *E. coli* and *Haemophilus influenzae*. It is active against a variety of intracellular microorganisms, including *Mycoplasma pneumoniae*, *Ureaplasma urealyticum*, *Chlamydia trachomatis*, *Chlamydophila psittaci*, and *Chlamydophila pneumoniae* [376].

Minocycline is indicated for the treatment of the following infections: gonorrhoea, non-gonococcal urethritis, prostatitis, moderate to severe acne, acute and chronic bronchitis, bronchiectasis, lung abscess, pneumonia, ear, nose and throat infections, UTIs, pelvic inflammatory disease (e.g., salpingitis, oophoritis), SSTIs, ophthalmic infections, nocardiosis, prophylactic treatment of asymptomatic meningococcal carriers, pre- and post-operative prophylaxis of infection. It is a potential option for the treatment of infections caused by certain strains of MRSA and MDR *Acinetobacter baumannii*, although notably used in combination with other classes of antimicrobials (e.g., carbapenems) [376].

Alternative and safer options are available.

Importance for animal health

Minocycline is not authorised in veterinary medicine in the EU and cannot be used in food-producing species as it is not included in the Annex to the MRL Regulation (EU) 37/2010. Earlier generation tetracyclines are currently judged to be sufficiently efficacious to cover most of the bacterial infections encountered in veterinary medicine that specifically require treatment with tetracyclines (e.g. vector-borne diseases). However, second generation tetracyclines, doxycycline and minocycline could present an interest in specific infections when the bacterial pathogen has been shown to be resistant to earlier generation tetracyclines (e.g. *Staphylococcus pseudintermidius* infections in dogs). According to WAVD guidelines, minocycline may be a treatment for MRS infections that are resistant to other tetracyclines due to *tet*(K), and could be used dependent on the results of susceptibility testing using the breakpoints for doxycycline [334].

No cascade use of minocycline was quoted by stakeholders in the call for data.

Development, selection and transmission of resistance

Resistance mechanisms to minocycline are generally common to all tetracycline classes (except the newer generation TCs). Resistance to tetracyclines occurs in Gram-negative and Gram-positive bacteria due to several mechanisms, encoded by numerous *tet* genes borne on MGEs. The most common mechanisms are efflux systems and ribosomal protection. Resistance may also be due to enzymatic inactivation. The *tet*(B) gene is present in a wide range of Gram-negative bacteria and

encodes an efflux pump conferring resistance to tetracyclines including doxycycline and minocycline. Widespread distribution of specific *tet* genes, such as *tet*(B) or *tet*(M), supports exchange between different ecosystems including humans and animals [365, 366].

Owing to their long term and extensive use, acquired resistance to tetracyclines is widespread in Enterobacterales and other important pathogens. According to EFSA mandatory surveillance of AMR in zoonotic and indicator bacteria, prevalence of resistance to tetracyclines is generally high in *Salmonella* spp. from humans and pigs and moderate-high in isolates from poultry (except laying hens). In indicator *E. coli*, levels of resistance to tetracyclines are high in pigs and poultry and moderate in calves, although there is geographical variation and a decreasing trend in some countries. The occurrence of resistance to tetracyclines in *Campylobacter* spp. from humans and food-producing animals is generally high-extremely high in the EU. Likewise, levels of resistance to tetracyclines in key target animal pathogens are moderate to high e.g. in *Streptococcus* suis and respiratory pathogens (APP, *P. multocida*) from pigs; *E. coli* from gastrointestinal infections in pigs, calves and poultry and *Staph. pseudintermedius* from dogs [28].

Although minocycline is not authorised in VMPs, there is evidence for the selection and transfer of resistance from animals to humans or other animals, through zoonotic or target pathogens or commensals capable of transferring resistance to pathogens, if use in animals became established.

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to Minocycline.

Criterion (c) – availability of other treatments for animals

Where tetracyclines are specifically indicated in veterinary medicine, (e.g. for vector-borne diseases), first or second generation tetracyclines (e.g. oxytetracycline, doxycycline) are currently judged to be adequate.

Alternatives for *Staph. pseudintermedius* include amoxicillin-clavulanic acid and first generation cephalosporins. For MRS infections in companion animals, alternatives are dependent on the results of susceptibility testing. Topical treatments may be effective, or where systemic treatment is needed, rifampicin or amikacin could be alternatives for infections not susceptible to veterinary-authorised antimicrobials.

Criterion (d) – availability of other antimicrobial treatments for humans

Minocycline is nationally authorised in some of the EU member states for indications that include ear, nose and throat infections, RTIs such as pneumonia, bronchiectasis, lung abscess, acute and chronic bronchitis, prostatitis, venereal diseases (gonorrhoea), UTIs, pelvic inflammatory disease (salpingitis, oophoritis), SSTIs, can, ophthalmological infections, nocardiosis, prophylactic treatment of asymptomatic meningococcal carriers, preventative treatment before and after surgery, actinomycosis, anthrax patients, with a penicillin allergy. However, sufficient effective or safer alternative options exist for the treatment of the presented serious infections.

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

- Minocycline is authorised in human medicines in some EU member states; however, sufficient effective or safer alternative options exist for the treatment of the presented infections.
- No veterinary medicines containing minocycline are authorised in Europe. In the absence of MRLs, minocycline can only be used outside the terms of the marketing authorisation in non-food-producing animals. Minocycline is a potential option for treatment of infections caused by certain

intracellular microorganisms, although alternatives are available, and MRS that are resistant to earlier generation tetracyclines.

- Widespread high prevalence of resistance to tetracyclines including to minocycline in common pathogens has limited their usefulness in both human and veterinary medicine.
- It is considered that possible use outside the terms of the authorisation will be very rare.

Therefore, considering the points above relevant to criteria (b), (c) and (d), it is recommended that no conditions should be placed on the use of Minocycline outside the terms of the marketing authorisation, although responsible antimicrobial use principles should be applied.

4.18. Amphenicols

4.18.1. Background information

Examples of substances included in the class that are authorised in veterinary and human medicine in the EU

Examples of substances authorised for veterinary use	Examples of ATCvet codes
Chloramphenicol	QJ01BA1 QJ51BA01 QS01AA01 QS02AA01 QS03AA08 QD06AX02 QD10AF03 QG01AA05
Florfenicol	QJ01BA90 QJ51BA90
Thiamphenicol	QJ01BA02 QJ51BA02
Examples of substances authorised for human use	**Examples of ATC codes**
Chloramphenicol	J01BA01
Thiamphenicol	J01BA02

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

Substance	Species	MRL tissues	MRL milk	MRL eggs	Other provisions
Chloramphenicol	Prohibited substance in Table 2 - MRL cannot be established				
Florfenicol	All food-producing species	Yes	-	-	Not for animals from which milk is produced for human consumption Not for animals from which eggs are produced for human consumption
Thiamphenicol	All food-producing species	Muscle Fat Liver Kidney	Yes	-	Not for use in animals from which eggs are produced for human consumption

EU-authorised VMP formulations, based on sales reported to ESVAC

Species		Route of administration						
		Group		Individual				
		In-feed	In-water	Injection	Oral powder	Oral e.g. tablet, paste, powder	Topical/local (incl. intrauterine)	Intra-mammary
Major	**Cattle**		TAP	FF, TAP		TAP	TAP	TAP
	Sheep (for meat)	-	-	FF, TAP		TAP	TAP	
	Pigs	FF, TAP	FF, TAP	FF, TAP	FF		TAP	

	Chickens	FF, TAP	FF, TAP			FF		
	Dogs		CHL	CHL		TAP		
	Cats		CHL	CHL		TAP		
Limited market species *	**Turkeys**	_	TAP	TAP	_	FF		
	Poultry	_	FF		_			
	Ducks	_	FF, TAP		_			
	Geese	_	FF, TAP		_			
	Goats		_	TAP		TAP	TAP	
	Pigeons		CHL		CHL	CHL		
	Rodents	_	CHL		_			
	Fish	FF	_		_			
	Ornamental birds	_	CHL		_			
	Horses						TAP	
	Rabbits						TAP	
	Mink						TAP	

TAP (thiamphenicol), FF (florfenicol), CHL (chloramphenicol)

Summary of main indications and contra-indications for EU-authorised VMPs, based on selected SPCs

Main indications	**Chloramphenicol:** Dog, cat: Oral tablets and injections have limited availability. Eye drops - Treatment of bacterial eye infections such as conjunctivitis, keratitis, dacryocystitis and blepharitis, caused by chloramphenicol-susceptible bacteria pump spray Treatment of bacterial skin infections caused by chloramphenicol-susceptible bacteria Ear drops – Treatment of otitis externa associated with *Staph. pseudintermedius* Racing pigeons: Oral powder - Treatment of primary and secondary bacterial infections caused by chloramphenicol-susceptible bacteria (respiratory infection, e.g., coryza contagiosa, UTI, GIT e.g. salmonellosis, central nervous system, skin, eye, ear canal infections) **Thiamphenicol:** Cutaneous spray, solution Horses, cattle, goats, sheep, pigs, mink, rabbits: Treatment of superficial wound infections caused by micro-organisms susceptible to thiamphenicol. Cattle, goats and sheep: Treatment of infections of the claw and hoof such as foot rot, interdigital dermatitis, digital dermatitis caused by micro-organisms susceptible to thiamphenicol. Injection Cattle, sheep, pigs: septicaemia, intestinal infection, bronchopneumonia, secondary infection, urinary tract infections, metritis, pyometra, mastitis, nail infections and dermatitis Caused by: *Salmonella* spp., *Escherichia coli*, *Pasteurella* spp., *Clostridium* spp. and other microorganisms Oral powder for in-feed use Chickens (broilers) and pigs: respiratory and gastrointestinal infections due to susceptible microorganisms. Oral solution Calves: Treatment of respiratory infections caused by strains of *Mannheimia haemolytica* and *Pasteurella multocida*. Pigs: respiratory infections caused by *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, *Haemophilus parasuis*, *Streptococcus suis* and *Mycoplasma hyopneumoniae* Premix

	Chickens - Treatment of respiratory and gastrointestinal infections due to staphylococci (including *Staphylococcus aureus*), streptococci (including *Streptococcus progenes*), *Shigella* spp., *Pasteurella* spp., *E. coli*, *Proteus* spp., *Salmonella* spp. (*Salmonella typhi* and *Salmonella paratyphi*), chlamydia and mycoplasmas. **Florfenicol:** Chicken: Oral solution Treatment of bacterial infections caused by florfenicol-susceptible *E. coli* Cattle: Iinjectable Preventive and therapeutic treatment of respiratory tract infections due to strains of *Mannheimia haemolytica, Pasteurella multocida, Mycoplasma bovis* and *Histophilus somni* susceptible to florfenicol. Sheep: Injectable Treatment of ovine respiratory tract infections due to *Mannheimia haemolytica* and *Pasteurella multocida* susceptible to florfenicol. Pigs: oral, injectables Treatment and prevention of acute outbreaks of swine respiratory disease caused by strains of *Actinobacillus pleuropneumoniae* and *Pasteurella multocida* susceptible to florfenicol. Dogs Topical treatment of otitis externa caused by bacteria susceptible to florfenicol (*Staph. pseudintermedius*). Rainbow trout and salmon: Premix For the treatment and metaphylaxis of furunculosis caused by *Aeromonas salmonicida* susceptible to florfenicol in freshwater fisheries and other susceptible bacteria. Salmon – treatment of *Vibrio salmonicida*.
Contraindications	Do not use in adult bulls, rams and boars intended for breeding purposes. Do not use in broodstock. Do not administer intravenously. Do not use in case of hypersensitivity.

Examples of EU-authorised HMP formulations, from Article 57 database

Substance	Route of administration		
	Injection	Oral e.g. tablet, liquid	Topical/local
Chloramphenicol	x	x	x
thiamphenicol	x	x	x

Existing recommendations

WOAH recommendations

Amphenicols are categorised VCIA by WOAH (formerly OIE). *Specific comments:* The wide range of applications and the nature of the diseases treated make phenicols important for veterinary medicine. This class is of particular importance in treating some fish diseases, in which there are currently no or very few treatment alternatives. This class also represents a useful alternative in respiratory infections of cattle, swine and poultry. This class, in particular florfenicol, is used to treat pasteurellosis in cattle and pigs.

WHO classifications

WHO: HIA

- (C1: No) In certain geographic settings, Criterion 1 may be met: the class may represent one of the limited therapies for acute bacterial meningitis, typhoid and non-typhoid fever, and respiratory infections.
- (C2: Yes) May result from transmission of Enterobacterales, including *E. coli* and *Salmonella* spp., from non-human sources.

WHO AWaRe: Access: Chloramphenicol, Thiamphenicol

AMEG and CVMP recommendations

Amphenicols are included in the AMEG Category C: this category includes antibiotics for which there are alternatives in human medicine for their indications but which comply with one or both of the following criteria:

- For the veterinary indication under treatment, there are few or no alternatives belonging to Category D. Some examples of these indications are given in Table 4 of the AMEG advice [8], alongside the relevant (sub)class.
- The antibiotic selects for resistance to a substance in Category A through specific multiresistance genes.

Antibiotics placed in this category present a higher AMR risk for human and/or animal health than antibiotics placed in Category D. These antibiotics should only be used when there is no available substance in Category D that would be clinically effective.

CVMP referrals

Five referral procedures for VMPs containing amphenicols have been conducted by the CVMP since 2009. Referral procedures that led to revisions of the terms of marketing authorisation are briefly presented hereafter.

In 2009, the referral was related to a solution for injection for cattle containing florfenicol (300 mg/ml) where potential serious risk to the environment was questioned. At the end of the referral, in order to limit the environmental impact, it was concluded by the CVMP that the product should only be used for therapeutic treatment of respiratory tract infections in cattle due to *Mannheimia haemolytica, Pasteurella multocida* and *Histophilus somni*.

In 2013, the CVMP undertook a further referral for solutions for injection containing florfenicol (450 mg/ml). Concerns were raised that the clinical efficacy at a single intramuscular dose of 30 mg/kg bw in the treatment of swine respiratory disease was not satisfactorily demonstrated. The CVMP concluded that the observed high and variable clinical failure rates in the clinical field study were unacceptable and it could not be ruled out that a single intramuscular dose of 30 mg/kg bw of this time-dependent antimicrobial may not be sufficient to treat respiratory tract infections, in particular for pathogens associated with MIC values ≥1 µg/ml. Therefore, the CVMP concluded that the overall benefit-risk balance for the indication for the VMP under consideration was negative.

Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Disclaimer: The information in this section reflects reported use of antimicrobials outside the terms of a marketing authorisation. No evaluation is made in this section by the working group on the efficacy or safety of the reported uses, or on their potential impact on development and dissemination of AMR.

Information from published sources

CVMP's reflection paper on off-label use reported choramphenicol/florfenicol use in foals <4 months for septicemia, meningitis and osteomyelitis [9].

In the US, florfenicol is used for treatment of bovine interdigital phlegmon (foot rot, acute interdigital necrobacillosis, and infectious pododermatitis) associated with *Fusobacterium necrophorum* and *Bacteroides melaninogenicus* and for treatment of infectious bovine keratoconjunctivitis caused by *Moraxella bovis* [46].

Information from the open call for data on use of antimicrobials in animals

The information below is summarised from the open call for data. Inclusion in the table does not endorse use or imply that it is consistent with use according to legislative provisions in Articles 112 to 114.

Substance	Species	Indication	Alternatives	Consequences of unavailability
Chloramphenicol				
eye drops/ointments	dogs, cats, small mammals, reptiles, ornamental birds, birds of prey	eye infections, corneal ulcerations (chlamydia in cats)	few e.g. fusic acid, gentamycin protective gels but these are unlikely to be sufficient on their own. (can be supplemented with tetracycline orally, but usually does not give full cure)	deterioration of eye conditions, loss of sight (chronic and recurrent severe conjunctivitis, that can persist for months; will spread to other household cats, and on rare occasions also to humans)
eye drops	Horse	eye infections	only few other (e.g. FQs) antibacterials for eye treatments available	inability to treat leading to enucleation or euthanasia
ophthalmic	Equidae	corneal disease (ulcerative keratitis)	cloxacillin is authorised for use in horses but has a narrow spectrum of activity and is not effective against pseudomonas which is an important pathogen of the equine eye, FQs	horses could not be treated adequately, which would cause a serious violation of animal welfare and blindness
tablets	Equidae	infections with bacteria in challenging locations and limited susceptibility	none - has excellent penetration including to CSF with a spectrum of activity that makes it useful in equidae on occasional basis	horses could not be treated adequately, which would cause a serious violation of animal welfare and blindness
oral powder	rabbits (not for human consumption)	tularaemia	tetracycline	potential zoonotic disease transmission
Thiamphenicol				
topical use	Rabbits	wound infections	spiramycin	
topical aerosol spray	bovine	dermatitis digitalis	tetracyclines; chelated copper	digital dermatitis affects approximately 10% of dairy cows in UK at any single time point; it causes lameness through pain; it is not life threatening but effective licensed topical antimicrobial treatments are necessary from a welfare and production perspective; thiamphenicol is very useful for cases which do not respond to alternative licensed products
Florfenicol				
flowing powder	rainbow trout	rainbow trout fry syndrome (RTFS)	none	potential huge losses

solution for injection, oral powder, premix	fish, fish in aquaculture, ornamental fish	several bacterial diseases e.g. furunculosis, multidrug resistant bacteria	none, depends on bacterial resistance	lack in treatment of fish in aquaculture and ornamental fish, no therapy, death most likely, high mortalities in hatcheries, disaster
premix	Seabass	pasteurellosis, vibriosis	oxytetracycline	not many alternatives, so high risk of AMR development
	teleosts, elasmobranchs	bacterial infections		severe disease or death
	mink	diarrhea, pneumonia	none last option in certain cases of multidrug-resistant *E. coli* strains.	dramatically increased mortality, catastrophic for mink health and welfare
	peking duck parent flocks	bacterial infections (*mainly E.coli, Enerococcus cecorum, Pasteurella multocida, Riemerella anatipestifer*)	often none due to antibiotic resistance pattern	spread of diseases to fattening farms, increasing spread of diseases to fattening farms, increasing use of antimicrobial substances on fattening farms, high animal losses in parent flocks and in fattening flocks
oral	poultry (turkey)	colibacillosis	other antibiotics	necessity to use FQ with some *E. coli* multidrug-resistant
	ovine	genital disease		
injectables, oral solution	pigs	*Haemophilus parasuis* infections	yes, but other antimicrobials not licensed for this indication either. Vaccines available but present practical, cost and sometimes efficacy issues	poorer pig health and welfare, possibly little effect on food safety although, as a major cause of polyserositis, increased cases may well result in dressing difficulties at slaughter and increased carcase contamination by micro-organismsm such as salmonella, cascade restrictions could increase selection pressure on other classes of antimicrobial
	pigs	neonatal diarrhea caused by enterococci		
	pigs	genital infections	penicillin/streptomycin	mortality, decrease reproduction performances
in milk replacer	calf	*Mycoplasma bovis*	yes but not always so effective and this is a yellow AB only used in animals	more chronic resp. disease more mortality=community acquired pneumonia

4.18.2. Evaluation

Scope of permitted use according to the MRL Regulation

Chloramphenicol is included in Table 2 (prohibited substances) of the Annex to Regulation (EU) 37/2010 and hence cannot be used in any food-producing species.

Florfenicol and thiamphenicol are included in Table 1 (allowed substances) of the Annex to Regulation (EU) 37/2010 and hence can be used in all food-producing species in accordance with Articles 113 and 114 of Regulation (EU) 2019/6. 'Other provisions' important for use outside a marketing authorisation state that neither florfenicol nor thiamphenicol may be used in animals producing eggs for human consumption and florfenicol may not be used in animals producing milk for human consumption.

Amphenicols can be used in non-food-producing species in accordance with Article 112.

Examples of veterinary-authorised formulations/species

Formulations of chloramphenicol are authorised as eye drops for dogs and cats. Oral tablets and injections containing chloramphenicol have availability for dogs in limited MSs. An oral powder is authorised for racing pigeons, rodents and ornamental birds.

Thiamphenicol is available as an injectable formulation for pigs and ruminants. It is also available as a premix formulation for chickens and pigs, and as formulations for administration in drinking water and feed to poultry, pigs and calves. It is authorised as a cutaneous spray for horses, cattle, goats, sheep, pigs, mink and rabbits.

Florfenicol is authorised in injectable formulations for use in pigs and ruminants, as in-feed formulations for pigs, chickens and fish (trout and salmon) and drinking water preparations for pigs and poultry. Ear preparations containing florfenicol are available for dogs.

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Importance for human health

Amphenicols are a class of broad-spectrum, time-dependent bacteriostatic antibiotics that include thiamphenicol, chloramphenicol and florfenicol. Fluorinated amphenicols (e.g. florfenicol) are not used in human medicine. Thiamphenicol is a chloramphenicol analogue and has limited use in human medicine. Therefore, the information provided in this section concerns primarily chloramphenicol. Chloramphenicol inhibits protein synthesis by reversibly binding to the peptidyl transferase cavity of the 50S subunit of the bacterial 70S ribosome. This prevents the aminoacyl-tRNA from binding to the ribosome, thus terminating polypeptide chain synthesis. Chloramphenicol also inhibits mitochondrial protein synthesis in mammalian bone marrow cells in a dose-dependent manner.

Chloramphenicol is effective against a wide range of aerobic and anaerobic bacteria, including Gram-positive (e.g. *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Enterococcus faecalis)* and Gram-negative bacteria (*Bacteroides fragilis*, *Neisseria*-meningococci and gonococci, *Haemophilus* spp., *Salmonella Typhi*) [377].

Chloramphenicol was the first broad-spectrum antibiotic and has been in clinical use since 1949 [377]. Currently, it is no longer the antibiotic of choice for any specific infection and is not frequently used due to serious adverse effects (e.g. bone marrow toxicity). Chloramphenicol can be used for bacterial conjunctivitis (topical formulation), typhoid fever, meningitis (specifically in countries where access to recommended 3rd-generation cephalosporines is limited) or *S. aureus* infections (including VRSA) [377].

It is recommended by the WHO as an option for the treatment of meningitis, meningococcal sepsis, osteoarthritis, and pyomyositis in children in low-income countries, and is included on their model list of essential medicines [378].

Chloramphenicol is nationally approved in some of the EU Member States for the treatment of typhoid, meningitis caused by *H. influenzae* and for other serious infections; eyedrops are approved for the treatment of acute bacterial conjunctivitis.

Importance for animal health

Thiamphenicol is a derivative of chloramphenicol, in which the *p*-nitro group been replaced by a sulfomethxyl group. Thiamphenicol is generally 1–2 times less active than chloramphenicol, although it has equal activity against *Haemophilus, B. fragilis*, and streptococci. Florfenicol is a fluorinated derivative of thiamphenicol, in which the hydroxyl group at C-3 has been replaced with fluorine. The spectrum of activity for both florfenicol and thiamphenicol is similar (although thiamphenicol has greater activity against some anaerobes and mycoplasmas, whereas florfenicol against other clinically relevant bacteria) and includes most important enteric, respiratory and dermal/sepsis-related bacterial pathogens of food animals. Florfenicol is more widely used in feed and water because of lower minimal inhibitory concentration (MIC) values for most important pathogens (other than anaerobes and mycoplasmas), superior pharmacokinetics characteristics and reduced susceptibility to inactivation by chloramphenicol transacetylase enzymes.

The potential for idiosyncratic fatal aplastic anaemia in humans has led to prohibition of chloramphenicol use in food animals.

Florfenicol is authorised for treatment and prevention of bovine respiratory disease caused by *Mannheimia haemolytica, Pasteurella multocida, Mycoplasma bovis* and *Histophilus somni* susceptible to florfenicol, as well as treatment of ovine respiratory tract infections due to *Mannheimia haemolytica* and *Pasteurella multocida*. In pigs, florfenicol is authorised for the treatment and prevention of acute outbreaks of swine respiratory disease caused by strains of *Actinobacillus pleuropneumoniae* and *Pasteurella multocida* susceptible to florfenicol [385-387].

In third countries, florfenicol is authorised for the treatment of pododermatitis caused by *Fusobacterium necrophorum* and *Bacteroides melaninogenicus* and infectious bovine keratoconjunctivitis caused by *Morexella bovis*, however penicillin or oxytetracycline have a narrower antimicrobial spectrum and should be used first for these infections.

Florfenicol is authorised for the treatment of susceptible bacterial diseases of fish, including yersiniosis, pasteurellosis (*Pasteurella piscicida*), furunculosis (*Aeromonas salmonicida* subsp. *salmonicida*) in rainbow trout and salmon and vibriosis in salmon. In the USA, florfenicol is approved for *Flavobacterium columnare* and streptococcal septicemia associated with *Streptococcus iniae* in freshwater finfish, and for enteric septicaemia in catfish due to *Edwardsiella ictaluri* [379].

Thiamphenicol is authorised as an injection for cattle, sheep, pigs for treatment of a range of infections - septicaemia, intestinal, respiratory and urinary tract infections, metritis, pyometra, mastitis, nail infections and dermatitis. Indicated pathogens include *Salmonella* spp., *Escherichia* coli, *Pasteurella* spp., *Clostridium* spp. and other microorganisms. Oral group formulations are available for calves and pigs for respiratory infections due to susceptible microorganisms including *Mannheimia haemolytica* and *Pasteurella multocida* in calves and *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, *Haemophilus parasuis*, *Streptococcus suis* and *Mycoplasma hyopneumoniae* in pigs.

Amphenicols are authorised for chickens for treatment of respiratory and gastrointestinal infections due to susceptible pathogens including staphylococci (including *Staphylococcus aureus*), streptococci (including *Streptococcus pyogenes*), *Shigella* spp., *Pasteurella* spp., *E. coli*, *Proteus* spp., *Salmonella* spp. (*Salmonella typhi* and *Salmonella paratyphi*), chlamydia and mycoplasmas.

Fluoroquinolone antimicrobials for companion animals are the main alternative for amphenicols, but amphenicols (chloramphenicol, florfenicol) are still considered for some anaerobic infections, serious ocular infections, prostatitis, and otitis externa/media/interna in dogs and cats [46]. Amphenicol use in dogs and cats has increased due to the increase in MRSA and MRSP infections, but chloramphenicol is associated with more adverse effects (mainly gastrointestinal and bone marrow) than other treatment options such as doxycycline, clindamycin and amikacin [380].

Uses of amphenicols outside the terms of the MA reported to the open call for data included treatment of indications not authorised e.g. neonatal diarrhoea due to enterococcal infections in piglets, *Glaesserella parasuis* infections in pigs, and RTFS in trout. Use was also reported in unauthorised minor species e.g. horses, zoo animals, mink. There were also reports of use of specific formulations (administration routes) in species for which they are not authorised, in particular chloramphenicol eye drops to treat infections in minor species e.g. horses, reptiles, ornamental birds.

In addition, florfenicol can sometimes be used for the treatment of *Salmonella abortusovis* in sheep when antibiotic susceptibility tests are available, and resistance to other antibiotics (tetracyclines and sulfonamides) is identified. In these cases, its use is metaphylactic (parenterally) for all animals that have been exposed to the agent (generally contaminated water) and are pregnant (JMGS, personal communication).

Selection, development and transmission of resistance

The most common mechanism of bacterial resistance to chloramphenicol is enzymatic inactivation by acetylation by chloramphenicol acetyltransferases (CAT genes). Acetylation of the hydroxyl groups on chloramphenicol prevents drug binding to the 50S ribosomal subunit. Other mechanisms of resistance include efflux systems, inactivation by phosphotransferases, and mutations of the target site or permeability barriers [381]. CAT genes are commonly found on plasmids, transposons or gene cassettes in Enterobacterales and Pasteurellaceae, and most of these plasmids carry one or more additional resistance genes. The efflux of chloramphenicol from bacteria can be mediated by either specific transporters or multidrug transporters. Thiamphenicol cross-resistance with chloramphenicol is complete in bacteria that possess CAT genes. Due to the substitution of a hydroxyl group with a fluorine molecule, florfenicol is less susceptible to resistance from bacteria expressing CAT enzymes.

However, several other mechanisms of bacterial resistance to chloramphenicol and florfenicol have been identified [382, 383]. Florfenicol resistance in Gram-negative bacteria is related to plasmid transfer of the *floR* gene. This gene codes for a membrane-associated exporter protein that promotes efflux of chloramphenicol and florfenicol [381, 384]. Later, the *cfr* gene was identified mostly from staphylococci and enterococci and mediating resistance to florfenicol (all Phenicols), and other antimicrobial classes (Lincosamides, Oxazolidinones, Pleuromutilins, and Streptogramin A antibiotics). A novel gene *fexA* which encoded an efflux pump in Gram-positive cocci was found to confer resistance to florfenicol and chloramphenicol [385]. Also, many other florfenicol-associated resistance genes have been discovered regularly, such as the phenicol-specific exporter genes *fexB*, *pexA* [382], *AcrAB-Tok* multidrug efflux system *tolC* gene, *acrB* gene [386], *poxtA* (phenicols, oxazolidinone, tetracycline) and the novel ATP-binding cassette (ABC) transporter gene *optrA* (oxazolidones and phenicols) [387]. Most of the genes co-existed with bacterial mobile genetic elements, including plasmids or transposons, which contributed to the rapid spread of florfenicol resistance genes to numerous bacterial species through horizontal gene transfer (HGT).

Europe

According to the mandatory surveillance conducted by EFSA/ECDC, the median levels of resistance to chloramphenicol in indicator *E. coli* from pigs, calves, broilers and turkey in 2019/20 were 12.9, 12.6,

8.2 and 21.8%, respectively [28]. Monitoring of MRSA prevalence under CID 2013/652/EU is voluntary and data are provided by few member states. EFSA has reported that most *spa*-types from food and from investigations in animals were associated with LA-MRSA lineages. Linezolid-resistance was reported in two LA-MRSA isolates collected in 2016 from Belgian breeding pigs and three isolates collected from pigs in Portugal in 2019. The isolates were harbouring the *cfr* gene. It was noted that further molecular characterisation is needed to assess the public health significance of these isolates and more widespread testing to determine if the *cfr* gene is more widespread in MRSA in the animal population. No linezolid resistance was detected in isolates submitted from 2017/2018 surveillance [230].

Linezolid resistance has also been detected in enterococci in other European screening programmes. Under the EASSA program, 960 *E faecium* and 779 *E faecalis* isolates were collected at slaughter from cattle, pigs and chickens from 9 European countries during 2013-14. Of these, 7 porcine strains of Spanish origin were resistant to linezolid [388].

The DANMAP programme screened > 12,000 enterococci isolates submitted between 2004 to 2015 and found only five that were resistant to linezolid. These included two isolates from 2006 from Danish broiler meat and one isolate from 2015 from domestically reared veal; the remaining two isolates were from imported poultry. The *optrA* gene was identified from an *E faecium* from imported turkey meat and an *E faecalis* from Danish veal [389].

At the international level, *cfr, poxtA* and particularly *optrA* genes have all been identified from enterococci from various livestock species. Plasmids carrying these genes have the potential to exchange between enterococcal species and even across genera. These genes may both co-select and be linked to resistance genes for antibiotics that are commonly used in animals (e.g. phenicols, lincosamides), indicating the possibility that use of these substances can select MDR bacteria that are also resistant to oxazolidinones.

In addition, mobile genetic elements conferring resistance to florfenicol and other antibiotics have been increasingly identified in *Actinobacillus pleuropneumoniae* (APP) isolates from numerous sources, including the EU [390-395], reflecting a development towards less susceptible APP bacterial populations, in many different countries.

Nine out of 13 recent studies found less than 8% of *P. multocida* isolates resistant to florfenicol, with recent European isolates in general being susceptible to florfenicol (e.g. [396]). Also, a variety of mobile genetic elements conferring resistance to florfenicol and other antibiotics have been identified in *P. multocida* isolates from numerous sources, including the EU [397, 398].

Costa Hurtado *et al.* [399] found 10% florfenicol resistance among *Glaesserella parasuis* field strains isolated in Spain between 2014 and 2017.

De Smet *et al.* [400] investigated the impact on porcine commensal *E. coli* in the intestinal tract following the administration of florfenicol. The effect of various administration protocols on both plasma and gastrointestinal florfenicol concentrations in pigs were evaluated, including two IM injections of 15 mg FF/kg BW and 30 mg FF/kg BW, respectively. Ten hours after intramuscular administration of 30 mg florfenicol/kg BW, gastrointestinal concentrations of florfenicol were significantly elevated in comparison with the other treatment groups and ranging between 31.5 and 285.8 mg/g over the different gut segments. Both florfenicol susceptible (with minimal inhibitory concentration (MIC) values of 2–16 µg/mL) and florfenicol resistant (MIC > 256 µg/mL) *Escherichia coli* isolates were present in all treatment groups before treatment, while afterwards susceptible *E. coli* population were eradicated in all treatment groups.

This connection between florfenicol formulations and GI microflora exposure is further reflected in recent Danish data that show such a clear connection between indicator bacteria, *E. coli* isolated from pigs and florfenicol use [401, 402]. *E. coli* florfenicol resistance increased steadily from 2.1% in 2004–2007 to 3.4% in 2008–2011, 5.2% in 2012–2015, 11.9% in 2016, and finally 18.1% in 2017. This increase was also reflected in changes in MIC_{90} but not in MIC_{50}. Data from the Danish VetStat on consumption of florfenicol showed a temporal connection between usage and resistance for these compounds.

Animal produce in pet food may be a source of resistance genes for companion animals, just as it is for humans.

International

Asian countries are known to represent examples of higher florfenicol usage compared to Europe. A Korean study [394] found 34.3% of 102 APP isolates were florfenicol resistant. The authors speculated this higher level of resistance could be from recent national increasing florfenicol use. The results were supported by Kim et al. [393] whom found florfenicol resistance in 43.1% of 65 Korean porcine isolates.

Several Asian studies have reported between 16.3% and 91.9% *P. multocida* florfenicol resistance [398, 403, 404]. One Argentinian study [405] found 20% resistance among *P. multocida* isolates from pigs.

Li *et al.* [406] tested 62 clinical *Glaesserella parasuis* isolates (collected in China, between 2013 and 2014). Of the 62 isolates, three were resistant with high florfenicol MIC values of 8 mg/L and carried the florfenicol resistance gene *floR* on plasmid pHPSF1. This plasmid showed a high similarity with other *Pasteurellaceae* (*Pasteurella multocida, A. pleuropneumoniae*) plasmids, suggesting an exchange of genetic elements between species. This was further supported by a large molecular study by da Silva [407] investigating the relationship between mobile genetic elements (MGEs) and antimicrobial resistance in 345 publicly available *Pasteurellaceae* genomes. It appeared that MGEs are linked with 77.6% of AMR genes discovered, indicating their important involvement in the acquisition and global transmission of such genes.

The *cfr* gene has been reported in *S. aureus* and CoNS from pigs, cattle and poultry. The *cfr* gene was first described in 2000 in a bovine isolate of *Staphylococcus sciuri* [320]. It has also been identified on a plasmid in *S. suis* (S10) originating from a healthy pig in China. The high similarity of the genetic segment surrounding the *cfr* gene to that found in the *cfr*-carrying segment in *E. faecalis* suggested spread of the gene between the two genera [408].

OptrA has been identified in coagulase-negative staphylococci from pigs [294]. Enterococcal plasmids carrying *optrA* have been identified in poultry and swine [294]. Surveillance in China indicated that *optrA* occurs more commonly in enterococci from livestock (15.9%) than from humans (2.0%). It was suggested that this could have been due to selective pressure from use of florfenicol in livestock [387]. The *optrA* gene is located closely to resistance genes (e.g. *fexA, ermA*) that are selected by other antimicrobials commonly used in livestock [409]. In this study, *E. faecalis* from humans and pigs had similar PFGE patterns and the same MLST profile, suggesting an exchange of isolates. In addition, an *optrA* carrying plasmid was easily transferred between *E. faecium* in the presence of a plasmid carrying the *vanA* gene (vancomycin resistance). It has been suggested that *E. faecium* isolates of animal origin are not a direct hazard but act as donors of ARGs; whereas the same strains of *E. faecalis* have been identified from animals and humans [410].

The first identification of linezolid resistance in the USA in bacteria from cattle and pigs was published in 2018, when plasmids containing *optrA* were identified in two *E. faecalis* and one *E. faecium* isolates from the NARMS program. The plasmid in *E. faecium* also carried the *cfr* gene, whilst plasmids from both species also contained various combinations of resistance genes to phenicols, aminoglycosides and macrolides [411].

Identical *E. faecium* carrying *poxtA* have been identified in screening from cows' milk in Tunisia and hospital surveillance sample in Portugal [412].

Companion animals

In a study from Portugal analyzing raw frozen pet foods from international brands, 50% of samples contained linezolid-resistant *E. faecium* and *E. faecalis* carrying *optrA*, *poxtA* or *optrA*+*poxtA* [413].

In a survey of pets fed raw meat diets in China conducted in 2016, *optrA* genes were identified in enterococci and *Staph. sciuri* from dogs. Of 537 isolates from dogs and supermarket foods, 8.2% of enterococci and 2.5% of staphylococci were positive for *optrA*, but none carried *poxtA* or *cfr* genes. Some *E. faecalis* isolates from supermarket food products and companion animals were closely related in molecular and phenotypic characteristics, highlighting the possibility for spread of bacteria between fresh foods and dogs [414].

In a study that investigated 632 staphylococcal isolates from companion animals in Portugal obtained between 1999 to 2014, Couto, Monchique [233] reported the presence of the *cfr* and *fexA* genes in an *S. pseudintermedius* isolate taken from a dog that had been treated with florfenicol as last resort. The strain itself did not exhibit resistance to linezolid.

In conclusion,

- Due to its adverse effects, chloramphenicol has limited use in human medicine in the EU, being used mainly for topical treatment of conjunctivitis.
- Amphenicols are more widely used in veterinary medicine, mainly for food-producing species. They are important for treatment of common respiratory pathogens in pigs, ruminants and poultry, but also have broader indications including use for gastrointestinal infections in poultry. Amphenicols are used topically for eye and ear infections in companion animals and for wounds and foot infections in food-producing animals. Florfenicol is one of few antibiotics authorised for use in salmon and trout for treatment of furunculosis and vibriosis.
- The use of amphenicols in veterinary medicine may select for resistance genes and multi-resistant genes of public health concern (e.g. *cfr*, *optrA*, *poxtA*). In particular, these genes confer resistance to oxazolidinones which are of last resort for treatment of resistant Gram-positive infections in humans, including MRSA.
- There is evidence for the selection and transmission of resistance to amphenicols from animals to humans and other animals via zoonotic and target pathogens or commensal bacteria capable of transferring resistance to pathogens.

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to Amphenicols.

Criterion (c) – availability of other treatments for animals

In companion animals, fluoroquinolone antimicrobials are the main alternative for amphenicols, but for eye or ear infections, several other authorised formulations are available containing aminoglycosides,

polymyxins, steroid antibiotics, tetracyclines, etc. Doxycycline, clindamycin and amikacin are also alternatives to chloramphenicol for infections at other body sites.

Amphenicols are used mostly for respiratory diseases in cattle, swine and chickens. Alternatives include macrolides, aminopenicillins, pleuromutilins and cephalosporins (except chickens). For pododermatitis alternatives are mostly macrolides and tetracyclines.

Alternatives in salmon and trout for treatment of furunculosis and vibriosis include TMPS, tetracyclines and (fluoro)quinolones.

Criterion (d) – availability of other antimicrobial treatments for humans

There are alternative treatment options considered first choice such as fluoroquinolones to treat typhoid fever and conjunctivitis or 3rd-generation cephalosporines to treat meningitis and penicillinase-resistant penicillins and glycopeptides for *S. aureus* infections [377].

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

- In human medicine, chloramphenicol is nationally approved in some of the EU Member States for the treatment of typhoid, meningitis caused by *H. influenzae* and of other serious infections; eyedrops are approved for the treatment of acute bacterial conjunctivitis. However, it is no longer the antibiotic of choice for any specific infection and is not frequently used due to serious adverse effects (e.g. bone marrow toxicity).
- In veterinary medicine, the main use of amphenicols is in food-producing animals for treatment and prevention of respiratory diseases caused by susceptible bacteria such as *Mannheimia, Pasteurella* and *Histophilus, Actinobacillus pleuropneumoniae* and *M. hyopneumoniae, or E. coli*; although amphenicols also have a number of broader indications. They are used topically for treatment of pododermatitis in ruminants and eye and external ear infections in companion animals. Florfenicol is authorised for the treatment of susceptible bacterial diseases of fish, including furunculosis and vibriosis in salmon and trout.
- The most common mechanism of bacterial resistance to chloramphenicol/thiamphenicol is enzymatic inactivation by acetylation by chloramphenicol acetyltransferases (CAT genes) found on MGEs in Enterobacteriaceae and Pasteurellaceae, and most of these plasmids carry one or more additional resistance genes. Use can also select one or more of three multi-resistance genes of public health significance including the *cfr* gene (coding for resistance to all phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A antibiotics), *poxtA* (phenicols, oxazolidinone, tetracycline) and the novel ATP-binding cassette (ABC) transporter gene *optrA* (oxazolidones and phenicols). Oxazolidinones are an antibiotic of last resort in human medicine to treat infections due to MRSA, VRSA and VRE.
- Although amphenicols are rarely used systemically in companion animals, fluoroquinolone antimicrobials (AMEG Category B) are the main alternative. Florfenicol is used mostly for respiratory diseases in cattle, swine and chickens. Alternatives include macrolides, aminopenicillins, pleuromutilins and cephalosporins (except chickens). For pododermatitis alternatives are mostly macrolides and tetracyclines.
- In human medicine, there are alternative treatment options considered first choice such as fluoroquinolones to treat typhoid fever and conjunctivitis or 3rd-generation cephalosporines to treat meningitis and penicillinase-resistant penicillins and glycopeptides for *S. aureus* infections.
- Amphenicols are authorised for use in all major and several limited market species, in formulations for group and individual animal administration.

- The extent of amphenicol use outside the terms of the marketing authorisation is unknown. According to an open call for data, uses outside the marketing authorisation related mostly to unauthorised indications in major species and to use in unauthorised minor species.

Therefore, considering the points above relevant to criteria (b), (c) and (d), it should be considered if conditions or a prohibition should be placed on the use of Amphenicols outside the terms of the marketing authorisation.

Step 2. Considerations of conditions to be placed on use outside the terms of a marketing authorisation

Please refer to Section 3.1.2. of the main report for the general rationale behind the proposed conditions.

(i) Use for unauthorised indications

Conditions proposed: *For those indications not included in the SPC of the concerned product, use must be based on target pathogen identification and antimicrobial susceptibility testing that demonstrates that amphenicols are likely to be effective and that antimicrobials from a lower AMEG category would not be effective, unless it can be justified that this is not possible.*

Rationale: See Section 3.1.2(i) of this advice.

(ii) Use for unauthorised target species

Amphenicols are authorised for use in all major food-producing and companion animal species and several limited market species including various poultry and fish. Therefore, it is not proposed to place conditions on use in unauthorised species.

Rationale: See Section 3.1.2(ii) of this advice.

(iii) Administration by an unauthorised route or use of extemporaneous formulation

Amphenicols are authorised in formulations for administration to individual animals by injection, orally and by topical administration. They are also authorised for administration to groups of animals in-feed and in drinking water, including to aquaculture fish as a premix. No reports were identified relating to use of extemporaneous preparations. Therefore, it is not proposed to place conditions on use by unauthorised routes of administration.

Rationale: See also Section 3.1.2(iii) of this advice.

(iv) Use of a human medicinal product

Human formulations are authorised for administration via oral, injection and topical routes.

No conditions are proposed further to that mentioned above.

Rationale: Considering the availability of administration routes for veterinary formulations, it is not proposed to place additional conditions on use of HMPs.

Radtionale: See Section 3.1.2.(iv) of this advice.

(v) Use of a third country veterinary medicinal product

According to the Regulation, third country VMPs may only be used in the same species and for the same indication. No additional conditions are proposed to those above.

Rationale: See Section 3.1.2.(v) of this advice.

Consideration of Criteria (a) and (e) in view of proposed 'conditions':

Criterion (a) – risk to animal health or public health if the antimicrobial is used in accordance with Articles 112, 113 and 114

In animals, chloramphenicol toxicity is dependent on both the dose and duration of treatment, and cats are more likely than dogs to develop toxicity [415]. Chloramphenicol causes changes in the peripheral blood and bone marrow due to reversible, dose-related disturbances in red cell maturation. Administration for less than 10 days is less likely to cause toxicity in either dogs or cats, unless the animals have depressed hepatic microsomal enzyme activity or severely impaired renal function. Use in dogs for MRSA and MRSP infections is associated with frequent adverse gastrointestinal effects (vomiting, diarrhea, weight loss, nausea, anorexia and decreased appetite), as well as lethargy, shaking, increased liver enzymes, and anemia [380].

Transient diarrhoea or inappetence has been described in cattle treated with florfenicol. In swine, peri-anal inflammation and/or rectal eversion may occur in treated animals. Injectable florfenicol formulations for cattle and swine are only labelled for a maximum of 2 doses, so bone marrow suppression has not been reported with clinical use in these species. Potentially fatal bone marrow suppression, from suppression of protein synthesis in erythroid cells, has been documented with over dose or prolonged florfenicol administration [416, 417].

Contraindications described in the SPC of authorised products, include:

- Do not use in adult bulls, rams and boars intended for breeding purposes.
- Do not use in broodstock.
- Do not administer intravenously.

Target animal safety warnings in the SPCs of authorised VMPs should be followed.

Consumer safety is mitigated through the application of the statutory withdrawal period in accordance with Article 115.

Criterion (e) Impact on aquaculture and farming if the animal affected by the condition receives no treatment

Proposed condition: Amphenicols	**Potential impact on aquaculture and farming if animal affected by the condition receives no treatment**
For those indications not included in the SPC of the concerned product, use must be based on target pathogen identification and antimicrobial susceptibility testing that demonstrates that amphenicols are likely to be effective and that antimicrobials from a lower AMEG category would not be effective, unless it can be justified that this is not possible.	This condition does not preclude treatment. See Annex 1 of report for further discussion. Several alternatives are available for disease treated with amphenicols.

4. Final conclusion - recommendations made for Conditions to be placed on the use of class outside the terms of the marketing authorisation

- For those indications not included in the SPC of the concerned product, use must be based on target pathogen identification and antimicrobial susceptibility testing that demonstrates that amphenicols are likely to be effective and that antimicrobials from a lower AMEG category would not be effective, unless it can be justified that this is not possible.

4.19. Evaluation of Sulfonamides, Trimethoprim and their combinations

4.19.1. Background information

Examples of substances included in the class that are authorised in veterinary and human medicine in the EU

Sulfonamides

Examples of substances authorised for veterinary use	Examples of ATCvet codes
Formosulfathiazole	QA07AB90
Phthalylsulfathiazole	QA07AB02
Sulfacetamide	QJ01EQ21
Sulfachlorpyridazine	QJ01EQ12
Sulfaclozine	QP51AG04
Sulfadiazine	QJ01EQ10
Sulfadimethoxine	QJ01EQ09 QP51AG02
Sulfadimidine	QJ01EQ03 QP51AG01
Sulfadoxine	QJ01EQ13
Sulfaguanidine	QA07AB03
Sulfalene	QJ01EQ19
Sulfamerazine	QJ01EQ17
Sulfamethoxazole	QJ01EQ11
Sulfamethoxypyridazine	QJ01EQ15
Sulfamonomethoxine	QJ01EQ18
Sulfapyridine	QJ01EQ04
Sulfaquinoxaline	QJ01EQ16 QP51AG03
Sulfathiazole	QJ01EQ07
Examples of substances authorised for human use	**Examples of ATC codes**
Sulfadiazine	J01EC02
Sulfadimidine	J01EB03
Sulfafurazole	J01EB05
Sulfamethizole	J01EB02

Trimethoprim

Examples of substances authorised for veterinary use	Examples of ATCvet codes
Trimethoprim	QJ01EA01 QJ51EA01
Examples of substances authorised for human use	**Examples of ATC codes**
Trimethoprim	J01EA01

Trimethoprim-sulfonamide (TMPS) combinations

Examples of substances authorised for veterinary use	Examples of ATCvet codes
Combinations of sulfonamides and trimethoprim	QJ01EW30
Sulfachlorpyridazine and trimethoprim	QJ01EW12
Sulfadiazine and trimethoprim	QJ01EW10 QJ51RE01
Sulfadimethoxine and trimethoprim	QJ01EW09
Sulfadimidine and trimethoprim	QJ01EW03
Sulfadoxine and trimethoprim	QJ01EW13
Sulfamerazine and trimethoprim	QJ01EW18
Sulfamethoxazole and trimethoprim	QJ01EW11
Sulfamethoxypyridazine and trimethoprim	QJ01EW15
Combinations of sulfonamides	QA07AB20 QA07AB99 QJ01EQ30 QP51AG30
Sulfadimethoxine combinations with other antibacterial	QJ01EQ59
Sulfadimidine combinations with other antibacterial	QP51AG51
Sulfaquinoxaline combinations with other antibacterial	QP51AG53
Examples of substances authorised for human use	**Examples of ATCcodes**
Sulfadiazine and trimethoprim	J01EE02

Sulfamethoxazole and trimethoprim	J01EE01
Sulfametrole and trimethoprim	J01EE03

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

Substance	MRL tissues	MRL milk	MRL eggs	Other provisions
All substances belonging to the sulfonamide group	Yes (All food-producing species)	Yes (Bovine, ovine, caprine)	-	Not for use in animals from which eggs are produced for human consumption.
Trimethoprim	Yes (All food-producing animals)	Yes (All food-producing animals, except *Equidae*)	-	Not for use in animals from which eggs are produced for human consumption.

EU-authorised VMP formulations, based on sales reported to ESVAC

Sulfonamides

Species		Route of administration						
		Group		Individual				
		In-feed	In-water	Injection	Oral powder	Oral e.g. tablet, paste, powder	Topical/local (incl. intrauterine)	Intra-mammary
Major	**Cattle**	SDD, SG, SMPZ	PSTZ, PSTZ+SG, SDZ, SDM, SDM+SDD, SDD, SDD+SG, SDD+SQX, SG, SMPZ, SMM, SQX	SAC+SDD+STZ, SDZ+SDD+SMZ, SDZ+SDM, SDM, SDM+SDD+STZ, SDM+SDD, SDD, SDD+SMPZ, SDD+SMZ+STZ, SE, SMPZ, SMM, SP	PSTZ, SDM, SDD+STZ, SDD, SDD+SG, SG	FSTZ, SDZ+SDD+STZ, SDD, SG, SMPZ	SDD, SMM	
	Sheep (for meat)	SDM, SDD, SG	SDM, SDD, SDD+SG, SG, SMPZ	SAC+SDD+STZ, SDZ+SDD+SMZ, SDZ+SDM, SDM, SDM+SDD+STZ, SDD, SDD+SMZ+STZ, SE, SMPZ	PSTZ, SDD, SG	SDD, SMPZ		
	Pigs	FSTZ, SDM, SDD, SDD+SG, SG, SMZ, SMPZ	PSTZ, PSTZ+SG, SDZ, SDM, SDM+SDD, SDD, SDD+SG, SG, SMPZ, SMM	SAC+SDD+STZ, SDZ+SDD+SMZ, SDZ+SDM, SDM, SDM+SDD+STZ, SDM+SDD, SDD, SDD+SMPZ, SDD+SMZ+STZ, SE, SMPZ, SMM	PSTZ, SDD, SDD+SG, SG, SMZ	FSTZ, SDD, SG	SDD	
	Chickens	SDZ, SDM, SDD+SG	SCP, SCZ, SDZ, SDM, SDD, SMM, SQX	SDM, SDD+SMZ+STZ, SMM	SCZ, SDM, SDD	SDM		

	Dogs		PSTZ, SDM, SG, SE	SAC+SDD +STZ, SDZ+SDD +SMZ, SDZ+SDM, SDM, SDM+SDD +STZ, SDD, SDD+SMZ +STZ, SMPZ, SMM	PSTZ, SDM, SDD, SG	FSTZ, SDM, SDD, SG, SE, SMPZ		
	Cats		SDM, SG, SE	SDZ+SDM, SDM, SDD+SMZ +STZ, SMPZ, SMM	SDM, SDD, SG	FSTZ, SDM, SG, SE		
Limited market species	**Turkeys**		SCP, SCZ, SDZ, SDM, SDD, SQX	SMM	SCZ	SDM		
	Poultry	SDZ, SDM, SG	SCZ, SDZ, SDM, SDM+SDD, SDD, SDD+SQX, SG, SQX	SDM				
	Ducks							
	Geese		SQX					
	Guinea Fowl		SQX					
	Pheasant		SCZ, SQX		SCZ	SDM		
	Guinea-pig							
	Goats	SDM, SDD	SDM, SDD, SDD+SG, SG	SDZ+SDD +SMZ, SDZ+SDM, SDM, SMPZ	SDD, SG	SDD, SG		
	Rabbits	FSTZ, PSTZ+SDM, SDM, SDD, SDD+SG, SQX	SCP, SCZ, SDM, SDM+SDD, SDD, SDD+SQX, SQX	SDD+SMZ +STZ	SCZ, SDM			
	Horses		PSTZ, SDD+SG, SG	SAC+SDD +STZ, SDZ+SDD +SMZ, SDZ+SDM, SDM, SDM+SDD +STZ, SDD, SDD+SMPZ, SDD+SMZ +STZ, SE, SMPZ, SMM	PSTZ, SDD, SDD+SG	SDD, SG, SMPZ		
	Pigeons		SDM, SDM+SQX, SDD, SQX		SDM, SDD			
	Fish							
	Rodents		SDM					
	Reptiles							
	Ornamental birds		SDM, SDM+SQX, SDD, SQX		SDX			
	Fur animals	SDD+SG	SDD					
	Nutria							

FSTZ (formosulfathiazole), PSTZ (phthalylsulfathiazole), SAC (sulfacetamide), SCP (sulfachlorpyridazine), SCZ (sulfaclozine), SDZ (sulfadiazine), SDM (sulfadimethoxine), SDD (sulfadimidine), SDX (sulfadoxine), SG (sulfaguanidine), SE (sulfalene), SMZ (sulfamerazine), SMX (sulfamethoxazole), SMPZ (sulfamethoxypyridazine), SMM (Sulfamonomethoxine), SP (sulfapyridine), SQX (sulfaquinoxaline), STZ (sulfathiazole)

Trimethoprim

Species		Route of Administration						
		Group		Individual				
		In-feed	In-water	Injection	Oral powder	Oral e.g. tablet, paste, powder	Topical/local (incl. intrauterine)	Intra-mammary
Major	**Cattle**				T			
Limited market species	**Pigeons**		T		T			
	Ornamental birds		T					

T (trimethoprim)

Trimethoprim-sulfonamide combinations - TMPS

Species		Route of administration						
		Group		Individual				
		In-feed	In-water	Injection	Oral powder	Oral e.g. tablet, paste, powder	Topical/local (incl. intrauterine)	Intra-mammary
Major	**Cattle**	SDZ+T, SDM+T, SDD+T, SDD+STZ+T	SCP+T, SDZ+T, SDM+T, SDD+T, SDX+T, SMZ+T, SMPZ+T, SMM+T	SDZ+T, SDM+T, SDM+SDD+T, SDD+T, SDX+T, SMX+T, SMPZ+T, SMM+T	SDZ+T, SDM+T, SDD+T, SMM+T	SDZ+T, SMZ+T		SDZ+T, SDD+T
	Sheep (for meat)	SDZ+T, SDM+T	SDZ+T, SDM+T, SDD+T, SMPZ+T, SMM+T	SDZ+T, SDX+T, SMX+T, SMPZ+T	SDZ+T, SMM+T	SDZ+T, SMZ+T		
	Pigs	SDZ+T, SDZ+SMZ+T, SDM+T, SDD+T, SDD+STZ+T, SMX+T	SCP+T, SDZ+T, SDM+T, SDD+T, SDX+T, SMZ+T, SMX+T, SMPZ+T, SMM+T	SDZ+T, SDM+T, SDM+SDD+T, SDD+T, SDX+T, SMX+T, SMPZ+T, SMM+T	SDZ+T, SDM+T, SDD+T, SMX+T, SMM+T	SDZ+T, SMZ+T		
	Chickens	SDZ+T, SDM+T	SCP+T, SDZ+T, SDM+T, SDM+SDD+T, SDD+T, SMX+T, SMM+T, SQX+T	SDM+T	SDD+T, SMM+T			
	Dogs		SDD+T	SDZ+T, SDM+T, SDD+T, SDX+T, SMX+T	SDZ+T	SDZ+T, SMX+T, SMPZ+T		
	Cats			SDZ+T, SDM+T, SDD+T, SDX+T, SMX+T		SDZ+T, SDM+T, SMX+T, SMPZ+T		
Limited market species	**Turkeys**	SDZ+T, SDM+T	SCP+T, SDZ+T, SDM+T, SDM+SDD+T, SDD+T, SQX+T		SDD+T			
	Poultry	SDZ+T, SDM+T, SMX+T	SCP+T, SDZ+T, SDM+T, SDD+T, SMX+T, SMPZ+T,	SMPZ+T				

			SMM+T, SQX+T					
	Ducks	SDZ+T						
	Geese	SDZ+T	SCP+T					
	Guinea Fowl	SDZ+T						
	Pheasant	SDZ+T	SDD+T					
	Guinea-pig			SDX+T				
	Goats	SDM+T	SDZ+T, SDM+T, SMM+T	SDZ+T, SDM+T, SDX+T, SMX+T	SDZ+T, SDM+T, SMM+T	SDZ+T		
	Rabbits	SDZ+T, SDZ+SMZ+T, SDM+T	SDZ+T, SDM+T, SDD+T, SMX+T, SMPZ+T, SMM+T		SMM+T			
	Horses	SDM+T	SDZ+T, SMZ+T, SMPZ+T	SDZ+T, SDM+T, SDD+T, SDX+T, SMX+T, SMPZ+T	SDZ+T, SDM+T, SDD+T, SMZ+T	SDZ+T, SDM+T, SDD+T, SMZ+T, SMX+T		
	Pigeons		SDZ+T, SDM+SDD+T, SMX+T, SQX+T		SDZ+T			
	Fish	SDZ+T		SDX+T				
	Rodents		SDX+T	SDX+T				
	Reptiles		SMX+T					
	Ornamental birds	SDM+T						
	Fur animals		SDD+T	SDZ+T	SDZ+T	SDZ+T		
	Nutria					SDZ+T		

Summary of main indications and contra-indications for EU-authorised VMPs, based on selected SPCs

Main indications	Sulfonamide (unpotentiated) VMPs are available for use in all major and several minor species and are authorised for a variety of indications including infections of the gastrointestinal, respiratory and urinary tract, neonatal infections, SSTI, necrobacillosis and treatment of protozoal gastroenteritis (coccidiosis). Where named, bacterial pathogens include *E. coli*, staphylococci, streptococci, *Pasteurella* spp., *Fusobacterium necrophorum*. TMPS combinations are authorised in injectable and oral formulations with broad indications, which in some SPCs are not specified beyond 'infections caused by organisms susceptible to the combination …' When specified, diseases include: Respiratory tract infections, including rhinitis, pneumonia, bronchitis, pleurisy, strangles in horses. Urogenital tract infections, including cystitis, vaginitis, urethritis, nephritis and metritis. Alimentary tract infections, including neonatal diarrhoea and salmonellosis. Other infections, such as foul-in-the-foot, severe mastitis, bacterial agalactia of sows, infections of the eye, ear or mouth, wounds, septicaemia. The listed pathogens include Gram-positive and Gram-negative bacteria: *Actinobacilli*, *Actinomycae*, *Arcanobacterium* spp., *Bordetella* spp., *Brucella*, *Corynebacteria*, *Enterobacterales*, *Erysipelas rhusiopathae*, *Haemophilus* spp., *Klebsiella* spp., *Listeria monocytogenes*, *Nocardia* spp., *Pasteurellacea*, *Pneumococci*, *Proteus*, *Rhodococcus* equi, *Salmonella* spp., *Staphylococci*, *Streptococci.*

	Products for administration in drinking water or in-feed to groups of animals are authorised for: Pigs: post-weaning diarrhoea due to *E. coli*, bacterial respiratory infections caused by *Pasteurella multocida*, *Actinobacillus pleuropneumoniae*, *Bordetella bronchiseptica*, *Streptococcus* spp. and *Haemophilus parasuis* Broilers: colibacillosis, salmonellosis, coryza caused by *Avibacterium paragallinarum* Turkeys: salmonellosis, pasteurellosis Intramammary preparations are authorised in cows for treatment of mastitis due to susceptible Gram-positive and Gram-negative bacteria including *Streptococcus* spp., *Staphylococcal* spp., *Corynebacterium* spp. and *E. coli*. In farmed fish, trimethoprim-sulfadiazine is authorised as a premix for bacterial infections including: *Aeromonas salmonicida*, *Vibrio anguillarum*, *Yersinia ruckeri* and *Flexibacter columnaris* Some VMPs containing TMPS also include indications for treatment of protozoal gastrointestinal infections (coccidiosis).
Contraindications	Not to be used in animals with severe liver or kidney damage or blood dyscrasia.

Examples of EU-authorised HMP formulations, from Article 57 database

Sulfonamides

Substance	Route of administration		
	Injection	Oral e.g. tablet, liquid	Topical/local
Sulfadiazine	x	x	
Sulfadimidine			X
Sulfadoxine		x	
Sulfafurazole		x	
Sulfamethizole		x	

Trimethoprim

Substance	Route of administration		
	Injection	Oral e.g. tablet, liquid	Topical/local
Trimethoprim		x	

TMPS

Substance	Route of administration		
	Injection	Oral e.g. tablet, liquid	Topical/local
Sulfamethoxazole + TMP	x	x	
Sulfadiazine + TMP		x	
Sulfametrole + TMP	x	x	

Existing recommendations

WOAH recommendations

Sulfonamides, trimethoprim and the combinations are categorised VCIA by WOAH (formerly OIE). *Specific comments:* The wide range of applications and the nature of the diseases treated make sulfonamides extremely important for veterinary medicine. These classes (sulfonamides and trimethoprim) alone or in combination are critically important in the treatment of a wide range of diseases (bacterial, coccidial and protozoal infections) in a wide range of animal species.

WHO classifications

WHO: HIA (Sulfonamides, dihydrofolate reductase inhibitors and combinations)

- (C1: No) In certain geographic settings, Criterion 1 may be met: the class may be one of limited therapies for acute bacterial meningitis, systemic nontyphoidal *Salmonella* spp. infections, and other infections.
- (C2: Yes) May result from transmission of Enterobacterales, including *E. coli*, from non-human sources.

WHO AWaRe:

- *Sulfonamides* - Access: e.g. sulfadiazine, sulfamethoxazole, sulfadimidine
- *Trimethoprim* - AWaRe: Access: trimethoprim, brodimoprim
- *TMPS* - Access: Sulfadiazine-trimethoprim, Sulfamethizole-trimethoprim, Sulfamethoxazole-trimethoprim, Sulfametrole-trimethoprim, Sulfamoxole-trimethoprim

AMEG and CVMP recommendations

Sulfonamides, dihydrofolate reductase inhibitors and combinations are included together in one class in the AMEG Category D. There are alternative treatments in human and veterinary medicine for their indications and they do not select for resistance to Category A substances through specific multiresistance genes. Antibiotics in this category present a lower AMR risk than the antibiotics in the higher categories, A to C.

These antibiotics are not devoid of negative impact on resistance development and spread. To keep the risk from use of these antibiotic classes as low as possible it is important that responsible use principles are complied with in everyday practice. Unnecessary use and unnecessarily long treatment periods should be avoided and group treatment restricted to situations where individual treatment is not feasible.

Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Disclaimer: The information in this section reflects reported use of antimicrobials outside the terms of a marketing authorisation. No evaluation is made in this section by the working group on the efficacy or safety of the reported uses, or on their potential impact on development and dissemination of AMR.

Information from published sources

Considering the wide availability of authorised formulations for use in different species and non-specific nature of the authorised indications, most published uses would appear to be consistent with marketing authorisations.

In companion animals, human formulations are sometimes used e.g. topical formulations of silver sulfadiazine for treatment of otitis externa due to MDR *Pseudomonas aeruginosa* and sulfasalazine for chronic idiopathic colitis in dogs, in which case efficacy is assumed to be related to release of anti-inflammatory salicylate by colonic bacteria [33, 46]. Pyrimethamine/sulfonamide combination has been used to treat clinical toxoplasmosis in dogs and cats, but is associated with toxicity in cats particularly [125, 255].

Information from the open call for data on use of antimicrobials in animals

The information below is summarised from the open call for data. Inclusion in the table does not endorse use or imply that it is consistent with use according to legislative provisions in Articles 112 to 114.

Substance	Species	Indication	Alternatives	Consequences of unavailability
Sulfamethoxazole + TMP	Cats and dogs	Pyelonephritis and prostatitis	Fluoroquinolones	
Sulfadiazine + TMP	Aquacullture and ornamental fish	Enteric Redmouth and other bacterial diseases	None	
Sulfadiazine + TMP	Seabass	Pasterurellosis, vibriosis	Oxytetracyline, florfenicol	
TMPS Premix	Fish	Yersiniosis	Based on AST, oxytetracycline, florfenicol	
TMPS	Mink	Diarrhoea, pneumonia (*E. coli, Pseudomonas*), mastitis, metritis	None	Dramatically increased mortality, welfare issues, chronic disease
Sulfadiazine-TMP	Mink	Cystitis, urolithiasis, enteritis	None	Mortalities
TMPS	Dogs, cats, rodents, rabbits	Susceptible bacterial infections	None for oral use	
TMPS (injection)	Goats, sheep	Systemic infection and septicaemia ± diarrhoea in neonatal and juveniles	Enrofloxacin for neonatal septicaemia	
TMPS tablets	Dogs	Coccidiosis		Possible euthanasia
Sulfadimidine, TMPS	Ornamental birds	Susceptible infections e.g. gastrointestinal infection in chicks, coccidiosis	None	Animal welfare issues
Sulfamethoxazole	Cetaceans, teleosts	Susceptible protozoa,	Metronidazole	Severe disease, death
Trimethoprim-sufadiazine	Cetaceans	Cryptocariosis		
Silver-sulfadiazine (human medicine)	Horses	Bacterial skin infections – topical treatment		
Sulfadiazine tablets (human medicine)	Horse	Colitis/typhlitis, typhlocolitis		

4.19.2. Evaluation

Scope of permitted use according to the MRL Regulation

All substances belonging to the sulfonamide group and trimethoprim are included in Table 1 (allowed substances) of the MRL Regulation (EU) 37/2010 and they may be used in all food-producing species (separately or in combination) in accordance with Articles 113 and 114 of Regulation (EU) 2019/6. 'Other provisions' state that they are not for use in animals from which eggs are produced for human consumption.

Sulfonamides and trimethoprim can be used in non-food-producing species in accordance with Article 112.

Examples of veterinary-authorised formulations/species

Sulfonamides, dihydrofolate reductase inhibitors (Trimethoprim) and their combinations are approved for use in food-producing and companion animals. Formulations are authorised for use in group (in-feed, in-water) and individual animals, for systemic and local treatments.

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Importance for human health

Sulfonamides

Sulfonamides (e.g. sulfamethoxazole, sulfamethizole, sulfadiazine) are bacteriostatic and work by interfering with the synthesis of folic acid (an essential component for DNA and RNA formation). Sulfonamides show activity against Gram-positive (e.g., *Staphylococcus aureus*, *S. saprophyticus*, *Streptococcus pyogenes*, *S. pneumoniae*, *Bacillus anthracis*, *Clostridium tetani*, *C. perfringens*) and Gram-negative (*Enterobacterales*, *Neisseria*, *Brucella*), *Actinomyces*, *Nocardia*, *Chlamydia*, *Plasmodium* and *Toxoplasma* spp. Sulfonamides alone have been used to treat uncomplicated UTIs. Nowadays, their clinical use is diminishing due to emerging spread of resistance. Topical argentic sulfadiazine is used for wound infections [418].

Trimethoprim

Trimethoprim has a broad-spectrum bacteriostatic activity; it works by inhibiting the action of dihydrofolate reductase (DHFR), an enzyme that catalyses the last step of folic acid synthesis, and ultimately, DNA synthesis [419].

TMP is active against a range of bacterial species including Gram-positive (*Streptococcus*, *Staphylococcus*, *Corynebacterium*, *Listeria monocytogenes*) and Gram-negative bacteria (*E. coli*, *Enterobacter*, *Klebsiella*, *Proteus*, *Salmonella*, *Shigella*, *Providencia*, *Citrobacter*, *Hafnia*, *Edwardsiella*, *Serratia*, *Haemophilus influenzae*) [419].

TMP is approved for the treatment of initial episodes of uncomplicated urinary tract infections due to susceptible strains of the following organisms: *Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, Enterobacter* species and coagulase-negative *Staphylococcus* species, including *S. saprophyticus.* Nowadays, due to emerging spread of TMP-resistant organisms, the importance of this antibiotic is diminishing.

Sulfonamides in combination with Trimethoprim

Trimethoprim-sulfamethoxazole also known as co-trimoxazole, is a combination of two antimicrobial agents (sulfamethoxazole-SMX and trimethoprim-TMP) that act synergistically and have bactericidal effect against a wide variety of bacteria. Although other combinations of sulfonamides are available with trimethoprim, co-trimoxazole is by far the most widely used [420].

Co-trimoxazole is effective against aerobic Gram-positive (Staphylococcus spp., including MRSA) and Gram-negative (e.g., Enterobacterales) bacteria. It is also active against certain nosocomial acquired and/or infections seen in immunocompromised patients: e.g., *Burkholderia cepacia* (formerly *Pseudomonas cepacia*), *Stenotrophomonas maltophilia* (formerly *Xanthomonas maltophilia*), Serratia marcescens, and Nocardia spp.

Co-trimoxazole is also treatment of choice for *P. jirovecii* pneumonia (PjP) which is a potentially life-threatening fungal infection that occurs in immunocompromised individuals [420]. Co-trimoxazole is further among first choice agents for the treatment of MRSA infections, particularly those community acquired. Most importantly co-trimoxazole is a recommended treatment for nocardiosis [421]. Co-trimoxazole is the only available alternative against MDR *S. maltophilia* and *B. cepacia*.

Co-trimoxazole is nationally approved in the EU member states. Among the approved indications are the following: treatment and prevention of PjP, treatment and prophylaxis of toxoplasmosis, treatment

of nocardiosis. The following infections may be treated with co-trimoxazole where there is evidence of to support susceptibility and good reason to prefer the combination of antibiotics in co-trimoxazole to a single antibiotic: acute uncomplicated UTIs, acute otitis media, acute exacerbation of chronic bronchitis.

Importance for animal health

Sulfonamides

Several substances belonging to the sulfonamides class are approved for use in food-producing and companion animals, including minor species (e.g. sulfadiazine, sulfadimidine, sulfamethoxazole, sulfadimethoxine). Sulfonamides are of 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 and individual animals for systemic and local treatments [422]. Authorised uses include treatment of gastrointestinal, respiratory, urinary and skin and soft tissue infections due to bacterial pathogens which are often unspecified in SPC indications.

Trimethoprim

VMPs containing Trimethoprim-only have been identified as an oral powder for administration in drinking water to calves for treatment of respiratory disease due to *Pasteurella* spp., gastrointestinal infections due to *E. coli* and *Salmonella* spp. and UTI due to Gram-negative bacteria. There are also a formulations for administration in drinking water for pigeons and ornamental birds.

Sulfonamides in combination with Trimethoprim

The importance of TMPS combinations lies in their use as a first-line antibiotics, particularly in food-producing and minor species, for numerous Gram-positive and Gram-negative infections of respiratory, gastrointestinal and urogenital tracts, SSTI including bovine interdigital necrobacillosis and septicaemia (see authorised indications above). The possibility for oral administration of TMPS to horses without adverse effects supports its importance in this species [33]. In dogs and cats, use of TMPS has declined due to availability of alternatives that have fewer adverse effects in these species, especially when long treatment courses are required; however, the combination is included in the WSAVA List of essential medicines for treatment of bacterial cystitis, skin and other infections including bacterial and protozoal infections of the CNS [125, 178]. TMPS are one of few antibiotic classes authorised in the EU for use in fish, being important for treatment of e.g. *Aeromonas* spp., *Vibrio* spp., *Yersinia ruckerii*, *Streptococcosis* [26].

The indications as stated in the SPC are often broad and may not be specified beyond 'infections caused by organisms susceptible to the combination …'; hence it is difficult to determine which of the reported uses are outside the terms of the marketing authorisation. TMPS are also important as one of limited options for treatment of less common infections e.g. *Nocardia* spp. and certain protozoal infections e.g. toxoplasmosis, Equine Protozoal Myeloencephalitis (imported cases), *Neospora* [255]. In addition, MRSA isolated from companion animals may remain susceptible to TMPS.

In the open call for data, several reports indicate that TMPS has been used outside the marketing authorisation, for combinations of species and indications that may not have been in the SPC for a specific VMP, for example to treat septicaemia and diarrhoea in young small ruminants, bacterial diseases in aquaculture, ornamental birds and mink or coccidiosis in ornamental birds and dogs. In horses, use of human formulations was reported (tablets and topical silver-sulfadiazine). Use of specific human formulations has also been reported in dogs (topical silver-sulfadiazine and sulfasalazine tablets).

Development, selection and transmission of resistance

Both sulfonamides (S) and trimethoprim (TMP) affect bacterial folic acid synthesis. Sulfonamides inhibit dihydropteroate synthetase (DHPS), which catalyses the formation of dihydrofolate from para-aminobenzoic acid. In the subsequent step of the pathway, TMP inhibits dihydrofolate reductase (DHFR), which catalyses the formation of tetrahydrofolate from dihydrofolate.

Bacterial resistance to TMP and to sulfonamides is mediated by the following main mechanisms: changes to the permeability barrier and/or efflux pumps, naturally insensitive target enzymes, regulational changes in the target enzymes, mutational or recombinational changes in the target enzymes, and acquired resistance by drug-resistant target enzymes [423].

Resistance to TMPS can be chromosomal or the resistance genes may be located on MGE e.g. plasmids or transposons. Resistance in Gram-negative bacteria is mainly conferred by acquisition of *sul* genes and/or *dfr* genes [419]. Resistance in staphylococci to TMP is based on several *dfr* genes [294].

Sul and *dfr* genes have been detected in Enterobacterales from humans, food-producing and companion animals [294, 424]. The *dfrK* gene (linked to *tetL*) is widely disseminated on plasmids in LA-MRSA from food-producing species and has also been identified on a transposon in MSSA and *E. faecium* [425].

In *Salmonella* spp. and indicator *E. coli* isolates recovered from animals and food during the 2018–2019 routine EU monitoring, resistance to sulfonamides was generally high to very high. Resistance to TMP is at a lower level [59]. In LA-MRSA, extremely high levels of TMP resistance were detected in isolates from pigs [59], although the number of isolates tested is small.

Also, *sul1* genes and *dfrA* genes are part of class1 integrons. MDR in Enterobacterales, particularly among isolates with ESBLs, is likely to be a result of the coexisting nature of *sul1* and *sul2*, *dfr* genes encoded within ESBL and carbapenemase-encoding plasmids [285].

The resistance mechanisms in *P. jirovecii* have not been fully elucidated, but genetic mutations within DHFR probably play a role [426]. The majority of patients with PCP and DHPS mutations who are treated with trimethoprim-sulfamethoxazole respond to this treatment [426].

The mechanisms of resistance in *Nocardia* spp. are not fully understood, but *sul* and *dfr*A genes play a role [419]. Class 1 and 3 integrons carrying *sul* genes have been found in *Nocardia* spp.

Resistance to sulfonamides and trimethoprim is widespread in target pathogens, especially *E. coli*, from food-producing and companion animals reflecting the extensive use over decades [136, 154, 204, 295]. This has limited the usefulness of this class for many indications in veterinary medicine [125].

Clinical resistance to sulfonamides-trimethoprim does not appear to be a significant problem in *T. gondii* at present [3].

Transmission

Similar *sul* and *dfr* genes have been detected in Enterobacterales from food-producing and companion animals and humans [294, 424, 427]. Sul and *dfr* resistance genes are located on MGEs that have the potential to be transferred from animal commensal bacteria to pathogenic bacteria in humans and other animals. There is also the possibility for transmission of TMPS-resistance from animals to humans via zoonotic pathogens, e.g. LA-MRSA.

Pneumocystis is host-specific and there is no evidence to support potential transfer of TMPS-resistance from animals to humans.

In conclusion, there is evidence for the selection and transmission of resistance to sulfonamides and trimethoprim from animals to humans and other animals via zoonotic and target pathogens or commensal bacteria capable of transferring resistance to pathogens.

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to sulfonamides, trimethoprim and their combinations (TMPS).

Criterion (c) – availability of other treatments for animals

Sulfonamide-trimethoprim combinations (TMPS) are authorised in VMPs in the EU for a wide range of infections in food-producing and companion animals and are one of few classes authorised for use in minor species including turkeys, goats, rabbits, fish and children's pets. Alternatives are usually available for the major indications and major species, but may be in a higher AMEG category.

There are limited treatment options for the less common infections mentioned above e.g. *Nocardia* spp. and certain protozoal infections. Clindamycin is the preferred treatment for toxoplasmosis in cats; azithromycin have also been used (Ettinger 2018; Riviere 2018).

TMPS is the only authorised antibiotic for oral administration for horses in the EU. TMPS is one of few antibiotic classes authorised in the EU for use in fish, being important for treatment of e.g. *Aeromonas* spp., *Vibrio* spp., *Yersinia ruckerii*, Streptococcosis. Limited alternatives include amoxicillin, tetracyclines or substances from higher AMEG categories e.g. florfenicol or (fluoro)quinolones.

Criterion (d) – availability of other antimicrobial treatments for humans

For most of the approved indications, treatment alternatives are available. Alternative treatment options include:

- *P. jirovecii* pneumonia - for patients with moderate-to-severe disease: pentamidine/ primaquine plus clindamycin; for patients with mild-to-moderate disease dapsone plus trimethoprim, primaquine plus clindamycin or atovaquone;
- MRSA acute bacterial skin and skin structure infections: clindamycin, doxycycline, minocycline, linezolid, for complicated cases vancomycin, linezolid, daptomycin, telavancin;
- Nocardiosis: imipenem or meropenem, ceftriaxone, cefotaxime, amikacin, linezolid.

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

- Sulfonamides and trimethoprim are important as first-line antibiotics to treat a wide range of infections in humans and animals.
- In humans, TMPS is considered as an essential component of limited treatment alternatives specifically for life-threatening *P. jirovecii* pneumonia in immunocompromised patients and nocardiosis in immunosuppressed patients. This disease is not zoonotic.
- TMPS is authorised for use in all major animal species and many minor species. It is used for common, but sometimes serious, infections of respiratory, gastrointestinal and urogenital tracts, SSTI and also for coccidiosis and more rare protozoal infections.
- There is evidence for the selection and significant transmission of resistance to TMPS from animals to humans and between animals via zoonotic and target pathogens or commensal bacteria capable of transferring resistance to pathogens. The high prevalence of resistance to sulfonamides and trimethoprim in major bacterial pathogens in humans and animals has limited the clinical usefulness of these classes.

- TMPS are included in the AMEG Category D, acknowledging that in general there are alternative treatments in human and veterinary medicine for their indications.
- TMPS are authorised for use in all major food and non-food-producing species, and some limited market species, with indications that encompass a broad range of infections. They are also available in formulations for oral group and individual medication and for parenteral and topical use. Use outside the terms of a marketing authorisation is likely to account for a small proportion of overall TMPS use and it is unlikely that this use would contribute to the AMR risk substantially beyond authorised use.

Therefore, considering the points above relevant to criteria (b), (c) and (d), it is recommended that no conditions should be placed on the use of Sulfonamides, Trimethoprim or their combinations outside the terms of the marketing authorisation, although responsible antimicrobial use principles should be applied.

4.20. Quinolones, including fluoroquinolones

4.20.1. Background information

Examples of substances in the class that are authorised in veterinary and human medicine in the EU

Examples of substances authorised for veterinary use	Examples of ATCvet codes
Oxolinic acid	QJ01MB05
Flumequine	QJ01MB07
Danofloxacin	QJ01MA92
Difloxacin	QJ01MA94
Enrofloxacin	QJ01MA90
Marbofloxacin	QJ01MA93
Norfloxacin	QJ01MA06
Pradofloxacin	QJ01MA97
Examples of substances authorised for human use	**Examples of ATC codes**
Cinoxacin	J01MB06
Pipemidic acid	J01MB04
Ciprofloxacin	J01MA02
Delafloxacin	J01MA23
Levofloxacin	J01MA12
Lomefloxacin	J01MA07
Moxifloxacin	J01MA14
Nadifloxacin	D10AF05
Norfloxacin	J01MA06
Ofloxacin	J01MA01
Ozenoxacin	D06AX14
Pefloxacin	J01MA03
Prulifloxacin	J01MA17
Rufloxacin	J01MA10

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

Substance	Species	MRL tissues	MRL milk	MRL eggs	Other provisions
Oxolinic acid	All food-producing species	Yes	-	-	Not for use in animals from which milk or eggs are produced for human consumption.
Flumequine	All food-producing species	Yes	Yes	-	Not for use in animals from which eggs are produced for human consumption.
Danofloxacin	All food-producing species	Yes	Yes	-	Not for use in animals from which eggs are produced for human consumption.
Difloxacin	All food-producing species	Yes	-	-	Not for use in animals from which milk or eggs are produced for human consumption.

Enrofloxacin	All food-producing species	Yes	Yes	-	Not for use in animals from which eggs are produced for human consumption.
Marbofloxacin	Bovine, Porcine	Yes	Yes	-	-

EU-authorised VMP formulations, based on sales reported to ESVAC

Species		Route of administration					
		Group		Individual			
		In-feed	In-water	Injection	Oral e.g. tablet, paste, powder	Topical/local (incl. intrauterine)	Intra-mammary
Major	**Cattle**		ENR, FLU, OA	DAN, ENR, FLU, MAR	MAR, OA	ENR	
	Sheep (for meat)		ENR, FLU	ENR, FLU	FLU	ENR	
	Pigs	ENR, FLU	ENR, FLU, OA	DAN, ENR, FLU, MAR	ENR, FLU, OA	ENR	
	Chickens	ENR	DIF, ENR, FLU, OA	FLU	ENR		
	Dogs		ENR	ENR, FLU, MAR	ENR, FLU, MAR, PRA		
	Cats		ENR, PRA	ENR, FLU, MAR	ENR, MAR, PRA		
Limited market species	**Turkeys**	ENR	DIF, ENR, FLU		ENR		
	Poultry		ENR, FLU		ENR		
	Ducks		ENR, FLU				
	Geese		FLU				
	Pheasants		ENR, FLU		ENR		
	Guinea fowl		ENR				
	Quail		ENR, FLU		ENR		
	Partridges		FLU				
	Guinea pig				ENR		
	Goats		ENR, FLU	ENR, FLU	FLU	ENR	
	Rabbits		ENR, FLU	ENR, FLU	ENR, FLU		
	Buffalo		FLU	FLU			
	Horses		FLU	FLU			
	Pigeons		ENR, FLU, NOR		ENR		
	Fish	ENR, FLU, OA					
	Rodents		ENR	ENR	ENR		
	Reptiles		ENR	ENR			
	Ornamental birds		ENR, FLU	ENR	ENR		
	Fur animals		ENR				

ENR (enrofloxacin), FLU (flumequine), OA (oxolinic acid), DAN (danofloxacin), MAR (marbofloxacin), DIF (difloxacin), PRA (pradofloxacin), NOR (norfloxacin)

Summary of main indications and contra-indications for EU-authorised VMPs, based on selected SPCs

Main indications	*Food-producing species* – Treatment of septicaemia and alimentary tract infections caused by *Escherichia coli.* Treatment of: Cattle - Respiratory tract - *Pasteurella multocida, Mannheimia haemolytica, Histophilus somni* and *Mycoplasma* spp. Acute mycoplasma-associated arthritis due to *Mycoplasma bovis*. Acute mastitis due to *E. coli.* Sheep and Goats - Mastitis caused by enrofloxacin susceptible strains of *Staphylococcus aureus* and *Escherichia coli*. Goats - Respiratory tract *Pasteurella multocida* and *Mannheimia haemolytic*a. Pigs - Respiratory tract - *Pasteurella multocida, Mycoplasma* spp. and *Actinobacillus pleuropneumonia*e. Alimentary tract – salmonellosis (piglets). Metritis-mastitis-agalactia syndrome due to susceptible organisms.

	Chickens - *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, *Avibacterium paragallinarum*, *Pasteurella multocida*, *E. coli* Turkeys - Respiratory tract - *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, *Pasteurella multocida*, *E. coli* Horses (flumequine) – gastrointestinal infections due to *E. coli* and *Salmonella* spp. Fish, including Salmon and Trout - treatment of furunculosis (*Aeromonas* spp.), yersiniosis (Enteric Redmouth), vibriosis, streptococci and other susceptible bacteria. Rabbits - Respiratory tract - *Pasteurella multocida*, *E. coli.* Skin and wound infections due to *Staphylococcus aureus.* *Dogs and cats* – treatment of alimentary, respiratory and urogenital tract infections caused by the following bacteria: *Staphylococcus spp.*, *Pasteurella spp.*, *Bordetella spp.*, *Klebsiella spp.*, *E. coli*, *Pseudomonas spp.*, *Proteus spp.* Topical treatment of otitis externa due to susceptible bacteria. *Rodents, reptiles and ornamental birds* – alimentary and respiratory tract infections.
Contraindications	Do not use in growing horses and dogs due to possible damage to articular cartilage. Do not use in epileptic animals or animals with neurological conditions.

Examples of EU-authorised HMP formulations, from Article 57 database

Substance	Route of administration		
	Injection	Oral e.g. tablet, liquid	Topical/local
Cinoxacin		x	
Pipemidic acid		x	
Ciprofloxacin	x	x	X
Delafloxacin	x	x	
Levofloxacin	x	x	X
Lomefloxacin		x	
Moxifloxacin	x	x	X
Nadifloxacin			X
Norfloxacin		x	X
Ofloxacin	x	x	X
Ozenoxacin			X
Pefloxacin		x	
Prulifloxacin		x	
Rufloxacin		x	

Existing recommendations

WOAH recommendations

Non-fluorinated (1st-generation) quinolones are categorised VHIA by WOAH (formerly OIE). *Specific comments:* Quinolones of the first generations are used in the treatment of septicaemias and infections such as colibacillosis.

Fluoroquinolones (2nd-generation quinolones) are categorised VCIA by WOAH (formerly OIE). *Specific comments:* The wide range of applications and the nature of the diseases treated make fluoroquinolones extremely important for veterinary medicine. Fluoroquinolones are critically important in the treatment of septicaemias, respiratory and enteric diseases.

Additional WOAH recommendations for fluoroquinolones:

- Not to be used as preventive treatment applied by feed or water in the absence of clinical signs in the animal(s) to be treated;
- Not to be used as a first line treatment unless justified, when used as a second line treatment, it should ideally be based on the results of bacteriological tests; and
- Extra-label/off-label use should be limited and reserved for instances where no alternatives are available. Such use should be in agreement with the national legislation in force

WHO classifications

WHO: HPCIA

- (C1: Yes) Limited therapy for *Campylobacter* spp., invasive disease due to *Salmonella* spp., and MDR *Shigella* spp. infections.
- (C2: Yes) May result from transmission of *Campylobacter* spp. and Enterobacterales, including *E. coli* and *Salmonella* spp., from non-human sources.
- (P1: Yes) High absolute number of people affected by diseases for which the antimicrobial is the sole or one of few therapies available.
- (P2: Yes) High frequency of use in human medicine
- (P3: Yes) Transmission of resistant *Campylobacter* spp. and Enterobacterales, including *E. coli* and *Salmonella* spp., from non-human sources.

Non-fluorinated quinolones: WHO AWaRe: Watch: e.g. Flumequine, Oxolinic acid, Rosoxacin

Fluoroquinolones: WHO AWaRe: Watch: Ciprofloxacin, Delafloxacin, Enoxacin, Gatifloxacin, Levofloxacin, Lomefloxacin, Moxifloxacin, Norfloxacin, Ofloxacin, Sitafloxacin, Sparfloxacin, Tosufloxacin

AMEG and CVMP recommendations

Fluoroquinolones are included in the AMEG Category B, for which there is a higher AMR risk to public health. For these antimicrobials, the risk to public health resulting from veterinary use needs to be mitigated by specific restrictions. These restricted antimicrobials should only be used for the treatment of clinical conditions when there are no alternative antimicrobials in a lower category that could be effective. Especially for this category, use should be based on the results of antimicrobial susceptibility testing, whenever possible.

The CVMP published a public statement on the use of (fluoro-)quinolones in 2007 [428] which was accompanied by a reflection paper [429] making recommendations on responsible use guidance to be included in the SPCs of quinolone products, as follows:

- 'official and local antimicrobial policies should be taken into account when the product is used';
- 'whenever possible, (fluoro)quinolones should only be used based on susceptibility testing';
- 'use of the product deviating from the instructions given in the SPC may increase the prevalence of bacteria resistant to the (fluoro)quinolones due to the potential for cross-resistance';

and, additionally for fluoroquinolones, that:

- 'fluoroquinolones should be reserved for the treatment of clinical conditions which have responded poorly, or are expected to respond poorly, to other classes of antimicrobials'.

A referral for enrofloxacin products administered in drinking water to poultry [430] recommended removal of indications for the treatment of *Salmonella* spp. due to the lack of evidence to support the dosing regimen for elimination of the infection and considering the EU legislation in regard to national control programmes.

Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Disclaimer: The information in this section reflects reported use of antimicrobials outside the terms of a marketing authorisation. No evaluation is made in this section by the working group on the efficacy or safety of the reported uses, or on their potential impact on development and dissemination of AMR.

Information from published sources

Considering the broad range of indications stated in many SPCs and the wide range of animal species for which this class is authorised, it is not always possible to determine which of the published indications fall outside the terms of the marketing authorisation. Potential uses cited include for otitis media in calves, osteomyelitis, bovine anaplasmosis, feline bartonellosis, *Mycoplasma felis, Chlamydophila felis*. In individual animals with severe infections, fluoroquinolones may be used in combination with another antibiotic to provide broad-spectrum coverage e.g. in combination with penicillin G in horses and with metronidazole in dogs. There is limited availability of products authorised for use in horses, but enrofloxacin is reported to be used for treatment of *Staphylococcus* spp. infections and Gram-negative infections in this species. Some textbooks include dosing regimens for exotic species including reptiles, small mammals and ornamental birds and fish [33, 125]. Treatment guidelines recommend fluoroquinolones as part of the combination treatment regimen for mycobacterial disease in companion animals [244, 255].

Information from the open call for data on use of antimicrobials in animals

The information below is summarised from the open call for data. Inclusion in the table does not endorse use or imply that it is consistent with use according to legislative provisions in Articles 112 to 114.

Fluoroquinolones were quoted mainly for the treatment of individual animals; in most cases specific infectious disease conditions were not specified and as such, information on "alternative treatment classes" was not available. However, the survey did identify that, based on their efficacy and broad spectrum of activity, (fluoro)quinolones were used to treat a variety of infectious diseases in various body systems.

Substance	Species	Indication	Alternatives
marbofloxacin	Equine	multiresistant infection	Enrofloxacin
enrofloxacin	Cattle	Salmonellosis	Yes
ciprofloxacin	dog	Bacterial ear infections with perforated ear drums, susceptible to Ciprofloxacin (oftentimes Pseudomonas spp)	None to my knowledge
ciprofloxacin	Dogs Cats Horses	Treatment of eye surface infections due to *Pseudomonas aerugionsa*	0.3% Topical Tobramycin drops are used in Human ophthalmology: to date, the concentration seems to be less effective (see Gentamicin comment above).
ciprofloxacin	Dogs, cats, horses, rabbits, guinea pigs, chinchillas, degus, hamsters, gerbils, ferrets, mice, rats, reptiles, ornamental birds	Eye Infection with bacteria that are only sensitive to this antibiotic	other fluoroquinolones for use on the eye
moxifloxacin	Dogs, cats, horses	Eye Infection with bacteria that are only sensitive to this antibiotic	other fluoroquinolones for use on the eye
ofloxacin	Dogs, cats, horses, rabbits, guinea pigs, chinchillas, degus, hamsters, gerbils, ferrets, mice, rats,	Eye Infection with bacteria that are only sensitive to this antibiotic	other fluoroquinolones for use on the eye

	reptiles, ornamental birds		
ciprofloxacin	dog, cat	severe keratitis (Pseudomonas)	No
ofloxacin	dog, cat	severe keratitis (Pseudomonas)	No
norfloxacin	dog, cat	severe keratitis (Pseudomonas)	No
ciprofloxacin	Dogs, cats, horses, rabbits, guinea pigs, reptiles, ornamental birds, birds of prey	Eye Infection with bacteria that are only sensitive to this antibiotic	other fluoroquinolones for use on the eye
levofloxacin	Dogs, cats, horses, rabbits, guinea pigs, reptiles, ornamental birds, birds of prey	Eye Infection with bacteria that are only sensitive to this antibiotic, Therapy without delayed corneal healing required	other fluoroquinolones for use on the eye
moxifloxacin	Dogs, cats, horses, rabbits, guinea pigs, reptiles, ornamental birds, birds of prey	Eye Infection with bacteria that are only sensitive to this antibiotic like septic keratitis	other fluoroquinolones for use on the eye
ofloxacin	Dogs, cats, horses, rabbits, guinea pigs, reptiles, ornamental birds, birds of prey	Eye Infection with bacteria that are only sensitive to this antibiotic, Therapy without delayed corneal healing required	other fluoroquinolones for use on the eye

4.20.2. Evaluation

Scope of permitted use according to the MRL Regulation

(Fluoro)quinolones are included in Table 1 (allowed substances) of the Annex to Regulation (EU) 37/2010 and can be used accordingly in all food-producing species in compliance with Articles 113 and 114 of Regulation (EU) 2019/6. Other than for marbofloxacin, 'Other provisions' state that (fluoro)quinolones should not be used in animals from which eggs are produced for human consumption.

All (fluoro)quinolones can be used in non-food-producing species in accordance with Article 112.

Examples of veterinary-authorised formulations/species

(Fluoro)quinolones are available in formulations for administration in the drinking water to groups of animals including all major food-producing species and several limited market species e.g. horses (flumequine in one member state), goats, rabbits, turkeys, gamebirds, fur animals and exotic species. They are also available for administration to farmed fish via medicated feed. Formulations for administration to individual animals by injection or orally are available for all major species and some limited market species. VMPs containing fluoroquinolones are also available for intrauterine administration to ruminants and pigs.

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Importance for human health

Fluoroquinolones overall have activity against aerobic Gram-negative enteric bacilli (e.g. Enterobacterales, including MDR *E. coli*, *Klebsiella* spp., *Proteus* spp., *Salmonella* spp., *Shigella* spp. and *Campylobacter* spp., and many common human respiratory pathogens (e.g. *Streptococcus*

pneumoniae (including MDR strains), *Haemophilus influenzae*, *Moraxella catarrhalis*). In addition, some fluoroquinolones are active against *Pseudomonas* species, some Gram-positive organisms (including MRSA), and have excellent activity in vitro against *Mycobacterium tuberculosis* [431]. The non-fluorinated quinolones (e.g. nalidixic acid) and older fluoroquinolones (e.g., norfloxacin, ciprofloxacin) have activity against Gram-negative bacteria but not against Gram-positives. The non-fluorinated quinolones have generally been replaced in clinical practice. Newer fluoroquinolones (e.g., levofloxacin, sparfloxacin, moxifloxacin) have enhanced activity against Gram-positive bacteria as well as good activity against *Mycoplasma* and *Chlamydia*. The newest fluoroquinolones (e.g., moxifloxacin) have the most potent activity against anaerobic bacteria [432].

The spectrum of activity and potency of fluoroquinolones has led to a wide range of clinical indications in humans, including treatment of UTIs, RTIs, SSTIs, infectious diarrhoea, bone and joint infections and infections of the ear and eyes.

Fluoroquinolones are used as second-line agents to treat TB in the context of resistance and/or intolerance to first-line agents, and similarly campylobacteriosis in HIV infection. Very relevant indications for which fluoroquinolones are first line are the post-exposure prophylaxis and the treatment of inhalational anthrax [297].

Most fluoroquinolones are nationally approved in the EU, for a broad range of indications. EMA confirmed that the use of fluoroquinolones should be restricted to cases when it is considered inappropriate to use other antibacterial agents that are commonly recommended for the initial treatment of the infections for which they are indicated (in particular in view of the risk of disabling sequelae) risk of serious adverse effects involving the musculoskeletal and nervous system).

Importance for animal health

Quinolones are classified by OIE as VCIAs and are in AMEG Category B. The first-generation quinolones (nalidixic acid, oxolinic acid, flumequine) have activity against Enterobacterales, only, and less favourable pharmacokinetics compared with the fluoroquinolones. Based on ESVAC, in 2020 the sales of 1st-generation quinolones made up only around 7% of the total sales of quinolones. Fluoroquinolones are essential in many animal species for the treatment of various serious infections due to their broad spectrum of activity, widespread distribution throughout the body and generally low toxicity. They are authorised for indications including gastrointestinal, respiratory and urogenital infections (e.g. pyelonephritis, prostatitis, endometritis), severe mastitis and septicaemia. According to SPCs and the AMEG B categorisation, fluoroquinolones should only be used in animals for the treatment of clinical conditions when there are no alternative antibiotics in a lower category that could be clinically effective.

Fluoroquinolones are among few alternatives for treatment of diarrhoea in piglets (*E. coli*) and sepsis caused by Enterobacterales in various animal species. *E. coli* infections (e.g. septicaemia, meningitis, severe enteritis) are a major cause of morbidity and mortality in neonatal and juvenile livestock, and the most common cause of peracute mastitis leading to bacteraemia and fatality in adult cattle [53, 175-177, 205, 433].

Fluoroquinolones are also important for treatment of *Mycoplasma* spp. infections, including *M. gallisepticum* and *M. synoviae* in poultry, *M. hyopneumoniae* in pigs and *M. bovis* in cattle. *M. bovis* is a cause of mastitis and arthritis and a frequent and challenging cause of enzootic pneumonia with potentially high morbidity/mortality in young calves. *M. bovis* is often resistant to tetracyclines and macrolides with alternatives otherwise being limited to florfenicol [55, 237, 269, 278].

In cats and dogs, fluoroquinolones are recommended in international and EU treatment guidelines for respiratory infections due to *Pseudomonas aeruginosa* and empirical treatment of pneumonia accompanied by sepsis and pyothorax, whilst awaiting AST results [72, 107].

UTI in dogs and cats are increasingly associated with pathogens resistant to first-line antibiotics [104, 402, 434, 435]. According to ISCAID guidelines, fluoroquinolones may be one of limited options for pyelonephritis, often due to Enterobacterales, a disease requiring prompt empirical treatment. 3rd-generation cephalosporins are the alternative [104]. In the case of severe sepsis, early treatment with a broad-spectrum bactericidal antimicrobial is critical for survival and fluoroquinolones are recommended alone or in combination.

In aquaculture, quinolones and fluoroquinolones are authorised and are the only or one of few effective treatment options for certain infections of farmed fish (e.g. *Yersinia ruckeri*, *Aeromonas salmonicida* and *Flavobacterium spp.*) [26].

In rabbits and rodents, fluoroquinolones are used because of their good oral bioavailability and the need to avoid narrow spectrum antibiotics such as beta-lactams that cause toxicity by disruption of the normal gut microbiota [33, 96].

Veterinary literature includes numerous reports of the use of fluoroquinolones outside the terms of marketing authorisations. This may be for severe and/or atypical infections that are not included in the SPC e.g. fluoroquinolones are one of the few effective treatment options in animals for infections caused by atypical bacteria such as *Mycobacteria* spp., *Chlamydia* spp., *Bartonella* spp. and *Rickettsia* spp.. They are also often used to treat limited market species, including reptiles, zoo and other exotic species (see above). Fluoroquinolones are used outside the terms of the marketing authorisation in horses to treat serious Gram-negative infections when use of the authorised alternative, gentamicin, is compromised by renal dysfunction [33, 125]. According to the open call for data, there were several reports of the use of topical formulations to treat eye infections, in particular keratitis associated with *Pseudomonas aeruginosa* infection in companion animal species.

Development and selection of resistance

In Enterobacterales, resistance to fluoroquinolones is most commonly acquired by step-wise mutations. One mutation in the *gyr*A gene mediates full resistance to first generation quinolones such as nalidixic acid and flumequine and reduced susceptibility to fluoroquinolones. Further mutations in either *gyr*A or topoisomerase IV genes are needed to mediate progressively increasing resistance levels to fluoroquinolones [428]. In addition, the *qnr* gene is a plasmid-borne resistance mechanism in Enterobacterales which confers low level resistance and is selected by both quinolones and fluoroquinolones. Combinations of the *qnr* mechanism with mutational mechanisms can variably impact on bacterial fitness [436]. Plasmid-mediated quinolone resistance genes (PMQR) (qnr oqxAB, aac(6')-Ib-cr, qep and *crp*P genes) are increasing, with several reports of the isolation of quinolone-resistant microorganisms in the absence of target mutations, including in a Salmonella Rissen isolate [437]. The PMQR aac(6')-Ib-cr gene also confer decrease susceptibility to aminoglycosides (kanamycin, tobramycin, and amikacin) [438] and oqxAB to antimicrobials, disinfectants and detergents [439].

PMQR genes (mainly *qnr* genes) have been identified in isolates from food-producing and companion animals in the EU [86, 440, 441]. However, studies in companion animals have shown resistance in Enterobacterales isolates mostly associated with chromosomal mutations in quinolone resistance-determining region (*gyr*A, *par*C, *par*E) [440, 442].

Co-selection of resistance to other antimicrobials might occur due to the frequent location of PMQR on plasmids carrying resistance genes to other antimicrobials.

In *Campylobacter* spp., a single mutation in *gyr*A imparts high-level resistance to fluoroquinolones. The CmeABC efflux pump also contributes significantly to fluoroquinolone resistance in *Campylobacter jejuni*. Fluoroquinolone resistance develops rapidly in *Campylobacter* spp. in poultry following exposure [443].

Resistance in *Pseudomonas* spp. is due to decreased permeability, over-expression of efflux pumps and mutations in topoisomerases which have been detected in isolates from dogs [444].

There is evidence for the selection and spread of resistance to fluoroquinolones due to the use of these antimicrobials in food-producing animals [437, 445].

ECDC/EFSA mandatory surveillance shows very high levels of resistance to fluoroquinolones in *Salmonella* spp. from humans and poultry, indicator *E. coli* from poultry and in *Campylobacter* spp. from humans and food-producing animals. However, macrolides remain first-choice for Campylobacter infections that require treatment in humans and combined resistance to both ciprofloxacin and erythromycin in *C. jejuni/C.coli* from humans is very low/low [59].

EFSA does not routinely monitor for antimicrobial resistance in aquaculture food production.

Based on the literature reviews performed by EFSA of publications since 2010 and national AMR monitoring reports, the mean overall EU level of resistance to fluoroquinolones in pathogenic *E. coli* from poultry, pigs and calves remains low compared with resistance to other antibiotic classes [55, 108, 295], with exceptions in individual countries (See Annex 3. EFSA Animal Health Law Scientific opinions). The overall EU level of resistance to fluoroquinolones in *Pseudomonas aeruginosa* from dogs and cats was 56.4% [range 8-67.7% across MSs] [136]. According to EFSA *Aeromonas hydrophila, Aeromonas salmonicida, Flavobacterium columnare* and *Flavobacterium psychrophilum* are highly relevant pathogens in relation to fish species kept in the EU. Evidence was found of widespread resistance to tetracyclines and quinolones in these species [446].

Transmission of resistance

In the EU, non-typhoidal *Salmonella* and *Campylobacter* spp. infections in humans are predominantly foodborne. A significant association has been shown between fluoroquinolone-resistance in *C. jejuni* isolates from poultry and from humans [89].

Epidemiological studies have shown similarity between fluoroquinolone-resistant *E. coli*, fluoroquinolone-resistant *Salmonella* and *Campylobacter* spp. isolates from humans and chickens [82, 86, 447, 448]. There is also evidence for association between several PMQR carrying isolates and food-producing animals [437]. Fluoroquinolone-resistant MDR *E. coli* of the ST131 clone of importance in human medicine has been isolated in dogs [449].

In conclusion, there is evidence for the selection and significant transmission of resistance to fluoroquinolones from animals to humans via zoonotic pathogens or commensal bacteria capable of transferring resistance to human pathogens. Fluoroquinolone-resistance can also be transmitted between animals via pathogenic and commensal *E. coli* [450].

In conclusion,

- Fluoroquinolones are of high importance in human medicine to treat a wide range of infections including RTI, UTI and SSTI. They are important for treatment of Gram-negative infections that are resistant to other antibiotic classes and as second-line treatment for MDR tuberculosis. Uses are restricted due to safety concerns in humans.

- In animals, (fluoro)quinolones are reserved for use as last resort to treat serious gastrointestinal, respiratory and urogenital infections, including those due to MDR Gram-negative bacteria. They are used outside the terms of the marketing authorisation for treatment of atypical microbial infections, eye infections and in unauthorised species including horses and exotic species including zoo animals.
- Resistance to (fluoro)quinolones may be transferred from animals to humans and other animals.
- Use of first-generation quinolones can select for full resistance to fluoroquinolones in *Campylobacter* spp. and reduced susceptibility to fluoroquinolones in Enterobacterales; hence the AMR risk for both non- and fluorinated- quinolones has been addressed together.

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to non-fluorinated quinolones and fluoroquinolones.

Criterion (c) – availability of other treatments for animals

In pigs, vaccinations (sows or piglets) can be an effective way to reduce the occurrence of neonatal and post-weaning diarrhoea due to *E. coli*; however, it is necessary to use the appropriate vaccine for the most prevalent ETEC pathotype on the farm and to ensure that the vaccine is administered at the optimal time. In poultry, vaccination programmes for various viral diseases (e.g. ND, IB, IBD, Marek's) have greatly reduced the need for antibiotic treatments but there is a great diversity in APEC strains and fewer effective vaccines are available. Regardless, these preventive options cannot replace antibiotics when treatment is needed for sick animals.

Recent EFSA opinions noted high levels of resistance to first line antimicrobials (e.g. aminopenicillins, potentiated sulfonamides, tetracyclines), often involving multidrug resistance, in pathogenic *E. coli* from swine, poultry, calves, lambs and horses. This suggests the limited efficacy of first-line antibiotics against these infections in many EU countries [55, 108, 154, 204, 295]. Levels of resistance to fluoroquinolones have remained lower. Alternatives for resistant *E. coli* are limited to AMEG Category B substances: colistin (not foals) or 3rd- and 4th-generation cephalosporins (not poultry) or, depending on patient/disease suitability, aminoglycosides (Category C).

For dogs and cats suffering from resistant UTI, 3rd-generation cephalosporins may be the only alternative to fluoroquinolones. For sepsis, alternatives include combinations of e.g. aminopenicillins, clindamycin, or for septicaemias caused by MDR Gram-negative bacteria, aminoglycosides, although the latter are limited by nephrotoxicity [33, 255, 451]. EFSA has noted high levels of resistance to aminopenicillins, 3rd-generation cephalosporins and fluoroquinolones in *E. coli* isolates from dogs and cats [136], supporting that a range of antibiotic classes should be available for their treatment.

Although a vaccine is available for *A. salmonicida*, there are few authorised antimicrobials available for treatment of disease outbreaks in aquaculture.

Criterion (d) – availability of other antimicrobial treatments for humans

EMA has concluded that in view of the disabling and potentially permanent adverse effects associated with fluoroquinolones in humans, their use should be restricted to patients with serious infections or as last resort for milder infections where other therapeutic options are not effective or not tolerated [452]. For these cases there are very few or no alternatives available.

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

- Fluoroquinolones are important in human medicine to treat a wide range of serious infections including UTI, RTI, SSTI, in particular those caused by MDR Gram-negative bacteria, for which there are limited effective alternative antibiotics. Their use is limited by their association with severe and potentially permanent adverse events in humans, and this means that for milder infections (fluoro)quinolones are only used as last resort.
- In animals, fluoroquinolones are reserved for use as last resort (AMEG Category B), but are used to treat a wide range of serious infections, including those caused by MDR Gram-negative bacteria, in both food-producing and companion animals. Resistance to first-line antibiotics in pathogenic *E. coli* from many animal species is high, meaning that the only alternatives to fluoroquinolones may also be from AMEG Category B. Fluoroquinolones are used outside the terms of the marketing authorisation for treatment of atypical microbial infections and for use in horses and exotic species including zoo animals. First-generation quinolones have fewer applications but are useful for treatment of certain fish diseases.
- There is evidence for the selection and significant transmission of resistance to fluoroquinolones from animals to humans via zoonotic pathogens or commensal bacteria capable of transferring resistance to human pathogens. In particular, very high levels of resistance to fluoroquinolones have been identified in Campylobacter from poultry and a significant association has been shown between this resistance in *C. jejuni* isolates from poultry and from humans. Despite this, macrolides remain first-choice for campylobacteriosis in humans and combined resistance to both ciprofloxacin and erythromycin in *C. jejuni/C.coli* from humans is very low/low. Use of quinolones can select for full resistance to fluoroquinolones in *Campylobacter* spp. and reduced susceptibility to fluoroquinolones in Enterobacterales; hence there is a rationale to consider restrictions on quinolone use in parallel to those for fluoroquinolones.
- The extent of use of (fluoro)quinolones outside the marketing authorisation is unknown; although numerous reports were found in the literature, mostly relating to minor indications and minor or exotic species.

Therefore, considering the points above relevant to criteria (b), (c) and (d), it should be considered if conditions or a prohibition should be placed on the use of (fluoro)quinolones outside the terms of the marketing authorisation.

Step 2. Considerations of conditions to be placed on use outside the terms of a marketing authorisation

Please refer to Section 3.1.2. of the main report for the general rationale behind the proposed conditions.

(i) Use for unauthorised indications

Although (fluoro)quinolones are authorised for a wide range of indications and target pathogens, the following conditions are proposed in accordance with Section 3.1.2. (i) of this advice.

Condition proposed: *For those indications not included in the SPC of the concerned product, use must be based on target pathogen identification and antimicrobial susceptibility testing that demonstrates that (fluoro)quinolones are likely to be effective and that antimicrobials from a lower AMEG category would not be effective, unless it can be justified that this is not possible.*

Condition proposed: *Use of (fluoro)quinolones under Article 113 to treat salmonellosis should be restricted to use of injectable products in individual animals with potentially life-threatening infection.*

Rationale: The primary mechanisms for controlling Salmonella in pigs in the EU are through elimination or control and reduction programmes [156], including use of vaccination and husbandry measures outlined above [157]. Despite these measures, Salmonella can be re-introduced onto the farm through contaminated feed and water or wildlife such as rodents, birds and foxes. Clinical salmonellosis infection in pigs is usually due to host-adapted *S.* Choleraesuis (causing septicaemia) or non-host adapted *S*. Typhimurium (enterocolitis). Ubiquitous serotypes such as *S.* Typhimurium and *S.* Enteritidis generally cause human infections, but serious systemic illness in humans due to *S*. Choleraesuis is rare. A single product containing enrofloxacin is authorised in the EU for oral treatment of individual piglets with salmonellosis. Use of antibiotics has been justified to reduce severity of signs and prevent suffering in individual animals but does not reduce the prevalence or duration of shedding by sick or recovered animals, hence it has been concluded that use of antimicrobials for *Salmonella* control [metaphylaxis] in pigs should be discouraged due to the public health risk and use should be limited to individuals with life-threating salmonellosis (bacteriemia with high fever, depression and dyspnoea) [158, 159].

Salmonella infection in cattle can manifest as haemorrhagic enteritis, endotoxaemia, septicaemia, pneumonia and abortions. Host-adapted *S.* Dublin is the most common serotype in cattle and rarely causes infections in humans; *S.* Typhimurium is the second most common serotype. Control programmes are also implemented in some EU countries. Antimicrobial treatment is controversial due to the public health risk and possibility that cattle infected by *S*. Dublin may become chronic sub-clinical carriers that maintain infection in the herd. However, faecal shedding is a lesser problem in calves. Antimicrobial use in calves has been justified in case of enteritic salmonellosis to prevent development of bacteraemia and multiple organ disease, in which case systemic antimicrobial treatment is always needed [53, 160].

In terms of public health risk, most concern relates to serovars of *Salmonella* that have been associated with human foodborne diseases outbreaks. In the EU, data for 2020 show that most such outbreaks were due to *S*. Enteritidis (57.9%), S. Typhimurium, Monophasic *S*. Typhimurium, *S.* Infantis and *S.* Derby. *S.* Enteritidis was primarily linked to broilers and layers/eggs, S. Typhimurium to broilers and pig sources, Monophasic S. Typhimurium to pigs and broilers, *S.* Infantis to broilers and *S.* Derby to pigs and turkeys [28]. EFSA/ECDC monitoring data from 2019-2020 show overall high resistance levels to ampicillin, sulfonamides and tetracyclines (ASuT) in *Salmonella* spp. isolates from human cases and moderate-very high ASuT resistance in *Salmonella* isolates from food-producing species in most member states, limiting first-line treatment options. Resistance to fluoroquinolones was also very high amongst *Salmonella* spp. isolates from poultry and moderately high in isolates from human cases; whilst it was moderate in isolates from calves (12.5%) and pigs (5.8%). Invasive salmonella infections in humans are treated by preference with 3rd-generation cephalosporins, fluoroquinolones or, in children, azithromycin. Combined resistance to cefotaxime and ciprofloxacin is very low in both animal and human isolates, with the exception of *S.* Kentucky and *S.* Infantis serovars.

Conditions proposed: *Must not be used for the treatment or metaphylaxis of Salmonella spp. in poultry.*
Considering the zoonotic risk related to zoonotic salmonella in poultry, antimicrobial use in national control programmes is already restricted in accordance with Commission Regulation EC 1177/2006 and the principal control strategy is elimination by testing and culling of infected flocks. In regard to treatment of *S.* Pullorum and *S.* Gallinarum, eradication should be the principal control strategy.

Although there is lower potential for transmission of resistant salmonella clones from other food-producing animals to humans, this is an on-going public health concern [82]. In conclusion, there may be justification for antibiotic use to reduce severity of signs of salmonellosis and prevent suffering in

individual animals with potentially life-threatening infection, considering that many member states do not have 'stamping out' policies for salmonellosis other than in poultry.

As fluoroquinolones have been authorised for a wide range of indications including systemic, respiratory, urogenital and soft tissue infections, it is not considered necessary to further restrict indications.

(ii) Use for unauthorised target species

(Fluoro)quinolones are authorised for use in all major and many limited market species

No conditions are proposed.

Rationale: See Section 3.1.2.(ii) of this advice.

(iii) Administration by an unauthorised route or use of extemporaneous formulation

Authorised VMPs containing (fluoro)quinolones are available for administration orally including group medication in-feed and drinking water, via injection, intramammary and topical (auricular) use. In addition, formulations are available for administration to farmed fish in-feed.

Condition proposed: *When the proposed route of administration is outside the terms of the SPC, or when using an extemporaneous formulation, the product should be administered to individual animals, only.*

Rationale: See Section 3.1.2.(iii) of this advice.

(iv) Use of a human medicinal product

HMPs are available for administration by injection, inhalation/nebulisation, orally and topically (auricular, ocular, cutaneous).

Condition proposed: *HMPs should be administered to individual animals, only.*

Rationale: Considering the additional inhalational/nebulisation route of administration available for HMPs. No further conditions to those mentioned above. See also Section 3.1.2.(iv) of this advice.

(v) Use of a third country veterinary medicinal product

According to Articles 112(2), 113(2) and 114(4), third country VMPs may only be used in the same species and for the same indication. No further conditions are proposed in addition to those mentioned above.

Rationale: See Section 3.1.2.(v) of this advice.

Step 3. Consideration of Criteria (a) and (e) in view of proposed conditions to be placed on use outside the terms of a marketing authorisation

Criterion (a) – risk to animal health or public health if the antimicrobial is used in accordance with Articles 112, 113 and 114

SPCs and textbooks advise that care should be taken when using fluoroquinolones in growing animals due to potential effects on cartilage development in various species. Dose-related blindness has been reported in cats treated with enrofloxacin. Fluoroquinolones may also have neurological adverse effects and care should be taken when treating animals with epilepsy. Target animal safety warnings in the SPCs of authorised VMPs and HMPs should be followed.

Consumer safety is mitigated through the application of the statutory withdrawal period in accordance with Article 115.

Criterion (e) Impact on aquaculture and farming if the animal affected by the condition receives no treatment

Proposed condition	**Potential impact on aquaculture and farming if animal affected by the condition receives no treatment**
For those indications not included in the SPC for the product, use must be based on target pathogen identification and antimicrobial susceptibility testing that demonstrates that (fluoro)quinolones are likely to be effective and that antimicrobials from a lower AMEG category would not be effective, unless it can be justified that this is not possible.	This condition does not preclude treatment. See Annex 1 of report for further discussion.
Use of (fluoro)quinolones under Article 113 to treat salmonellosis should be restricted to use of injectable products in individual animals with potentially life-threatening infection.	An EU baseline survey conducted by EFSA in 2008 [161] found *Salmonella Typhimurium* on approximately 6% of pig production and breeding holdings in the EU overall, with much lower prevalence of *S. Choleraesuis*. The findings of a systematic review of studies published between 2000 – 2017 estimated a prevalence of Salmonellae in healthy cattle in Europe of 2% [158, 162, 163]. However, prevalence of Salmonellae on farm or in healthy animals at slaughter does not give a full picture of the prevalence of outbreaks of clinical disease, for which evidence is difficult to find for the EU. In the longer term, outbreaks can be minimised by use of attention to biosecurity, husbandry and use of vaccination where available; however, eradication is not always feasible [53, 158]. Salmonellae are often resistant to many first-line antibiotics used in food-producing animals (ASuT resistance pattern); and second-line treatment options may be limited e.g. aminoglycosides, florfenicol, fluoroquinolones. Abortions, septicaemia, meningitis, encephalitis and death are potential sequelae to infection. Lack of effective antibiotics for group administration for treatment and metaphylaxis may result in more rapid spread of disease in the herd and hence higher morbidity and mortality. The proposed conditions do not prevent treatment of individual animals in order to protect animal welfare.
Must not be used for the treatment or metaphylaxis of Salmonella spp. in poultry.	According to Regulation EC 1177/2006, antimicrobials shall not be used as part of national control programmes for zoonotic salmonella in poultry. In regard to treatment of *S.* Pullorum and *S.* Gallinarum, eradication should be the

	principal control strategy; therefore, a legal restriction on such use is unlikely to have a significant impact on poultry farming.
When the proposed route of administration is outside the terms of the SPC, or when using an extemporaneous formulation, the product should be administered to individual animals, only. *HMPs should be administered to individual animals, only.*	There is little or no evidence supporting the efficacy or need for alternative routes of administration in relation to (fluoro)quinolones in farmed animals. Therefore, although the impact on farming/aquaculture of restriction to individual animal use cannot be foreseen, it is not expected to be significant.

Step 4. Final conclusion - recommendations made for conditions to be placed on use outside the terms of a marketing authorisation

Based on the discussion above, the following conditions are proposed:

- For those indications not included in the SPC of the concernedproduct, use must be based on target pathogen identification and antimicrobial susceptibility testing that demonstrates that (fluoro)quinolones are likely to be effective and that antimicrobials from a lower AMEG category would not be effective, unless it can be justified that this is not possible.
- Use of (fluoro)quinolones under Article 113 to treat salmonellosis should be restricted to use of injectable products in individual animals with potentially life-threatening infection.
- Must not be used for the treatment or metaphylaxis of *Salmonella* spp. in poultry.
- When the proposed route of administration is outside the terms of the SPC, or when using an extemporaneous formulation, the product should be administered to individual animals, only.
- HMPs should be administered to individual animals, only.

4.21. Nitrofuran derivates

4.21.1. Background information

Please note that this evaluation primarily relates to the use of nitrofurans as antibiotics/antibacterial agents. Use of nitrofurans as antiprotozoals is addressed more fully in Section 7.

Examples of substances in the class that are authorised in veterinary and human medicine in the EU

Examples of substances authorised for veterinary use	**Examples of ATCvet codes**
Furazolidone	QG01AX06
Nifurpirinol	QJ01XE91
Furaltadone	QJ01XX93
Examples of substances authorised for human use	**Examples of ATC codes**
Furazidin	J01XE03
Furazolidone	G01AX06
Nifuratel	G01AX05
Nifuroxazide	A07AX03
Nitrofural	P01CC02 S01AX04 S02AA02 B05CA03 D08AF01 D09AA03
Nitrofurantoin	J01XE01

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

According to Regulation 37/2010, Annex, Table 2: MRLs cannot be established for Nitrofurans (including furazolidone); therefore nitrofurans are prohibited from use in food-producing animals in the EU.

EU-authorised VMP formulations, based on sales reported to ESVAC

Species		**Route of administration**						
		Group		**Individual**				
		In-feed	**In-water**	**Injection**	**Oral e.g. tablet, paste**	**Topical/local (incl. intrauterine)**	**Intra-mammary**	**Oral powder**
Major	**Cattle**							
	Sheep (for meat)							
	Pigs							
	Chickens							
	Dogs	FZD			FZD			
	Cats	FZD			FZD			
Limited market species As listed in SPCs	**Pigeons**	FZD	FTD, FZD		FTD, FZD			FTD
	Ornamental birds	FZD	FTD, FZD		FZD			FZD
	Minks	FZD			FZD			
	Ferrets	FZD			FZD			
	Ornamental fish				NP			

FTD (furaltadone), FZD (furazolidone), NP (nifurpirinol)

Summary of main indications and contra-indications for EU-authorised VMPs, based on selected SPCs

Main indications	Furazolidone is available as a premix (in one MS) (in combination with metronidazole, oxytetracycline) for dogs, cats, polecats, mink, ornamental birds, homing pigeons for treatment and prevention of enteropathy due to bacterial and flagellate protozoal infections. Furazolidone and furaltadone are available in a few MSs, often in combination with other antibiotics (e.g. sulfonamides, tetracyclines) to treat ornamental birds and homing pigeons via drinking water and as tablets. They are stated as being effective against *Clostridium* spp., *Salmonella* spp., *Staphylococcus* spp., *E. coli* and protozoa (*Eimeria*, *Histomonas* and *Trichomonas* spp.).
Contraindications	The mutagenicity and carcinogenicity of furazolidone has been demonstrated in laboratory animals. Should not be used in presence of azotaemia, during pregnancy or in neonates or for infections due to *Proteus* or *Pseudomonas* spp.

Examples of EU-authorised HMP formulations, from Article 57 database

Substance	Route of administration		
	Injection	Oral e.g. tablet, liquid	Topical/local
Furazidin		x	
Furazolidone		x	
Nifuratel		x	X
Nifuroxazide		x	
Nitrofural			X
Nitrofurantoin		x	

Existing recommendations

WOAH recommendations

Nitrofurans are not classified by WOAH (formerly OIE).

WHO classifications

WHO: IA

- (C1: No)
- (C2: No)

WHO AWaRe: Access: Nitrofurantoin, Nifurtuinol, Furazidin

AMEG and CVMP recommendations

Nitrofurans are included in the AMEG Category D. There are alternative treatments in human and veterinary medicine for their indications and that do not select for resistance to Category A substances through specific multiresistance genes.

These antibiotics are not devoid of negative impact on resistance development and spread. To keep the risk from use of these antibiotic classes as low as possible it is important that responsible use principles are complied with in everyday practice. Unnecessary use and unnecessarily long treatment periods should be avoided, and group treatment restricted to situations where individual treatment is not feasible.

Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Disclaimer: The information in this section reflects reported use of antimicrobials outside the terms of a marketing authorisation. No evaluation is made in this section by the working group on the efficacy or safety of the reported uses, or on their potential impact on development and dissemination of AMR.

Information from published sources

It is reported in textbooks that human formulations of nitrofurantoin are used in dogs and cats for treatment of resistant UTI in particular, lower UTI [46, 125, 453].

Furazolidone is used mostly for treatment of protozoal infections.

Information from the open call for data on use of antimicrobials in animals

The information below is summarised from the open call for data. Inclusion in the table does not endorse use or imply that it is consistent with use according to legislative provisions in Articles 112 to 114.

Substance	Species	Indication	Alternatives	Consequences of unavailability
Nitrofurantoin (human product)	Dogs and cats	UTI	None	Inability to treat infection
Nitrofurantoin	Ornamental fish	Bacterial infections	None	Mortalities

4.21.2. Evaluation

The use of nitrofurans to treat protozoal infections is primarliy addressed in *Section 7. Evaluation of Antiprotozoals* of this advice.

Scope of permitted use according to the MRL Regulation

Nitrofurans (including furazolidone) are included in Table 2 (prohibited substances) of the Annex to Regulation (EU) 37/2010 and hence cannot be used in any food-producing species.

Nitrofurans can be used in non-food-producing species in accordance with Article 112.

Examples of veterinary-authorised formulations/species

Nitrofurans are available in premix, drinking water and tablet formulations for treatment of cats, dogs, mink, homing pigeons, ornamental birds and various other limited market species.

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Importance for human health

The most important members of the nitrofurans class with respect to the use in humans include nitrofurantoin, furazolidone and nitrofurazone.

Nitrofurantoin is nationally approved in some EU member states for the treatment of acute uncomplicated cystitis, chronic recurrent UTIs associated with antimicrobial-resistant bacteria and for long-term antibiotic prophylaxis [454-456]. Indications include UTIs that arise spontaneously or in association with surgical interventions.

Nitrofurantoin is specifically indicated for those urinary tract infections caused by susceptible strains of *E. coli*, staphylococci (including methicillin-resistant), enterococci, *Citrobacter*, *Klebsiella* and *Enterobacter* spp. Nitrofurantoin is often indicated to treat UTIs caused by ESBL-producing *Enterobacter* spp. Nitrofurantoin is not recommended for the treatment of UTIs due to *Proteus mirabilis* or *Pseudomonas* spp., due to the intrinsic resistance reported in such pathogens [453, 457, 458].

Prescribing rates for nitrofurantoin have increased in recent years due to the low reported incidence of pathogen resistance (1-2%), high clinical and bacterial cure rates (80-93%) and the low impact on the GI microbiota [454, 459-462].

The pharmacokinetic (PK) profile of nitrofurantoin in humans shows high concentrations in urine, but relatively low concentrations in serum and tissue. Humans administered 100 mg nitrofurantoin *per os* had serum concentrations ≤1.3 µg/mL at 1-3 hours post-dose *versus* urinary concentrations of 40-209 µg/mL at 1.3-8 hours post-dose [453, 463]. This PK profile has resulted in a significant shift towards nitrofurantoin as first line therapy for the treatment of lower UTIs in place of either beta-lactams, potentiated sulphonamides or fluoroquinolones. This shift has been further driven by the enhanced safety profile since the introduction of the newer macrocrystalline formulation (in contrast to the previous microcrystalline) with a significant reduction in the incidence of the previously reported hepatic, pulmonary and CNS adverse events [453, 464, 465]. Kidney function, however, does impact the elimination profile of nitrofurantoin, thereby limiting use in patients with significant renal damage.

An *in vitro* synergistic effect against Gram-negative bacteria has been demonstrated when nitrofurans were combined with vancomycin and deoxycholate [466]. In addition, collateral sensitivity has been demonstrated in situations in which tigecycline or beta-lactam resistance in certain bacterial strains promoted enhanced sensitivity to nitrofuran therapy in the same strains [467].

Furazolidone is a component of combination therapy for *Helicobacter pylori* infections [468]. It has also been used to treat bacterial diarrhoea in some parts of the world e.g. *Shigella* dysentery [469].

Nitrofurans also have an activity against protozoal infections - giardiasis.

Nitrofurazone has been mainly used for topical chemotherapy of wounds, burns, and skin infections, and for infections in skin grafts [470].

Importance for animal health

Furazolidone and furaltadone are authorised in a few member states for treatment of bacterial enteropathies e.g. due to Enterobacterales and *Clostridium* spp., and protozoal infections (*Eimeria* spp., *Histomonas* spp. and *Trichomonas* spp.). Products are mostly for use in ornamental birds and homing pigeons, but are also authorised for cats, dogs, mink etc. They are presented in combination with other antibiotics e.g. oxytetracycline, metronidazole, sulfonamides.

There were reports to the Open call for data of the use of human formulations of nitrofurantoin to treat resistant UTI in dogs and cats, and use of nitrofurantoin to treat bacterial infections in ornamental fish.

The most prevalent use of nitrofurantoin relates to oral administration in dogs for the treatment of recurrent lower UTIs in patients with underlying comorbidities that may have contributed to resistance to other antimicrobial classes [471].

Nitrofurantoin in dogs has a similar PK profile to humans with urinary concentrations providing effective therapeutic concentrations of drug in the lower urinary tract [472, 473]. Nitrofurantoin administered orally at 4-5 mg/kg in dogs achieved drug concentrations of <2 µg/mL in serum *versus* >60 µg/mL in urine at 4 hours post dose [474]. Common uropathogens isolated from dogs *exhibited favourable in vitro susceptibility* to nitrofurantoin based on the observed MIC values and, whilst caution must be advised, the CLSI breakpoints extrapolated from human isolates [474]. More specifically, nitrofurantoin displayed a broad spectrum of activity for the common Gram-positive (e.g. *Enterococcus* spp., *Staphylococcus* spp.) and Gram-negative (*e.g. E. coli* and *Klebsiella* spp.) pathogens isolated from canine UTIs.

The low levels of nitrofurantoin evident in the serum and tissues of dogs significantly precludes its use in the treatment of other soft tissue or osseous bacterial infections.

Leuin *et al.* [453] reported an overall cure rate of 86% when nitrofurantoin was prescribed in dogs with lower UTIs at a median dose of 4.3 mg/kg PO every 8 hours ranging from 7 to 28 days. The bacteriuria

in all dogs sampled was associated with either MDR bacteria or *Enterococcus* spp. that was resistant to multiple antimicrobial agents, including penicillins. These authors reported that nitrofurantoin proved effective in the study population despite the recurrent nature of the animal's UTIs, previous antibiosis and the MDR profile reported above. In addition to a clinical cure, all dogs sampled post-treatment displayed a bacteriological cure. The efficacy of this dose rate was further supported by similar results reported in dogs with UTIs [475].

Nitrofurans (primarily nitrofurantoin) are reported to be very occasionally used in non-food producing horses for the treatment of lower urinary tract infections, although the most common use of nitrofurans (particularly nitrofurazone) in equidae relates to topical preparations for infections or wounds affecting the eyes, ears and skin [476].

Although nitrofurans are commonly used in bath treatments for ornamental fish, absorption into the body may be limited, thereby rendering this class more effective in the treatment of superficial (rather than deep) infections [477].

Selection, development and transmission of resistance

Nitrofuran compounds are prodrugs that are activated in organisms such as *E. coli* via reduction by type I oxygen-insensitive nitroreductase enzymes, specifically NfsA and NfsB [478]. Mutations of *nfsA* and *nfsB* gene loci are the major mechanism for gaining resistance to nitrofurans reported in clinical isolates of *E. coli*. An in-frame deletion in the *ribE* gene that encodes for an enzyme involved in flavin biosynthesis (an essential NfsA/NfsB cofactor) has also been reported [479], but has not been documented in *E. coli* clinical isolates to date.

While the overall prevalence of nitrofuran resistance amongst human *E. coli* isolates in recent surveys is still relatively low [469], hyper-resistant isolates with MICs ≥128 µg/mL have been reported [479, 480]. As such high levels of resistance are not consistent with mutations in the *nfsA*, *nfsB*, and *ahpF* gene loci, other determinants may be at play in pathogenic strains. In one report from the UK, nitrofurantoin resistance was documented in *E. coli* urinary tract isolates arising from a mutated version of the ESBL enzyme, CTX-M-14. Experimental studies demonstrated that this mutated enzyme (when recombinantly overexpressed in *E. coli*) was capable of inducing hyper-resistance to nitrofurantoin in addition to maintaining resistance to beta-lactam antimicrobials [481].

Khamari *et al.* [482] studied the antibiotic susceptibility profiles of 100 *Enterobacteriaceae* isolates (45 nitrofurantoin-resistant, 21 intermediately resistant and 34 nitrofurantoin-susceptible) against nitrofurantoin and 17 other antimicrobial agents across eight different classes. Significant co-resistance was observed between nitrofurantoin and other tested antibiotics (beta-lactam, cephalosporin, carbapenem, aminoglycoside and tetracycline). A strong correlation was observed between nitrofurantoin resistance and the presence of bla PER-1, bla NDM-1, bla OXA-48, ant(2) and oqxA-oqxB genes.

High-level resistance to nitrofurantoin was recorded in 31/36 human isolates (89.6%) obtained from urine samples or patients following invasive procedures or in an ICU setting [483]. Efflux pump inhibitors had little effect on the nitrofurantoin MIC values in this study, though the *oqxAB* gene was prevalent in most isolates.

Leuin *et al.* reported a relatively low incidence of resistance to nitrofurantoin in canine UTI isolates [453].

Data on the potential transmission of nitrofuran resistance from animals to humans is lacking in the scientific literature, with most reported studies simply comparing the incidence of nitrofurantoin resistance between common isolates derived from humans and dogs. Rubin *et al.* [484] investigated a

total of 126 *Staphylococcus aureus* isolates from humans (n = 99) and dogs (n = 27) in a study to determine MIC values to a panel of 33 antimicrobials used in human and veterinary medicine. No resistance to nitrofurantoin was found in any of the isolates. Sannes *et al.* [485] assessed the prevalence and patterns of AMR among *E. coli* strains isolated from the urine of women with cystitis (n=82) or pyelonephritis (n=170) and from faecal samples from dogs (n=45) and healthy humans (n=76). None of the isolates were resistant to nitrofurantoin.

In conclusion,

- Nitrofurans are important first line antimicrobials for the treatment of potentially serious UTI in humans, in particular infections due to ESBL-producing bacteria. Based on the pharmacokinetic profiles of authorised nitrofurans, other alternatives to treat resistant UTIs may be limited. In addition, nitrofurans are an important component of combination chemotherapy to treat *Helicobacter pylori* in humans and protozoal gastrointestinal infections.
- Although group treatment in non-food-producing avian species is authorised in limited circumstances, the use of nitrofurans in veterinary medicine is predominantly limited to treatment of UTIs in individual companion animals only (use prohibited in food-producing species).
- Resistance to nitrofurans in both human and companion animal isolates has been reported to only occur at relatively low levels.

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to Nitrofurans.

Criterion (c) – availability of other treatments for animals

Alternative treatments for uncomplicated UTIs in companion animals include Beta-lactams +/- BLIs, potentiated sulphonamides and fluoroquinolones.

Criterion (d) – availability of other antimicrobial treatments for humans

There are several alternatives for treatment of uncomplicated UTIs (fosfomycin trometamol, co-trimoxazole, trimethoprim and beta-lactam antibiotics) as well as for bacillary dysentery (ciprofloxacin, pivmecilinam, ceftriaxone, azithromycin). Nitrofurantoin is one of a few alternatives to treat UTI caused by vancomycin-resistant *Enterococcus faecium* [486].

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

- Nitrofurans are important first line antimicrobials for the treatment of uncomplicated cystitis and potentially serious UTI in humans, in particular infections due to ESBL-producing bacteria. They are also an important component of combination chemotherapy to treat *Helicobacter pylori* in humans.
- In veterinary medicine, nitrofurans are mainly used as a first-line option for treatment of recurrent lower UTI in companion animals. However, alternative antimicrobials for the treatment of MDR urinary tract infections in companion animals are limited or would involve the use of classes belonging to higher AMEG categories. Nitrofurans are also used for treatment of bacterial and protozoal infections in minor species including ornamental birds and fish. They are prohibited from use in food-producing animals.
- Although nitrofuran resistance pathways have identified in the scientific literature, available data indicate that resistance amongst both human and companion animal isolates to nitrofurans is low; as such, the risk for the development and transmission of AMR to humans from animal use is low.

- The current authorisations for nitrofurans mostly relate to use in minor exotic species. Human-authorised nitrofurantoin is used in dogs and cats, for which the pharmacokinetic profile largely restricts clinical use to either gastrointestinal or urinary tract infections. Use outside the terms of the marketing authorisation is expected to be limited.

Therefore, considering the points above relevant to criteria (b), (c) and (d), it is recommended that no conditions should be placed on the use of Nitrofurans outside the terms of the marketing authorisation, although responsible antimicrobial use principles should be applied.

4.22. Nitroimidazoles (antibiotic substances)

Please note that this evaluation primarily relates to the use of nitroimidazoles as antibiotics/antibacterial agents. Use of nitroimidazoles as antiprotozoals is addressed more fully in Section 7.

4.22.1. Background information

Examples of substances included in the class that are used in veterinary and human medicine in the EU

(Note: The ATC(vet) codes below relate only to nitroimidazoles when used as antibiotics)

Examples of substances authorised for veterinary use	**Examples of ATCvet codes**
Metronidazole	QA01AB17 QJ01XD01 QG01AF01
Ornidazole	QJ01XD03
Examples of substances authorised for human use	**Examples of ATC codes**
Metronidazole	A01AB17 D06BX01 G01AF01 J01XD01

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

According to Regulation (EU) 37/2010, Annex, Table 2: MRL cannot be established for dimetridazoles, metronidazole and ronidazole.

EU-authorised VMP formulations, based on sales reported ESVAC

Species		**Route of administration**						
		Group		**Individual**				
		In-feed	**In-water**	**Injection**	**Oral e.g. tablet, paste**	**Topical/local (incl. intrauterine)**	**Intra-mammary**	**Oral powder**
Major	**Cattle**							
	Sheep (for meat)							
	Pigs							
	Chickens							
	Dogs	M			M			
	Cats	M			M			
Limited market species* As listed in SPCs	**Pigeons**	M	M		M			
	Minks	M			M			
	Ferrets	M			M			
	Ornamental birds	M	M		M			

M (metronidazole)

Examples of EU-authorised HMP formulations, from Article 57 database (excluding substances used as antiprotozoals)

Substance	**Route of administration**		
	Injection	**Oral e.g. tablet, liquid**	**Topical/local**
Metronidazole	x	x	X (cutaneous, vaginal, rectal)

Summary of main indications and contra-indications for EU-authorised VMPs, based on selected SPCs

Main indications	Metronidazole is available as an oral suspension and tablets for dogs and cats for treatment of gastrointestinal infections due to *Giardia* and *Clostridium* spp. (*C. perfringens, C. difficile*), and for treatment of infections of the genitourinary system, oral cavity, throat and skin caused by obligate anaerobic bacteria (e.g. *Clostridium* spp.). Metronidazole is also authorised in combination with spiramycin in tablets for dogs and cats for a wider range of infections due to aerobic and anaerobic bacteria including infections of the oral cavity, otitis, respiratory infections, urogenital infections, skin and anal gland infections. It is available in a premix formulation for treatment of enteropathies (and flagellate protozoa) in dogs, cats, and ornamental fish and birds.
Contraindications	Contraindication from use in dogs with hepatic disease.

Existing recommendations

OIE recommendations

Nitroimidazoles are not classified by WOAH (formerly OIE).

WHO classifications

WHO: IA

- (C1: No) In certain geographic settings, Criterion 1 may be met: the class may be one of limited therapies for anaerobic infections including *C. difficile.*
- (C2: No)

WHO AWaRe: Access: Metronidazole (IV) and Metronidazole (oral); Watch: spiramycin-metronidazole combination

AMEG and CVMP recommendations

Nitroimidazoles are included in the AMEG Category D. There are alternative treatments in human and veterinary medicine for their indications and that do not select for resistance to Category A substances through specific multiresistance genes.

These antibiotics are not devoid of negative impact on resistance development and spread. To keep the risk from use of these antibiotic classes as low as possible it is important that responsible use principles are complied with in everyday practice. Unnecessary use and unnecessarily long treatment periods should be avoided and group treatment restricted to situations where individual treatment is not feasible.

Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Disclaimer: The information in this section of the report reflects reported use of antimicrobials outside the terms of a marketing authorisation. No evaluation is made in this section by the working group on the efficacy or safety of the reported uses, or on their potential impact on development and dissemination of AMR.

Information from published sources

Published textbooks refer to the use of metronidazole in non-food-producing horses to treat pulmonary infections [33].

Metronidazole is frequently used in reptiles to treat anaerobic and protozoal infections.

Metronidazole is included in the WSAVA List of Essential Medicines for Cats and Dogs [178] for management of selected bacterial and protozoal infections and for some cases of diarrhoea. It is also included for management of hepatic encephalopathy and modulation of the microbiota in the colon.

Metronidazole has been used in the treatment of inflammatory bowel disease in dogs and cats. It is proposed that changes in the intestinal microbiota may prevent colonisation by pathogenic bacteria, or down-regulate exaggerated host immune responses to commensal bacteria. Suppression of cell-mediated immunity has also been suggested as a mechanism of action [255, 487]. Metronidazole is also used as part of combination therapy for symptomatic *Helicobacter* spp. gastritis in dogs and cats [488].

Information from the open call for data on use of antimicrobials in animals

The information below is summarised from the open call for data. Inclusion in the table does not endorse use or imply that it is consistent with use according to legislative provisions in Articles 112 to 114.

Substance	Species	Indication	Alternatives	Consequences of unavailability
Metronidazole	Dogs and cats	Digestive tract infections, giardia	Giardia – fenbedazole, unless resistant	Chronic diarrhoea
Metronidazole (human IV formulation)	Dogs, cats, non-food horses and other spp. Dogs	Serious anaerobic infections (e.g. endocarditis, cholongiohepatitis, peritonitis, deep abscess, septicaemia) *Clostridium tetani* infections	Amoxi-clav, although this is less effective; clindamycin For *Cl. tetani* – penicillin, but lower efficacy	Mortalities, uncontrolled infections
Metronidazole	Ornamental birds	Histomonosis, Trichomonosis, flagellates, *Clostridium perfringens*	None	Increased mortalities, welfare issues, extended use of CIA
Metronidazole (human tablets)	Horses	Giardia, (pleuro)pneumonia due to anaerobic infections, Clostridial diarrhoea	Other nitroimidazoles None for e.g. *Bacteroides fragilis*	
Metronidazole	Teleosts	Susceptible protozoal and bacterial infections	None	Severe disease, mortality

4.22.2. Evaluation

Scope of permitted use according to the MRL Regulation

Dimetronidazole, metronidazole and ronidazole are included in Table 2 (prohibited substances) of the Annex to Regulation (EU) 37/2010 and hence are prohibited from use in food-producing species. There are no other nitroimidazoles with MRL status and therefore they cannot be used in food-producing animals, including in accordance with Articles 113 and 114 of Regulation (EU) 2019/6.

Nitroimidazoles can be used in non-food-producing species in accordance with Article 112.

Examples of veterinary-authorised formulations/species

Metronidazole is available in oral formulations (tablets, suspension, in-feed and in-water) for dogs, cats and limited market species (non-food pigeons, ornamental birds, mink, ferrets).

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Importance for human health

Metronidazole is active against a wide range of pathogenic microorganisms notably species of *Bacteroides*, *Fusobacteria*, *Clostridia*, *Eubacteria*, anaerobic cocci and *Gardnerella vaginalis*. Nitroimidazoles (e.g. metronidazole) are effective in management of a wide range of anaerobic infections such as bacterial vaginosis, septicaemia, endocarditis, bone and joint infections, central nervous system infections, RTIs, skin and skin-structure infections [489]. Metronidazole has been the antibiotic of choice for the treatment of *Bacteroides* infection and remains reliable for this use [489]. It is considered first line therapy in the paediatric population for the treatment of mild *Clostridioides difficile* infections. Metronidazole is widely used as a therapeutic agent for *Helicobacter pylori* infection in the human gut, primarily as part of a combined treatment regimen (e.g. in combination with omeprazole, clarithromycin, and amoxicillin), and for intra-abdominal infections (in combination with fluoroquinolones) [489].

Indications authorised for metronidazole include: the prevention of post-operative infections due to anaerobic bacteria, particularly species of *Bacteroides* and anaerobic streptococci; the treatment of septicaemia, bacteraemia, peritonitis, brain abscess, necrotising pneumonia, osteomyelitis, puerperal sepsis, pelvic abscess, pelvic cellulitis, and post-operative wound infections from which pathogenic anaerobes have been isolated; bacterial vaginosis (also known as non-specific vaginitis, anaerobic vaginosis or *Gardnerella* vaginitis); acute ulcerative gingivitis; anaerobically-infected leg ulcers and pressure sores; acute dental infections (e.g., acute pericoronitis and acute apical infections).

Importance for animal health

Metronidazole is authorised in VMPs intended for use in companion animals (dogs, cats, mink, ferrets, ornamental birds) within the EU, for treatment of infections of gastrointestinal and urogenital tracts, mouth, pharynx and skin caused by obligate anaerobes (e.g. *Clostridium* spp. and *Clostridioides* difficile spp.). It is also authorised for treatment of *Giardia* spp. in dogs and cats, and for trichomoniasis and histomoniasis in (non-food-producing) pigeons (see Antiprotozoals in Section 7.).

Nitroimidazoles are among few alternatives for treatment of anaerobic infections in non-food-producing animals. Metronidazole has good tissue penetration and rapid bactericidal activity against *Clostridium* spp., *Clostridioides difficile*, *Prevotella*, *Bacteroides* and *Fusobacterium* spp. and in companion animals (including non-food horses) is important for treatment of life-threatening sepsis, peritonitis, intra-abdominal abscesses (horses), pleuropneumonia, osteomyelitis and central nervous system (CNS) infections, and for gastrointestinal and periodontal infections [490-496].

There are published reports of the use of metronidazole outside the terms of the marketing authorisation for unauthorised indications e.g. treatment of inflammatory bowel disease and *Helicobacter gastritis* in dogs and cats (see information from published sources above).

According to the Open call for data, the human IV formulation of metronidazole is used in companion animals for serious anaerobic infections. Metronidazole is also used in unauthorised species e.g. non-food-producing horses, teleosts, reptiles, for treatment of protozoal and bacterial infections.

Development and selection of resistance

The mechanisms of nitroimidazole resistance are complex and have not been extensively studied. Mechanisms described include reduced rate of uptake, by efflux or by reducing the rate of metronidazole reductive activation. Increased efficiency of DNA repair provides an additional mechanism [497]. Resistance to nitroimidazoles can be mediated by *nim* genes, which encode nitroimidazole-reductases responsible for antibiotic inactivation. *nim* genes can be located on the chromosome or on a plasmid [498]. *nim* genes have been described in a variety of anaerobic genera encompassing the four main groups of Gram-negative and Gram-positive bacilli and cocci (e.g. *Bacteroides* spp.) [498, 499].

C. difficile can harbour a plasmid-borne resistance capable of horizontal transfer [500].

C. difficile clones commonly associated with human diseases, such as ribotype 078 are found in food-producing animals, companion animals and humans.

Resistance to nitroimidazoles is reported from human and food-producing and companion animal isolates worldwide, but generally at low levels [498, 501-506]. There is no mandatory routine monitoring of nitroimidazole susceptibility in animal isolates at EU level.

Transmission of resistance

There is evidence for the selection of resistance to nitroimidazoles in companion animal isolates and there is a transmission pathway for this resistance from animals to humans via zoonotic pathogens or commensal bacteria (e.g. *C. difficile* isolates) [507-509].

Conclusion relating to criterion "*b*":

- Nitroimidazoles are important first line antimicrobials for the treatment of various indications including serious/life-threatening anaerobic infections in both human and veterinary medicine. Based on available formulations and pharmacokinetic profiles, other alternatives to treat anaerobic infections in animals may be limited.
- Although the use of nitroimidazoles in veterinary medicine is limited to treatment of companion animals only (use prohibited in food-producing species), evidence exist for a potential transmission pathway of resistance from animals to humans. However, resistance to nitroimidazoles in companion animal isolates has been reported to occur at low levels.

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to Nitroimidazoles.

Criterion (c) – availability of other treatments for animals

Alternatives for treatment of infections caused by anaerobic bacteria include aminopenicillin-BLIs, clindamycin (not horses) and 3rd-generation cephalosporins; however, depending on the nature of the infection, alternatives may have less favourable pharmacokinetics, lower activity against the target pathogen(s) due to intrinsic or acquired resistance, or derive from a higher AMEG category [510-515].

Criterion (d) – availability of other antimicrobial treatments for humans

Alternative antimicrobial agents to treat anaerobic infections in humans include carbapenems (e.g. meropenem, ertapenem), chloramphenicol, aminopenicillin-BLIs, tigecycline, cefoxitin and clindamycin [516]. Metronidazole is no longer considered an appropriate first-line agent to treat *C. difficile* infections in adult humans; instead, vancomycin and fidaxomicin are alternative treatment options with a higher success rate in such infections. Relapsing or refractory cases of *C. difficile* infection in humans are treated with monoclonal antibody (bezlotuxumab) therapy or faecal microbiota therapy.

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

- Nitroimidazoles are a first line treatment for a wide range of anaerobic infections in humans, including serious intra-abdominal infections and septicaemia.
- There are no nitroimidazoles with MRLs and they are prohibited from use in food-producing animals. In companion animals, metronidazole is authorised for treatment of anaerobic infections of the oral cavity, gastrointestinal and urogenital tracts and human formulations for parenteral use are used for serious acute anaerobic infections e.g. septicaemias.
- Alternative antimicrobials are available in human medicine, in particular to treat severe *C. difficile* infections, hence nitroimidazoles are included in AMEG Category D. Options to treat anaerobic infections in companion animals are more limited and would include substances from a higher AMEG category.
- Although there is a potential transmission pathway for resistance from companion animals to humans or other animals, data from the scientific literature indicate that resistance to nitroimidazoles amongst companion animal isolates is low.
- Limited evidence was found for use of metronidazole outside the terms of a marketing authorisation and related mainly to use in exotic or zoo species and use of human formulations for parenteral use in companion animals.

Therefore, considering the points above relevant to criteria (b), (c) and (d), it is recommended that no conditions should be placed on the use of Nitroimidazoles outside the terms of the marketing authorisation, although responsible antimicrobial use principles should be applied.

4.23. Rifamycins

4.23.1. Background information

Examples of substances included in the class that are authorised in veterinary and human medicine in the EU

Examples of substances authorised for veterinary use	Examples of ATCvet codes
Rifaximin	QA07AA11 QG51AA06
Examples of substances authorised for human use	**Examples of ATC codes**
Rifabutin	J04AB04
Rifampicin	J04AB02
Rifamycin	A07AA13 J04AB03 D06AX15
Rifaximin	A07AA11

The name 'rifampin' is used in the USA, whereas 'rifampicin' is used in Europe and Australia.

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

Substance	Species	MRL tissues	MRL milk	MRL eggs	Other Provisions
Rifaximin	Bovine	-	Yes	-	No entry
	Bovine	No MRL required for all tissue except milk		-	For intramammary and intrauterine use only
	All mammalian food-producing species	No MRL required	No MRL required	-	For topical use only.

EU-authorised VMP formulations, based on sales reported to ESVAC

Species		Route of administration					
		Group		Individual			
		In-feed	In-water	Injection	Oral e.g. tablet, paste, powder	Topical/local (incl. intrauterine)	Intra-mammary
Major	**Cattle**					R	R
	Sheep (for meat)					R	
	Pigs					R	
	Chickens						
	Dogs					R	
	Cats					R	
Limited market species As listed in SPCs	**Goats**					R	
	Horses					R	
	Buffalo					R	R
	Rabbits					R	
	Bison						R

R (Rifamixin)

*Limited market species includes species defined under Article 4(29)(b)s.

Summary of main indications and contra-indications for EU-authorised VMPs, based on selected SPCs

Main indications	**Rifaximin** Intramammary formulations for bovine (cattle, bison and buffalo) • Infections sensitive to rifaximin.

	• Curative treatment for subclinical mastitis due to: *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis* • Preventive treatment of new infections during the dry period. • Prevention of acute mastitis by rifaximin-sensitive pathogens: *Staphylococcus aureus* (including penicillin-resistant strains), *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Actinomyces pyogenes*. Cattle, sheep, goats, pigs, horses, rabbits, cats and dogs - cutaneous formulations are indicated for treatment of skin and nail infections, including interdigital dermatitis, pyoderma and wounds. In dogs and cats, topical combinations including rifaximin are available for treatment of otitis externa. Cows, buffalo, mares, sows, ewes and goats - intrauterine formulations are authorised for treatment of metritis, endometritis, vulvovaginitis (*Actinomyces* spp., *Staphylococcus* spp., *Streptococcus* spp. and some gram-negative bacteria e.g. *E.coli*, *Pasteurella* spp., *Salmonella* spp., *Bacteroides* spp., *Fusobacterium necrophorus*).
Contraindications	None.

Examples of EU-authorised HMP formulations, from Article 57 database

Substance	Route of administration		
	Injection	Oral e.g. tablet, liquid	Topical/local
Rifabutin		x	
Rifampicin	x	x	
Rifamycin	x	x	x
Rifaximin		x	

Existing recommendations

WOAH recommendations

Rifamycins are categorised VHIA by WOAH (formerly OIE). *Specific comments:* This antimicrobial class is authorised only in a few countries and with a very limited number of indications (mastitis) and few alternatives. Rifampicin is essential in the treatment of *Rhodococcus equi* infections in foals. However, it is only available in a few countries, resulting in an overall classification of VHIA.

WHO classifications

WHO: CIA (as ansamycins)

- (C1: Yes) Limited therapy as part of treatment of mycobacterial diseases including tuberculosis; single drug therapy may select for resistance.
- (C2: Yes) May result from transmission of *Mycobacterium* spp. from non-human sources and MDR *Staphylococcus aureus* through the food chain.
- (P1: Yes) High absolute number of people affected by diseases for which the antimicrobial is the sole or one of few therapies available.
- (P2: Yes) High frequency of use in human medicine.
- (P3: No)

WHO AWaRe: Watch: e.g. rifampicin, rifamycin, rifaximin

AMEG and CVMP recommendations

AMEG: Rifamycins (except rifaximin): Category A; rifaximin: Category C

Rifamycins (except rifaximin) are included in the AMEG Category A: these classes are not authorised in veterinary medicine but are authorised in human medicine in the EU. These antibiotic classes may only be used exceptionally in individual companion animals in compliance with the prescribing "cascade".

Substances in these classes cannot be used for food-producing animals in the absence of established maximum residue limits.

Rifaximin is included in the AMEG Category C: this category includes antibiotics for which there are alternatives in human medicine for their indications but which comply with one or both of the following criteria:

- For the veterinary indication under treatment, there are few or no alternatives belonging to Category D. Some examples of these indications are given in Table 4 of the AMEG advice [8], alongside the relevant (sub)class.
- The antibiotic selects for resistance to a substance in Category A through specific multiresistance genes.

Antibiotics placed in this category present a higher AMR risk for human and/or animal health than antibiotics placed in Category D. These antibiotics should only be used when there is no available substance in Category D that would be clinically effective.

Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Disclaimer: The information in this section reflects reported use of antimicrobials outside the terms of a marketing authorisation. No evaluation is made in this section by the working group on the efficacy or safety of the reported uses, or on their potential impact on development and dissemination of AMR.

Information from published sources

In companion animals, rifampicin is reported to be used as part of a treatment strategy for meticillin-resistant staphylococci [334, 375], and in some cases, for tuberculosis and other mycobacterial infections in companion animals. The ABCD guidelines on management of mycobacterioses in cats were first published 2013 [517] and later updated in 2015 [244]. In these guidelines, rifampicin is recommended for cats for the treatment of mycobacteria involved in tuberculosis complex, non-tuberculous mycobacteria including *Mycobacterium avium-intracellullare* complex (MAC) and feline leprosy.

The most common use outside of a marketing authorisation, and likely highest consumption of rifampicin for animals, is for *Rhodococcus equi* in foals (also known as *Prescottella equi*, and *Rhodococcus hoagie*). Treatments for several months are not only applied to clinically affected animals but also preventatively against outbreaks and to presumptive cases identified only by thoracic ultrasonography. Specifically, the combination of a macrolide (e.g. erythromycin, clarithromycin, or azithromycin) with rifampicin has become a traditional approach for foals infected with *R. equi* globally for nearly 40 years [243], or more recently use of a doxycycline/rifampicin combination has been reported in studies conducted in Germany [280, 518, 519].

Information from the open call for data on use of antimicrobials in animals

The information below is summarised from the open call for data. Inclusion in the table does not endorse use or imply that it is consistent with use according to legislative provisions in Articles 112 to 114.

Substance	Nature of Cascade use
Rifampicin	Horses: *Rhodococcus equi* infections, used to prevent uncontrolled disease/deaths
	Dogs, cats: MDR infections, e.g. pyoderma MRSP, to prevent euthanasia.

	Cats: mycobacterial infections in combination
	Pinnipeds, cetaceans: Mycobacteriosis Small odontocetes, bottlenose dolphin, and beluga, killer whale: ref textbook

4.23.2. Evaluation

Scope of permitted use according to the MRL Regulation

Only rifaximin is included in Table 1 (allowed substances) of the Annex to Regulation (EU) 37/2010 and hence can be used in all food-producing species in accordance with Articles 113 and 114 of Regulation (EU) 2019/6. 'Other provisions' restrict administration of rifaximin in food-producing animals to intramammary, intrauterine and topical use only.

Substances/indications in equines out of scope of evaluation for conditions due to listing in Regulation (EC) 1950/2006, as amended by Regulation (EU) 122/2013

Rifampicin is listed for the treatment of *Rhodococcus equi* infections.

Rifamycins can be used in non-food-producing animals in accordance with Article 112.

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Importance for human health

Rifamycins are used in human medicine for a variety of infections, including serious and life-threatening (e.g. mycobacteria, invasive staphylococci, *Neisseria meningitidis, C. difficile*) as well as zoonotic infections (e.g. brucellosis, MRSA and *R. equi* infections) [279, 520]. Notably, rifampicin (in combination) is used as an essential component of first-line therapy for mycobacterial infections*: Mycobacterium tuberculosis* (tuberculosis, TB), *M. leprae* (leprosy) and *M. avium* complex. Rifampicin is used for invasive staphylococcal infections due to its high volume of distribution, reaching high concentrations throughout the body (e.g. bone, CSF), as well as high level of activity in sessile *Staphylococcus aureus* growth (biofilm) that is particularly useful for foreign body infections [521].

Rifaximin is also used for non-infectious indications in human medicine (e.g. inflammatory bowel disease, hepatic encephalopathy), which can be classified as serious diseases.

Rifampicin and rifabutin are approved in the EU either as monoagents or part of combination human medicinal products. Indications for these rifamycins include the treatment of serious infections caused by mycobacteria, both *M. tuberculosis* and *M. avium-intracellulare* complex and other atypical mycobacteria, according to WHO guidelines. They are also approved for prophylaxis against *M. avium-intracellulare* colonization in patients with acquired immunodeficiency syndrome (AIDS). Rifaximin is approved in the EU for the treatment of adult patients with traveller's diarrhoea caused by non-invasive intestinal pathogens.

Importance for animal health

Rifaximin is the only rifamycin authorised in veterinary medicines in the EU. Intramammary VMPs containing rifaximin are authorised for either the treatment of intramammary infections during lactation or (prevention and treatment) of dry cow intramammary infections. SPC indications for the various products include:

- Infections sensitive to Rifaximin.

- Curative treatment for subclinical mastitis due to: *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*
- Preventive treatment of new mastitis infections during the dry period.
- Prevention of acute mastitis by rifaximin-sensitive pathogens: *Staphylococcus aureus* (including penicillin-resistant strains), *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Trueperella* (*Actinomyces) pyogenes*.

Topical preparations of rifaximin for cutaneous application are indicated for treatment of skin and nail infections, including interdigital dermatitis, pyoderma and wounds, in various food-producing and companion animals. Intrauterine formulations are authorised for treatment of metritis and endometritis due to Gram-positive and some Gram-negative bacteria in ruminants, mares and sows.

No specific evidence could be found for use of rifaximin outside the terms of a marketing authorisation in the EU.

Rifampicin is the only human-authorised antibiotic of the rifamycin class for which evidence was found relating to use in animals. Rifampicin is employed in companion, equine and exotic animal species to address a variety of specific infections.

In companion animals, rifampicin is used for rare serious infections due to mycobacteria, and occasionally bacteria resistant to authorised antibiotics (e.g. MRSP). MRSP is most commonly implicated in canine recurrent pyoderma, but may also be involved with life-threatening surgical wounds, urinary and respiratory tract infections [334, 375]. Tuberculosis complex and non-tuberculous mycobacterial infections are rare in cats and dogs but are usually serious/life-threatening [522]. Rifampicin is part of the recommended treatment, in combination with a macrolide and a fluoroquinolone. Rifampicin is also included in combination treatments for leproid mycobacterial infections [244, 255, 523].

The majority of rifampicin use in animals in the EU probably occurs for foals against *Rhodococcus equi*, in combination with either a macrolide (e.g. clarithromycin, azithromycin, erythromycin, etc) or other antibiotics (e.g. doxycycline, gentamicin). Rifampicin is used routinely for suspected cases of *R. equi* as well as preventatively. *R. equi* in foals is a common cause of severe pneumonia and well-known throughout Europe. Use of rifampicin for the prevention of *R. equi* in equines is considered to be in scope of this evaluation.

Based on the Open call for data, use in exotic animals includes mycobacterial infections in pinnipeds and cetaceans.

Rifamycins most often used in aquaculture in third countries, include rifampicin and rimamycin [524]. However, their effectiveness in fish and shellfish is declining, even when used in combination with tetracyclines, due to the development of resistant bacterial strains [525]. Rifamycin use in aquaculture is known in China, the Philippines and Vietnam.

No evidence was found for use of rifabutin, rifapentine or other rifamycins in animals.

Development and selection of resistance

In mycobacteria, *Rhodococcus* spp. and staphylococci, rifamycin resistance is known to develop quickly, predominantly from single-point mutations of the chromosomal *rpoB* gene, which alters the binding site on the RNA polymerase, thereby reducing rifamycin binding affinity [526, 527]. Therefore, rifamycins are most often used in combination with other antimicrobials, with the exception of rifaximin which is used in human and veterinary medicine as monotherapy [528]. Rifampicin-resistance

associated with the *rpo* gene has been reported in LA-MRSA from pigs, MRSP from dogs and *R. equi* from horses [529-531]. Rifampicin resistance has also been detected in *M. bovis* from cattle and humans [532, 533]. Although rifaximin is used as monotherapy, experience in human medicine has found that rifaximin use can lead to rifampicin-resistance in *staphylococci* spp. [534]. Also, rifampicin-resistant staphylococci can persist for several weeks after therapy with rifaximin [535].

Transmission of resistance

The only known potential route for transfer of rifamycin resistance to humans from non-human sources is via zoonotic bacteria. In Europe, EFSA/ECDC monitors rifampicin resistance only for MRSA isolates in animals. Monitoring of MRSA under EFSA/ECDC surveillance is voluntary and data are provided by few member states. Based on low numbers of isolates, rifampicin resistance was detected sporadically in MRSA isolates from dairy cows, fattening pigs and broilers submitted in 2018, 2019 and 2020, respectively [28, 59].

Food is generally not considered to be a significant source of MRSA in humans [63, 64]. MRSA is mainly transmitted by direct contact from food-producing animals [65]. In geographical areas with high density of farms, the livestock associated MRSA (LA-MRSA) contribution to the burden of MRSA disease could be significant [94, 95]. There is the potential for transmission of rifamycin-resistant staphylococci including *S. aureus* and MRSA/P from companion animals to humans [90].

Mycobacterial infections (e.g. *M. bovis, M. avium, M. tuberculosis*) historically have been reported rarely in companion animals in Europe [522, 536], however current prevalence data are not available. In regard to TB complex group, a risk assessment conducted in the UK following a cluster of nine *M. bovis* cases in cats in 2012-13 identified two cases of cat-to-human transmission and considered the risk as very low [537]. *M. tuberculosis* occurs sporadically in dogs and is mostly considered a reverse zoonosis (zooanthroponosis). There have been occasional reports of spread of *M. bovis* between cats housed at the same premises and of nosocomial spread [538, 539]. In regard to non-tuberculous mycobacteria (NTM), *Mycobacterium avium* Complex (MAC) is said to carry a very low risk of zoonotic infection to immunocompromised humans, but most human and companion animal infections appear to arise from an environmental reservoir. Other NTM and leprosy occurring in companion animals (e.g. *M. lepraemurium*) are regarded as opportunistic saprophytes and are not zoonotic or transmitted between pet animals if hygiene is followed [255, 540-542].

Transmission of rifampicin-resistant *R. equi* from foals to humans could occur. Nevertheless, human infection due to *R. equi* is a rare occurrence [543].

In conclusion,

- Rifamycins are important as first-line antibiotics to treat a wide range of infections in humans, but in particular are a mainstay of treatment for mycobacterial infection and are important for treatment of staphylococcal infections. In animals, rifaximin is authorised for local treatment of mastitis, metritis (food-producing species) and for skin infections (various species); whereas rifampicin is used outside the marketing authorisation for specific uses in companion animals.
- There is evidence to support the selection and a potential transmission of resistance to rifamycins from animals to humans and between animals via zoonotic and target pathogens (e.g. *Staphylococcus* spp.). There is a lack of dedicated rifamycin resistance surveillance for all relevant zoonotic and target pathogens in animal species. There are sporadic reports of transmission of mycobacterial infections from companion animals to humans in close contact and other animals, hence there is a potential pathway for transmission of rifamycin-resistant mycobacteria.

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to Rifamycins.

Criterion (c) – availability of other treatments for animals

Rifamycins are split in the AMEG categorisation into Category C (rifaximin) and category A (rifamycins, except rifaximin). In general, there are alternative antimicrobials for the authorised indications (e.g. macrolides, fluoroquinolones, doxycycline, beta-lactams) dependent on the specific disease, pathogen and target animal species under treatment. According to circumstances, alternatives may be from a lower or higher AMEG category. Target pathogens for the intramammary rifaximin VMP include staphylococci, streptococci, Actinomycetes, colibacilli and enterococci. Alternatives for these target pathogens include beta-lactam drugs (including cephalosporins), macrolides and lincosamides.

Efficacy of antimicrobials for severe *R. equi* pneumonia has not been investigated in the peer-reviewed scientific literature, and rifampicin in combination with a macrolide therefore remains the recommended treatment strategy [243]. For subclinical, mild and moderate *R. equi* pneumonia, alternatives to rifampicin are confirmed from randomised, controlled clinical trials [280, 518, 519, 544-546].

For dogs, MRSP is most commonly implicated in canine recurrent pyoderma, but may also be involved with life-threatening surgical wounds, urinary and respiratory tract infections. Selection of appropriate antibiotic should be based on susceptibility testing as MRSP is often susceptible to a few antimicrobials e.g. rifampicin, amikacin, doxycycline. Human last resort antimicrobials (e.g. oxazolidinones, lipopeptides) can no longer be used in animals according to Regulation (EU) 2022/1255.

Rifampicin is part of the recommended treatment combination for tuberculosis complex and non-tuberculous mycobacterial infections in dogs and cats [244, 255, 523]. Use of human first-line TB drugs (ethambutol and isoniazid) has been described rarely in companion animals (including pet birds) in international texts [33, 46, 255]. Euthanasia may be considered as an alternative due to the guarded prognosis or zoonotic potential (*M. bovis*) [244, 255].

Criterion (d) – availability of other antimicrobial treatments for humans

For most of the approved indications in human medicine, treatment alternatives are available: Mycobacterial infections can be treated with other classes of antibiotics (drugs used solely for tuberculosis and other mycobacteria, riminofenazines, sulfones); although rifampicin (or modern rifamycins e.g. rifabutin, rifapentine) in combination is the mainstay first-line treatment for drug-susceptible TB according to WHO guidelines [547]. For treatment of invasive infections with *Staphylococcus* spp., alternatives include last resort antimicrobials e.g. lipoglycopeptides, oxazolidinones, daptomycin.

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

- Rifamycins are important as first-line antibiotics to treat a wide range of infections in humans. The main therapeutic value of rifamycins is due to the PK/PD characteristics (broad spectrum, high volume of distribution, activity within biofilms). In humans, rifamycins are particularly important for treatment of invasive staphylococcal infections and as first-line for mycobacterial infections. The treatment of mycobacterial infections, especially in humans, typically involves combination antimicrobial treatments for several months, whereby alternatives to rifamycins tend to result in more adverse events over the longer treatment course. Alternatives for treatment of serious *Staphylococcus* spp. infections may be last resort antibiotics.

- In veterinary medicine, rifaximin is authorised for local treatment of mastitis and metritis and topical treatment of skin infections. It can only be administered by local routes in food-producing animals and there is little evidence for use of this specific substance outside the marketing authorisation in these species.
- The use of rifampicin for rhodococcal infections in foals and for treatment of mycobacterial infections in companion animals is widely documented; although there may be alternatives, rifamycins are the preferred option according to treatment guidelines. For MRS(P) in companion animals, rifampicin is one of few alternatives for systemic treatment.
- The prevalence of resistance to rifamycins in animal isolates is unclear due to lack of comprehensive surveillance. Resistance to rifamycins develops due to chromosomal mutations occurring during treatment; hence transmission from animals to humans or other animals would most likely be due to transfer of zoonotic or contagious target pathogens e.g. LA-MRSA from pigs to farm workers and MRSA/P between owners and pets in the same household. There is a potential pathway from transmission of rifampicin-resistant zoonotic mycobacterial species between companion animals and humans or other animals.
- No evidence was found for use of rifaximin outside the terms of the marketing authorisation. Based on expert opinion, most rifampicin use in animals in the EU probably occurs for foals against *Rhodococcus equi*. Rifampicin is also used for mycobacterial infections in companion animals, although treatment for these diseases is rare, and for MRSP according to susceptibility.

Therefore, considering the points above relevant to criteria (b), (c) and (d), it should be considered if conditions or a prohibition should be placed on the use of rifamycins outside the terms of the marketing authorisation.

Step 2. Considerations of conditions to be placed on use outside the terms of a marketing authorisation

Please refer to Section 3.1.2. of the main report for the general rationale behind the proposed conditions.

(i) Use for unauthorised indications

Applies to rifaximin VMPs, only. No evidence was found for use of rifaximin outside a marketing authorisation for unauthorised indications. VMPs are authorised for topical and local use only.

Conditions proposed: None.

Rationale: See Section 3.1.2.(i) and Annex 1, special note on the use of AST for pathogens treated topically or locally, of the advice.

(ii) Use for unauthorised target species

Applies to rifaximin VMPs, only. Rifaximin is authorised for use in all major food-producing mammalian species and several minor species. No evidence was found for use of rifaximin outside a marketing authorisation for unauthorised target species.

Conditions proposed: None.

Rationale: See Section 3.1.2.(ii) of the advice.

(iii) Administration by an unauthorised route or use of extemporaneous formulation

Authorised VMPs containing rifaximin are available for administration as topical, intrauterine and intramammary formulations.

According to Regulation (EU) 37/2010, Other Provisions restrict the use of rifaximin in food-producing animals to the intramammary, intrauterine and topical routes only. Hence rifaximin can only be administered to individual food-producing animals outside the terms of the marketing authorisation.

Other rifamycins can only be used in non-food-producing animals. The provisions relating to use of HMPs, below, should also apply to the use of extemporaneous formulations.

Conditions proposed: None.

Rationale: Section 3.1.2.(iii) of the advice.

(iv) Use of a human medicinal product

According to 'Other provisions' in Annex 1 to Regulation (EU) 37/2010, use of rifaximin in food-producing animals is restricted to intramammary, intrauterine and topical use only.

In the absence of inclusion in Annex 1 to Regulation (EU) 37/2010, rifamycins other than rifaximin can only be used in non-food-producing animals, under Article 112 of Regulation (EU)2019/6.

Proposed conditions

- Use must be based on target pathogen identification and antimicrobial susceptibility testing that demonstrates that rifamycins are likely to be effective and that antimicrobials from a lower AMEG category would not be effective, unless it can be justified that this is not possible. (See further notes relating to mycobacterial infections in Annex 1.).
- For treatment of mycobacteria and MDR staphylococci, only.
- Not to be used for prophylaxis of *R. equi* infection.
- To be used in individual animals only.

Rationale: Recognising the importance of rifamycins for human health and the need to use rifampicin for treatment of specific diseases in animals, as well as the fact that rifamycin resistance develops quickly and easily, it is proposed that conditions are placed on use under Article 112.

The use of rifampicin for the treatment of *Rhodococcus equi* infections in equines is out of scope of the evaluation of the need for conditions.

The risk of *R. equi* infection is highly variable, where a complex of risk factors include the type of management on farms (types of stalls, stocking density), seasonal conditions (dry, hot spring/summer), foal characteristics (immunity, etc…). Consequences of *R. equi* infection are also highly variable. Foals progress through different stages of infection (subclinical, mild, moderate, severe), where self-cure is possible or other antibiotics can be highly effective at stages before severe pneumonia.

Currently, there is no evidence that prophylactic rifampicin (or in combination) will prevent severe rhodococcal pneumonia in foals. Alternatives for prophylaxis include administering hyperimmune plasma, cranial lung lobe ultrasound screening/monitoring, farm management changes.

Previous experience in the USA of mass antimicrobial administration on *R. equi* endemic farms for both prevention and treatment of subclinical *R. equi* led to evidence of emerging antimicrobial resistance in *R. equi* with concerning prevalence [548]. Due to the strong environmental connections of *R. equi*, then resistant clinical strains can persist year-after-year on endemic farms. Around year 2001, many

endemic farms with recurring *R. equi* in foals implemented a policy of prophylactic antimicrobial use as well as thoracic ultrasonography for earlier detection of *R. equi* lung lesions (coupled with treating all subclinical foals with macrolides (with or without rifampicin)). Unfortunately, this approach came with negative consequences, including emergence of macrolide- and rifampicin-resistant *R. equi* after mass antimicrobial use was instituted for subclinical foals identified by thoracic ultrasonography [549]. Evidence exists, including from the EU, that many foals with subclinical pneumonia will recover spontaneously without the use of antimicrobials [518, 550], indicating that mass antimicrobial prophylaxis of pneumonia is not warranted.

With regard to mycobacterial infections in companion animals, it is noted that there is regional variability in access to diagnostic laboratories and the testing facilities available at those sites. Some mycobacterial species may either take 1-3 months to culture or not culture at all and different testing methods may be appropriate according to pathogen characteristics [551-554]. Please refer to the special note on diagnosis of mycobacterial infection in Annex 1. However, due to the length of treatment required, the need to select an effective treatment regimen, the potential for resistance to develop during treatment, the risk for transmission of resistant infections from treated animals to humans and other animals, it is recommended that before initiating treatment, steps should be taken for accurate target pathogen identification and susceptibility testing, according to the caveat stated above.

See also Section 3.1.2.(iv) of the advice.

(v) Use of a third country veterinary medicinal product

According to the Regulation, third country VMPs may only be used in the same species and for the same indication. No additional conditions are proposed to those above.

Step 3. Consideration of Criteria (a) and (e) in view of proposed conditions to be placed on use outside the terms of a marketing authorisation

Criterion (a) – risk to animal health or public health if the antimicrobial is used in accordance with Articles 112, 113 and 114

Gastrointestinal effects and hepatotoxicity associated with elevated liver enzymes may occur in dogs and cats administered rifampicin. Rifampicin induces cytochrome P450 enzyme activity. Pancreatitis has also been reported.

Target animal safety warnings in the SPCs of authorised VMPs should be followed.

Consumer safety relating to use of rifaximin under Articles 113 and 114 is mitigated through the application of the statutory withdrawal period in accordance with Article 115.

Criterion (e) Impact on aquaculture and farming if the animal affected by the condition receives no treatment

No conditions are proposed for use of VMPs containing rifaximin outside the terms of the marketing authorisation. Under Article 112 of Regulation (EU)2019/6, other rifamycins might be used in equine species declared as not intended for human consumption.

Proposed condition	**Potential impact on aquaculture and farming if animal affected by the condition receives no treatment**

Use must be based on target pathogen identification and antimicrobial susceptibility testing that demonstrates that rifamycins are likely to be effective and that antimicrobials from a lower AMEG category would not be effective, unless it can be justified that this is not possible. (See further notes relating to mycobacterial infections in Annex 1.).	This condition does not preclude treatment. See Annex 1. of report for further discussion.
For treatment of mycobacteria and MDR staphylococci, only. *Not to be used for prophylaxis of R. equi infection.* *To be used in individual animals only.*	Potential impact on equine species declared as not intended for human consumption, only. Although impacts of this condition cannot be fully foreseen, they are not expected to be significant considering the current uses identified. See Step 2 (iv) above. Previous experiences of mass antimicrobial administration on *R. equi* endemic farms for prevention of *R. equi* led to evidence of emerging antimicrobial resistance in *R. equi.* Evidence exists that many foals with subclinical pneumonia will recover spontaneously without the use of antimicrobials. Although the impact on equine farming of restriction to individual animal use cannot be foreseen, there is not expected to be significant negative impact.

Step 4. Final conclusion - recommendations made for conditions to be placed on use outside the terms of a marketing authorisation

Conditions apply to use of human medicinal products, extemporaneous preparations and VMPs authorised in third countries, only. They do not apply to EU-authorised VMPs containing rifaximin. In addition, they do not apply to the use of rifampicin for the treatment of *Rhodococcus equi* infections in equines:

- Use must be based on target pathogen identification and antimicrobial susceptibility testing that demonstrates that rifamycins are likely to be effective and that antimicrobials from a lower AMEG category would not be effective, unless it can be justified that this is not possible. See *'Special note regarding the diagnosis of mycobacterial infections in companion animals and antimicrobial susceptibility testing'* in Annex 1.
- For treatment of mycobacteria and MDR staphylococci, only.
- Not to be used for prophylaxis of *Rhodococcus equi* infection.
- To be used in individual animals only.

4.24. Substances used solely to treat tuberculosis or other mycobacterial diseases

Substances used solely to treat tuberculosis or other mycobacterial diseases are authorised in human medicinal products in the EU. At present they are not authorised in veterinary medicinal products in the EU.

4.24.1. Background information

Examples of substances included in the class that are authorised in human medicine in the EU

Examples of substances authorised for human use	Examples of ATC codes
Bedaquiline	J04AK05
Calcium aminosalicylate	J04AA03
Capreomycin	J04AB30
Cycloserine	J04AB01
Delamanid	J04AK06
Ethambutol	J04AK02
Ethionamide	J04AD03
Isoniazid	J04AC01
Para-aminosalicylic-acid	J04AA01
Protionamide	J04AD01
Pyrazinamide	J04AK01
Sodium aminosalicylate	J04AA02
Terizidone	J04AK03

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

Substances used solely to treat tuberculosis or other mycobacterial diseases are not included in the Annex to the MRL Regulation (EU) 37/2010 and cannot be used in food-producing animals in the EU.

Examples of EU-authorised HMP formulations, from Article 57 database

Substance	Route of administration		
	Injection	Oral e.g. tablet, liquid	Topical/local
Bedaquiline		x	
Calcium aminosalicylate		x	
Capreomycin	x		
Cycloserine		x	
Delamanid		x	
Ethambutol	x	x	
Ethionamide		x	
Isoniazid	x	x	
Para-aminosalicylic-acid		x	
Protionamide		x	
Pyrazinamide		x	
Sodium aminosalicylate		x	x
Terizidone		x	

Existing recommendations

WOAH recommendations

Substances used solely to treat tuberculosis or other mycobacterial diseases are not classified by WOAH (formerly OIE).

WHO classifications

WHO: CIA (bedaquiline, calcium aminosalicylate, capreomycin, cycloserine, delamanid, ethambutol, ethionamide, isoniazid, morinamide, para-aminosalicylic-acid, protionamide, pyrazinamide, terizidone, thioacetazone and tiocarlide)

- (C1: Yes) Limited therapy for tuberculosis and other *Mycobacterium* spp. disease; for many of these drugs, single drug therapy may select for resistance.
- (C2: Yes) May result from transmission of *Mycobacterium* spp. from non-human sources.
- (P1: Yes) High absolute number of people affected by diseases for which the antimicrobial is the sole or one of few therapies available.
- (P2: Yes) High frequency of use in human medicine.
- (P3: No)

WHO AWaRe: Not in scope

AMEG recommendations

Drugs used solely to treat tuberculosis or other mycobacterial diseases are included in the AMEG Category A: these classes are not authorised in veterinary medicine but are authorised in human medicine in the EU. These antibiotic classes should only be used exceptionally in individual companion animals in compliance with the prescribing "cascade". Substances in these classes cannot be used for food-producing animals in the absence of established maximum residue limits.

Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Disclaimer: The information in this section reflects reported use of antimicrobials outside the terms of a marketing authorisation. No evaluation is made in this section by the working group on the efficacy or safety of the reported uses, or on their potential impact on development and dissemination of AMR.

Information from published sources

Use of human first-line TB drugs included in this class (e.g. ethambutol and isoniazid) has been described in companion animals (including pet birds) for treatment of mycobacterium tuberculosis complex and *M. avium-intracellulare* [33, 46, 255]. However, alternatives exist and are more commonly used e.g. rifamycins, aminoglycosides, macrolides and fluoroquinolones [244, 517]. A literature search did not reveal use of any second-line or other anti-TB drugs in animals.

Information from the open call for data on use of antimicrobials in animals

The information below is summarised from the open call for data. Inclusion in the table does not endorse use or imply that it is consistent with use according to legislative provisions in Articles 112 to 114.

Substance	Species	Indication	Alternatives	Consequences of unavailability
isoniazid	Pinnipeds, cetaceans	Mycobacteriosis	No	-
ethambutol	Pinnipeds, cetaceans	Mycobacteriosis	No	-
isoniazid	Gray seal (Halichoerus grypus)	CRC Marine Mammal Handbook		-

4.24.2. Evaluation

Scope of permitted use according to the MRL Regulation

Substances used solely to treat tuberculosis or other mycobacterial diseases ('TB drugs') are not included in the Annex to the MRL Regulation (EU) 37/2010 and **cannot be used in food-producing animals in the EU**.

TB drugs can be used in non-food-producing animals in accordance with Article 112.

Examples of veterinary-authorised formulations/species

No authorised veterinary medicinal products were identified in the EU.

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Importance for human health

The group of substances used solely to treat mycobacterial infections is a specialised group represented by several antimicrobial classes. The majority are for the treatment of tuberculosis (TB). This can include active and latent infections, as well as MDR-TB and XDR-TB. Many of these substances are active only against mycobacteria. They may be bacteriostatic or bactericidal and have different modes of action (RNA, mycolic acid suppression, ATP, etc).

Tuberculosis is caused by one of several genetically related mycobacterial species that belong to the *Mycobacterium tuberculosis* complex. Best known TB pathogens include *M. tuberculosis, M. africanum* and *M. bovis*. The other member of the complex, *M. microti*, is primarily a rodent pathogen. *M. tuberculosis* is the most important of the human pathogens. Typical protocols for mycobacterial infections, as recommended by the WHO, involve combination therapy, over several months. These substances should be considered together because combination use is essential for successful treatment owing to development of resistance [555].

Isoniazid +rifampicin (or modern rifamycins e.g. rifabutin, rifapentine) is the mainstay first-line treatment combination for TB; however, they are often used in combination with other agents (e.g. pyrazinamide, ethambutol). For treatment of MDR-TB, regimens may include aminoglycosides, fluoroquinolones and other second-line TB drugs e.g. bedaquiline, delamanid; however, these regimens are often more toxic and involve prolonged treatment courses. There are few treatment options for XDR-TB (resistant to isoniazid, rifampicin, fluoroquinolones and at least one second-line substance) and in some cases it is incurable.

These medicines are approved in the EU either via centralised or national procedures.

Importance for animal health

Mycobacteria affecting companion animals are classified in three groups (i) Tuberculosis complex group (*M. tuberculosis, M. bovis, M. microti*), (ii) atypical (non-tuberculous) mycobacteria that are further divided into slow-growing species (e.g. *M. genavese*) and rapidly-growing species (e.g. *M. avium, M. fortuitum* and *M. avium-intracellulare* - MAC), and (iii) lepromatous mycobacteria.

Use of human first-line TB drugs included in this class (ethambutol and isoniazid) has been described in companion animals (including pet birds) for treatment of *Mycobacterium tuberculosis* complex and disseminated *M. avium-intracellulare* [33, 46, 255]. However, alternatives exist and are more commonly used e.g. rifamycins, aminoglycosides, macrolides and fluoroquinolones [244, 517].

A literature search did not reveal use of any second-line or other anti-TB drugs in animals.

Diagnosis of mycobacterial disease is based initially on cytology/histology, with culture being required to identify the species involved. Culture can only be done in a specialist laboratory; growth may take 2-3 months and culture fails in a high proportion of cases. PCR testing followed by DNA sequencing allows more rapid speciation but has limited availability. Interferon gamma and other immunoassays also have limited availability [551, 556].

Owing to differences in susceptibility patterns, treatment is most successful when the causative mycobacterium has been speciated so that the drug regimen can be tailored to known inherent resistance and susceptibility patterns. Drug susceptibility testing can be based on culture-based phenotypic methods, which are the gold standard but are time-consuming and require specialist laboratories. Alternatively, rapid genotypic tests can be used [553].

Although low, there is a potential zoonotic risk for all TB complex mycobacteria and *M. avium-intracellulare* complex (MAC). Owing to this risk, it is often recommended not to treat pets, especially if any member of the household is immunocompromised.

Considering the potential zoonotic risk, long treatment courses required and associated risk of development of drug resistance, it is proposed that all reasonable attempts should be made to achieve speciation of the mycobacterial infection; however, it is acknowledged that treatment may need to be started before results are available.

According to the Open call for data, TB drugs are used in pinnipeds and cetaceans to treat mycobacteriosis

Development and selection of resistance

Most antimicrobial resistance mechanisms appear unique to the substances used solely for treatment of mycobacterial infections. They are chromosomally encoded, inactivating essential enzymatic house-keeping systems. Resistance to TB antibiotics can develop rapidly [557, 558].

Cross-resistance between these antibiotics could be observed. Resistance to isoniazid from mutations of the *kat*G gene and/or *inhA*/*nph* genes confers resistance to ethionamide. Furthermore, mutations of ethA gene can lead to multi-resistance to isoniazid, ethionamide (protionamide), tiocarlide (Thiocarlide / Isoxyl) and thiacetazone. Mutations of the *thyA* gene can confer resistance to both para-amino salicylic acid (as well as Calcium Aminosalicylate and Sodium Aminosalicylate) as well as cyclic peptides (Capreomycin, Viomycin). Mutations in the transcriptional regulator Rv0678, leading to upregulation of efflux pump MmpL5, can cause cross-resistance involving both clofazimine and bedaquiline [559, 560].

Assessing the likely risk of transmission of resistance to TB antibiotics in mycobacteria is confounded by the knowledge that TB antibiotics are used as specialised combinations to delay the emergence of resistance and to enhance antimycobacterial efficacy, both in animals and humans. Thus, TB substances should be considered together because their combination use is essential for the successful treatment of mycobacterial infections.

There is no established mandatory European-wide surveillance of AMR in mycobacterial infections in either companion or food-producing animals.

There is a report of isolation from a pet dog in Portugal of pre-MDR *M. tuberculosis* that was resistant to isoniazid, ethambutol and streptomycin; however, the resistance was not linked to use of these antimicrobials in the dog. Such cases are usually suspected to be of human origin [561].

Transmission of resistance

The major cause of tuberculosis in humans is *M. tuberculosis*, with human-to-human transmission accounting for the vast majority of cases [562].

Food-producing animals are mostly affected by *M. bovis* and transmission of infection to humans is rare in the EU due to the widespread pasteurisation of milk and the long-established eradication programmes whereby all animals testing positive are removed from the food chain [563, 564]. Treatment of mycobacterial infections in food-producing animals is unlikely and illegal in most EU countries; therefore, the potential for emergence and transmission of resistance to TB antibiotics linked to their use in food-producing animals is low/negligible.

Mycobacterial infections (e.g. *M. bovis, M. avium, M. tuberculosis*) historically have been reported rarely in companion animals in Europe [517, 536], however current prevalence data are not available. In regard to TB complex group, a risk assessment conducted in the UK following a cluster of nine *M. bovis* cases in cats in 2012-13 identified two cases of cat-to-human transmission and considered the risk as very low [537]. There have been occasional reports of suspected spread of *M. bovis* between cats housed at the same premises and of nosocomial spread [538, 539]. In regard to non-tuberculous mycobacteria (NTM), Mycobacterium Avium Complex (MAC) is said to carry a very low risk of zoonotic infection to immunocompromised humans, but most human and companion animal infections appear to arise from an environmental reservoir. Other NTM occurring in companion animals are regarded as opportunistic saprophytes and are not zoonotic or transmitted between pet animals if hygiene is followed [255, 540-542].

In conclusion, based on current evidence and frequency of use, although there is the potential for emergence of resistance to TB antibiotics in isolates from companion animals if their use became established, transmission of resistant mycobacteria to humans and other animals is not likely to be significant at present. (Limited evidence).

In conclusion,

- First-line TB drugs e.g. isoniazid, ethambutol and pyrazinamide are the mainstay of combination treatments for the mycobacterial diseases in humans. Second-line drugs e.g. bedaquiline, are important for the treatment of drug-resistant infections, which are a public health threat in some eastern and central European countries [565].
- TB drugs are not authorised in veterinary medicines and there are a few references to their rare use outside the terms of a marketing authorisation in companion and zoo animals to treat life-threatening mycobacterial infections.
- There are sporadic reports of transmission of mycobacterial infections from companion animals to humans in close contact and other animals, hence there is a potential pathway for transmission of TB-drug resistant mycobacteria, although acknowledging that use of these drugs in animals is very rare.

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to Substances used solely to treat tuberculosis or other mycobacterial diseases.

Criterion (c) – availability of other treatments for animals

Use of human first-line TB drugs e.g. ethambutol, isoniazid, pyrazinamide, has been described in companion animals for treatment of mycobacterium tuberculosis complex and *M. avium-intracellulare*. Alternatives e.g. rifamycins, aminoglycosides, macrolides and fluoroquinolones are mentioned in

treatment guidelines and are more likely to be used; however, considering that long courses are required for treatment, it is recommended that mycobacteria should be speciated and that antimicrobial treatment should be selected on susceptibility testing or according to genotypic testing.

Criterion (d) – availability of other antimicrobial treatments for humans

As noted above, isoniazid (+rifampicin) is the mainstay first-line treatment combination for TB; however, resistance can develop rapidly, and they are often used in combination with other agents (e.g. pyrazinamide, ethambutol). For treatment of MDR-TB, regimens may include fluoroquinolones and second-line TB drugs e.g. bedaquiline, delamanid; however, these regimens are often more toxic and involve prolonged treatment courses. There are few treatment options for XDR-TB (resistant to isoniazid, rifampicin, fluoroquinolones and at least one second-line drug) and in some cases it is incurable. There are essentially no alternative anti-mycobacterial agents beyond those listed in this group that can be used as treatment option.

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

- First-line TB drugs e.g. isoniazid, ethambutol and pyrazinamide are the mainstay of combination treatments for the mycobacterial diseases in humans. Second-line drugs e.g. bedaquiline, are essential for the treatment of drug-resistant infections, which are an important public health threat in some eastern and central European countries. There are no alternatives to those antimycobacterial drugs listed in this class.
- TB drugs are not authorised in veterinary medicines and there are a few references to their rare use in companion and zoo animals to treat certain life-threatening mycobacterial infections. Although alternatives, including some veterinary-authorised antibiotics, are more likely to be used by preference, due to the long treatment courses required and the associated risk of development of resistance, it is important that an effective antibiotic treatment is used, based on knowledge of the likely susceptibility pattern of the mycobacterial pathogen under treatment.
- Owing to the potential zoonotic risk for all TB complex mycobacteria and *M. avium-intracellulare* complex (MAC), it may be recommended that infected animals should be euthanised. Reports of transmission of mycobacterial infections from companion animals to humans in close contact and other animals are sporadic, hence there is a potential pathway for transmission of TB-drug resistant mycobacteria, although use of these drugs in animals is likely to be very rare.
- In conclusion, TB drugs are highly important to treat life-threatening mycobacterial infections in humans. Evidence suggests that they are also rarely used in companion animals to treat mycobacteria. There is a potential pathway for transmission of drug-resistant mycobacteria between animals and to humans via zoonotic mycobacteria, and on the rare occasions when animals are treated, it is important that the pathogen is susceptible to the selected antibiotics.

Therefore, considering the points above relevant to criteria (b), (c) and (d), it should be considered if conditions or a prohibition should be placed on the use of Substances used solely to treat tuberculosis or other mycobacterial diseases outside the terms of the marketing authorisation.

Step 2. Considerations of conditions to be placed on use outside the terms of a marketing authorisation

Please refer to Section 3.1.2. of the main report for the general rationale behind the proposed conditions.

In the absence of authorised VMPs containing Substances used solely to treat tuberculosis or other mycobacterial diseases, (i) and (ii) are not addressed.

(iii) Administration by an unauthorised route or use of extemporaneous formulation

Human formulations of TB-drugs are available mainly as injections and oral formulations. Considering the long duration of treatment and suitability of tablet size for companion animals, extemporaneous formulations may ease administration.

No conditions proposed.

(iv) Use of a human medicinal product

Conditions:

- Use must be based on target pathogen identification and antimicrobial susceptibility testing that demonstrates that TB drugs are likely to be effective. (See *'Special note regarding the diagnosis of mycobacterial infections in companion animals and antimicrobial susceptibility testing'* in Annex 1.
- To be used in individual animals only

Rationale: Owing to differences in susceptibility patterns, treatment is most successful when the causative mycobacterium has been speciated so that the drug regimen can be tailored to known inherent resistance and susceptibility patterns. Seed discussion under 'Importance for animal health' above.

In regard to mycobacterial infections in companion animals, it is noted that there is regional variability in access to diagnostic laboratories and the testing facilities available at those sites. Some mycobacterial species may either take 1-3 months to culture or not culture at all and different testing methods may be appropriate according to pathogen characteristics [551-554]. Please refer to the 'Special note regarding the diagnosis of mycobacterial infections in companion animals and antimicrobial susceptibility testing' special note on diagnosis of mycobacterial infection in Annex 1. 2/AST. However, due to the length of treatment required, the need to select an effective treatment regimen, the potential for resistance to develop during treatment, the risk for transmission of resistant infections from treated animals to humans and other animals, it is recommended that before initiating treatment steps should be taken for target pathogen identification and susceptibility testing, according to the caveat stated above.

(v) Use of a third country veterinary medicinal product

According to Articles 112(2), 113(2) and 114(4), third country VMPs may only be used in the same species and for the same indication.

No further conditions proposed to those mentioned above.

Step 3. Consideration of Criteria (a) and (e) in view of proposed conditions to be placed on use outside the terms of a marketing authorisation

Criterion (a) – risk to animal health or public health if the antimicrobial is used in accordance with Articles 112, 113 and 114

Isoniazid should not be used in animals with hepatic disease. Adverse effects include hepatotoxicity, CNS effects, neuropathy and thrombocytopenia. Ethambutol should be used with care in animals with renal dysfunction. Optic neuritis has been reported in human patients. In humans, dose-related hepatotoxicity has been reported with pyrazinamide [46, 119, 342].

Criterion (e) Impact on aquaculture and farming if the animal affected by the condition receives no treatment

TB drugs can be used in non-food-producing equines in accordance with Article 112; the proposed conditions do not prevent treatment of such animals.

Step 4. Final conclusion - recommendations made for conditions to be placed on use outside the terms of a marketing authorisation

Based on the discussion above, the following conditions are proposed:

- Use must be based on target pathogen identification and antimicrobial susceptibility testing that demonstrates that TB drugs are likely to be effective. See *'Special note regarding the diagnosis of mycobacterial infections in companion animals and antimicrobial susceptibility testing'* in Annex 1.
- To be used in individual animals only.

4.25. Riminofenazines

Riminofenazines are authorised in human medicinal products in the EU. At present they are not authorised in veterinary medicinal products in the EU.

4.25.1. Background information

Examples of substances included in the class that are authorised in human medicine only

Examples of substances authorised for human use	Examples of ATC codes
Clofazimine	J04BA01

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

Riminofenazines are not included in the Table 1 (allowed substances) of the Annex to the MRL Regulation (EU) 37/2010 and cannot be used in food-producing animals in the EU.

Examples of EU-authorised HMP formulations, from Article 57 database

Substance	Route of administration		
	Injection	Oral e.g. tablet, liquid	Topical/local
Clofazimine		x	

Existing recommendations

WOAH recommendations

Riminofenazines are not classified by WOAH (formerly OIE).

WHO classifications

WHO: HIA

- (C1: Yes) Limited therapy for leprosy.
- (C2: No)

WHO AWaRe: -

AMEG recommendations

Riminofenazines are included in the AMEG Category A: these classes are not authorised in veterinary medicine but are authorised in human medicine in the EU. These antibiotic classes may only be used exceptionally in individual companion animals in compliance with the prescribing "cascade". Substances in these classes cannot be used for food-producing animals in the absence of established maximum residue limits.

Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Disclaimer: The information in this section reflects reported use of antimicrobials outside the terms of a marketing authorisation. No evaluation is made in this section by the working group on the efficacy or safety of the reported uses, or on their potential impact on development and dissemination of AMR.

Information from published sources

The WSAVA List of essential medicines for cats and dogs makes no mention of clofazimine or other Riminofenazines [178]. Clofazimine is known to be used in dogs and cats as part of a multidrug

therapy for mycobacterial granulomas or leprosy for up to 6 weeks [566]. Clofazimine has also been used as a topical formulation in petroleum jelly as an adjunct to oral antibiotics in mycobacterial granulomas [523, 567].

Clofazimine has also been described in horses for the treatment of fistulous withers [568]. Fistulous withers is a chronic inflammatory disease of the supraspinatus bursa and associated tissues. Although infection by *Brucella abortus* has been typically associated with the condition, other infectious organisms and trauma can also cause the disease (e.g. *Streptococcus zooepidemicus*, *Streptococcus equi*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Proteus mirablis*, *Actinomyces bovis*, *Bacteroides fragilis*, *Escherichia coli*, *Pasteurella spp*. and *Corynebacterium spp*.).

Information from the open call for data on use of antimicrobials in animals

No information on use outside the terms of a marketing authorisation was provided in the open call for data.

4.25.2. Evaluation

Scope of permitted use according to the MRL Regulation

Riminofenazines are not included in the Annex to the MRL Regulation (EU) 37/2010 and cannot be used in food-producing animals in the EU.

Clofazimine can be used in non-food-producing animals in accordance with Article 112.

Examples of veterinary-authorised formulations/species

Riminofenazines have not been authorised for use in VMPs in the EU and there is no knowledge of their veterinary authorisation globally.

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Importance for human health

Riminofenazines are currently represented by one antimicrobial - clofazimine. Clofazimine is a lipophilic compound (C27H22Cl2N4) that primarily acts on the bacterial outer membrane. It has broad-spectrum activity against bacteria, parasites and fungi. The WHO placed clofazimine in the group of five medicines for the management of multi-drug resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) [569].

Clofazimine is active against Gram-positive organisms, while Gram-negative organisms are uniformly resistant. Clofazimine has demonstrated high activity against various mycobacterial species, and acts synergistically with other antimicrobial agents, such as amikacin and clarithromycin [570]. It is principally used as a treatment of leprosy (*Mycobacterium leprae* and *Mycobacterium lepromatosis*), a second-line treatment for rifampicin-resistant TB, as well as selected atypical mycobacterial infections (e.g., *Mycobacterium abscessus*).

The approved indication of clofazimine is for treatment of lepromatous leprosy, including cases resistant to dapsone treatment and cases complicated by erythema nodosum. First line treatment of leprosy consists of multiple-drug therapy to prevent development of resistance and includes dapsone, rifampin and clofazimine. Therefore, clofazimine is deemed critical in leprosy treatment. Clofazimine

has been granted orphan drug status in the EU for the treatment of non-tuberculous mycobacterial lung disease.

While clofazimine is not approved in the EU for the treatment of drug-resistant tuberculosis (MDR-TB), it has been considered by WHO as a critical medicine in the treatment of drug-resistant TB for years and its importance has been growing. The WHO has recommended the use of clofazimine in the shorter regimen used to treat DR-TB since 2016. The current WHO guidance on treatment of drug-resistant TB has also prioritized clofazimine moving it into Group B for the longer DR-TB regimens [555].

Importance for animal health

Clofazimine is known to be used in dogs and cats as part of a multidrug therapy for non-tuberculous mycobacterial granulomas or leprosy for up to 6 weeks [566]. Clofazimine has also been used as a topical formulation in petroleum jelly as an adjunct to oral antibiotics in mycobacterial granulomas [523, 567].

The ABCD guidelines on prevention and management of mycobacterioses in cats were first published in 2013 [517] and later updated in 2015. In that guideline, clofazimine is recommended for cats for the treatment of non-tuberculous mycobacteria e.g. *Mycobacterium avium-intracellullare* complex (MAC) and feline leprosy (e.g. *Mycobacterium lepraemurium).*

Clofazimine is part of a combination treatment and alternatives e.g. doxycycline (authorised as a VMP for cats), rifampicin and clarithromycin, are mentioned in treatment guidelines [244, 517, 566]. Clofazimine may be advantageous in terms of ease of administration over the required long treatment duration (Rory Breathnach, personal communication).

Clofazimine has also been described in horses for the treatment of fistulous withers [568]. Fistulous withers is a chronic inflammatory disease of the supraspinatus bursa and associated tissues. Although infection by *Brucella abortus* has been typically associated with the condition, other infectious organisms and trauma can also cause the disease (e.g. *Streptococcus zooepidemicus*, *Streptococcus equi*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Proteus mirablis*, *Actinomyces bovis*, *Bacteroides fragilis*, *Escherichia coli*, *Pasteurella spp*. and *Corynebacterium spp*.).

The volume of clofazimine use in animals is currently not recorded at the EU-level. Overall annual use depends on the frequency of infections mentioned above.

Development and selection of resistance

Resistance to clofazimine has not yet been fully characterized; however, mutations in the chromosomal transcriptional regulator genes appear likely causes of resistance for *M. tuberculosis* and *M. leprae* [570-572]. Although there are reports relating to resistance to ofloxacin and rifampicin in leprosy patients, little was found on the prevalence of resistance to clofazimine.

Cross-resistance between clofazimine and bedaquiline have been described in *M. tuberculosis* with mutations in the transcriptional regulator *Rv0678* [571, 573].

There is no established mandatory European-wide surveillance of AMR in mycobacterial infections in either companion or food-producing animals.

No reports were found of resistance to clofazimine in mycobacteria isolates from animals.

Transmission of resistance

The major cause of tuberculosis in humans is *M. tuberculosis*, with human-to-human transmission accounting for the vast majority of cases.

Mycobacterial infections historically have been reported rarely in companion animals in Europe [522, 536], however current prevalence data are not available.

In cats, clofazimine is recommended for treatment of disseminated *Mycobacterium avium-intracellulare* complex (MAC) infection, a serious disease with a poor prognosis, and for feline leprosy (e.g. *M. lepraemurium*) [244, 522, 566]. Clofazimine has also been reported sporadically for treatment of canine leproid granuloma [523].

Moreover, in companion animals leprosy has been associated with different mycobacterial species (e.g. *M. lepraemurium*) from that causing the disease in humans and there is no evidence that food-producing animals or companion animals could act as reservoirs of *M. leprae* or *M. lepromatosis* [574]. Clofazimine resistance has been only associated with chromosomal mutations, restricting any potential horizontal transmission of resistance between mycobacterial species.

Therefore, transmission of clofazimine resistance from animal sources is not demonstrated to be relevant for treatment of *M. leprae* in humans. In addition, MAC appears to have low zoonotic potential, with most human infections deriving from environmental sources [542].

In dogs and cats, non-tuberculous and mycobacteria associated with leprosy are acquired from environmental sources (saprophytic) or rodent bites. In conclusion, based on current evidences and frequency of use, although there is the potential for emergence of resistance to riminofenazines in isolates from companion animals if their use became established, transmission of riminofenazine resistant mycobacteria to humans and other animals is not likely to be significant at present.

In conclusion,

- Clofazimine is used in human medicine for the treatment of mycobacterial infections. It is principally used as a treatment of leprosy (*Mycobacterium leprae* and *Mycobacterium lepromatosis*), a second-line treatment for rifampicin-resistant TB, as well as for selected atypical mycobacterial infections.
- Clofazimine resistance has been only associated with chromosomal mutations, restricting any potential horizontal transmission of resistance between mycobacterial species. Therefore, transmission of clofazimine resistance from animal sources is not demonstrated to be relevant for treatment of non-zoonotic mycobacteria (e.g. *M. leprae*) in humans.
- There is the potential for emergence of resistance to riminofenazines in isolates from companion animals under treatment.
- In veterinary medicine, clofazimine is reported as used, outside a marketing authorisation, for treatment of leproid mycobacteria and for MAC infections.
- The causative mycobacteria for these infections are mostly acquired from the environment. MAC appears to have a very low zoonotic potential and leproid mycobacteria are not zoonotic.

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to Riminofenazines.

Criterion (c) – availability of other treatments for animals

Alternatives reported for treatment of MAC and leproid mycobacterial infections in companion animals include rifamycins, doxycycline, macrolides, fluoroquinolones, ethambutol and isoniazid [33, 244, 255, 517]. Although alternatives are available, considering that long courses are required for treatment, and that susceptibility varies between species, it is recommended that mycobacteria should be speciated and that antimicrobial treatment should be selected on susceptibility testing or according to genotypic

testing. Despite this, considering the availability and time taken for testing, it may be necessary to initiate treatment with the recommended treatment options before results are available.

Criterion (d) – availability of other antimicrobial treatments for humans

Alternative agents for treatment of leprosy include minocycline, ofloxacin, levofloxacin, clarithromycin, and moxifloxacin [575]. MDR tuberculosis can be treated with two regimens based on the length of the drug administration. The recommended treatment concerns 3 different groups of medicines (A, B and C) as well as other medicines. Medicines from group A are considered highly effective against MDR-TB (e.g., bedaquiline, levofloxacin, moxifloxacin, linezolid). Cycloserine or terizidone (group B) can be used as second line agents, but not in those patients who get only two medicines from the group A substances. When the medicines from group A and B cannot be used, then Group C is recommended (e.g., ethambutol, delamanid, pyrazinamide, imipenem-cilastatin or meropenem, amikacin, streptomycin, ethionamide or prothionamide, para-aminosalicylic acid). Clofazimine belongs to group B and is an integral part of the short-term regimen and some of these alternatives for the long- term regimen, are more toxic than clofazimine.

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

- Clofazimine is used both in human medicine and in companion animals for the treatment of mycobacterial infections. In humans, it is principally used as a treatment of leprosy, as a second-line treatment for rifampicin-resistant TB and for selected atypical mycobacterial infections. In veterinary medicine, clofazimine is reported as used, outside a marketing authorisation, for treatment of leproid mycobacteria and for MAC infections in companion animals.
- While in general, alternative treatment options exist both in human and veterinary medicine, the main therapeutic value of clofazimine is due to its PK/PD characteristics (high volume of distribution, extra-long half-lives, safety profile, anti-inflammatory properties) and very low resistance rates. The susceptibility to different anti-mycobacterial substances varies between mycobacterial species and strains, and choice of treatment should ideally be based on test results.
- There are no EU-authorised VMPs containing clofazimine or other riminofenazines and in the absence of MRL status they can only be used in non-food-producing animals, outside of a marketing authorisation. Considering the incidence of mycobacterial infections in dogs and cats and that recommendations are limited to use for MAC and leprosy, the extent of use likely to be low. Based on the resistance mechanisms involved and the epidemiology of the mycobacterial species concerned, the AMR risk to public and animal health associated with use of riminofenazines in animals is likely to be very low.
- In conclusion, clofazimine is highly important to treat leprosy and mycobacterial infections in humans. Evidence suggests that it is also rarely used in companion animals to treat MAC and leprosy and that the AMR risk to public and animal health associated with this use is very low. However, when treating mycobacterial infections in animals, owing to the length of treatment course, and the importance of selecting an effective antimycobacterial treatment, appropriate diagnostic testing should be conducted.

Therefore, considering the points above relevant to criteria (b), (c) and (d), it should be considered if conditions or a prohibition should be placed on the use of Riminofenazines outside the terms of the marketing authorisation.

Step 2. Considerations of conditions to be placed on use outside the terms of a marketing authorisation

In the absence of authorised VMPs containing Riminofenazines, (i) and (ii) are not addressed.

(iii) Administration by an unauthorised route or use of extemporaneous formulation

Clofazimine has also been used as a topical formulation in petroleum jelly as an adjunct to oral antibiotics in mycobacterial granulomas.

(iv) Use of a human medicinal product

Use of a human medicinal product is the only known use of clofazimine in Europe.

Conditions:

- Use must be based on target pathogen identification and antimicrobial susceptibility testing that demonstrates that riminofenazines are likely to be effective. See 'Special note regarding the diagnosis of mycobacterial infections in companion animals and antimicrobial susceptibility testing' in Annex 1.
- To be used in individual animals only.

Rationale: In regard to mycobacterial infections in companion animals, it is noted that there is regional variability in access to diagnostic laboratories and the testing facilities available at those sites. Some mycobacterial species may either take 1-3 months to culture or not culture at all and different testing methods may be appropriate according to pathogen characteristics [551-554]. Please refer to the special note on diagnosis of mycobacterial infection in Annex 1. However, due to the length of treatment required, the need to select an effective treatment regimen, the potential for resistance to develop during treatment, it is recommended that before initiating treatment steps should be taken for target pathogen identification and susceptibility testing, according to the caveat stated.

(v) Use of a third country veterinary medicinal product

According to Articles 112(2), 113(2) and 114(4), third country VMPs may only be used in the same species and for the same indication. No further conditions proposed to those mentioned above.

Step 3. Consideration of Criteria (a) and (e) in view of proposed conditions to be placed on use outside the terms of a marketing authorisation

Criterion (a) – risk to animal health or public health if the antimicrobial is used in accordance with Articles 112, 113 and 114

Use of clofazimine was reported to induce photosensitisation in a cat [576]. It is reported that dose-related skin, eye and body fluid discolouration observed in humans can also occur in animals [119]. Gastrointestinal intolerance is also reported in humans [577]

Criterion (e) Impact on aquaculture and farming if the animal affected by the condition receives no treatment

No MRLs exist for clofazimine. Clofazimine can be used in non-food-producing equines in accordance with Article 112; the proposed conditions do not prevent treatment of such animals.

Step 4. Final conclusion - recommendations made for conditions to be placed on use outside the terms of a marketing authorisation

Based on the discussion above, the following conditions are proposed:

- Use must be based on target pathogen identification and antimicrobial susceptibility testing that demonstrates that riminofenazines are likely to be effective. See 'Special note regarding the diagnosis of mycobacterial infections in companion animals and antimicrobial susceptibility testing' in Annex 1
- To be used in individual animals only.

4.26. Sulfones

Sulfones are authorised in human medicinal products in the EU. At present they are not authorised in veterinary medicinal products in the EU.

4.26.1. Background information

Examples of substances in the class that are authorised in human medicine only in the EU

Examples of substances authorised for human use	Examples of ATC codes
Dapsone	J04BA02 D10AX05

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

Dapsone is contained in Table 2 of EU Council Regulation 37/2010; therefore, use is prohibited in food-producing species.

No other sulfones are included in the Annex to the MRL Regulation (EU) 37/2010 and therefore they **cannot be used in food-producing animals in the EU**.

Examples of EU-authorised HMP formulations, from Article 57 database

Substance	Route of administration		
	Injection	Oral e.g. tablet, liquid	Topical/local
Dapsone		x	

Existing recommendations

WOAH recommendations

Sulfones are not classified by WOAH (formerly OIE).

WHO classifications

WHO: HIA

- (C1: Yes) Limited therapy for leprosy.
- (C2: No)

WHO AWaRe: -

AMEG recommendations

Sulfones are included in the AMEG Category A: these classes are not authorised in veterinary medicine but are authorised in human medicine in the EU. These antibiotic classes may only be used exceptionally in individual companion animals in compliance with the prescribing "cascade". Substances in these classes cannot be used for food-producing animals in the absence of established maximum residue limits.

Use outside the terms of a marketing authorisation reported in the open call for data or in literature Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Disclaimer: The information in this section reflects reported use of antimicrobials outside the terms of a marketing authorisation. No evaluation is made in this section by the working group on the efficacy or safety of the reported uses, or on their potential impact on development and dissemination of AMR.

Information from published sources

The reports in equine patients primarily relate to the treatment of protozoal infections, most notably respiratory infections in foals caused by *Pneumocystis carinii* [578].

In companion animals, use primarily relates (in recent years) to use in dogs for the treatment of inflammatory or immune-mediated skin diseases that are either rare in occurrence (e.g. dermatitis herpetiformis) or that have failed to respond to other first-line and second-line immunosuppressive agents (e.g. pemphigus complex) [33, 46].

Previous reports of use of dapsone in cats to treat mycobacterial granulomas (e.g. feline leprosy) are largely outdated at this time point, as the drug is no longer recommended in this species due to the serious adverse effects reported with its use.

Information from the open call for data on use of antimicrobials in animals

No information on use outside the terms of a marketing authorisation was provided in the open call for data.

4.26.2. Evaluation

Scope of permitted use according to the MRL Regulation

Dapsone is included in Table 2 (prohibited substances) of the Annex to Regulation (EU) 37/2010 and hence cannot be used in any food-producing species. No other sulfones are included in the Annex to the MRL Regulation (EU) 37/2010 and therefore they **cannot be used in food-producing animals in the EU**.

Sulfones can be used in non-food-producing animals in accordance with Article 112.

Examples of veterinary-authorised formulations/species

There are no VMPs authorised in the EU that contain sulfones.

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Importance for human health

Sulfones include two substances (dapsone and sulfoxone) primarily used to treat leprosy caused by *Mycobacterium leprae* in humans. Dapsone (diaminodiphenyl sulfone-DDS) is the most effective sulfone derivative in the treatment of leprosy. It inhibits the synthesis of dihydrofolic acid through by competing with para-aminobenzoic acid for the active site of dihydropteroate synthetase.

Sulfoxone (aldesulfone sodium) is another representative of sulfones which was, but is no longer, used to treat leprosy.

Dapsone is active against many bacteria. Fully susceptible strains of *Mycobacterium leprae* are inhibited at very low minimum inhibitory concentrations (MICs). The antibiotic is primarily bacteriostatic.

Dapsone is also effective against some protozoa and fungi (e.g., malaria, *Pneumocystis jirovecii*).

The relevant indication is for the treatment of leprosy. Since this requires combination treatment, and since dapsone and clofazimine are the mainstays of the therapy, use of dapsone in leprosy is deemed critical.

Dapsone is approved in some EU Member States for a number of indications: as part of a multi-drug regimen in the treatment of all forms of leprosy, for the treatment of dermatitis herpetiformis and other dermatoses, for the prophylaxis of malaria in combination with pyrimethamine and for the prophylaxis of *Pneumocystis carinii* (now *jirovecii*) pneumonia in immunodeficient subjects, especially AIDS patients [579]. It can also be used (off-label) for the prophylaxis of toxoplasmosis (in combination with pyrimethamine) [580] and for the treatment (in combination with trimethoprim) of *P. jirovecii* pneumonia [581].

Importance for animal health

The use of dapsone in VMPs for food-producing animals has been prohibited in the EU since January 1994 following the conclusion of its evaluation for the potential establishment of maximum residue limits (MRLs) [582].

An individual case report documents the use of dapsone to successfully treat *Pneumocystis carinii* pneumonia in a foal that was unable to tolerate other treatments [578].

Dapsone was previously used in the treatment of feline leprosy (*Mycobacterium lepraemurium*) and other opportunistic mycobacterial granulomas in cats but is no longer recommended due to availability of safer alternatives for this species [125, 583].

Although some reports quote potential anti-inflammatory and immunosuppressive properties of dapsone in animals, most such data are derived from *in vitro* (and not controlled clinical) studies. Nevertheless, as in human dermatology, dapsone has been used in rare cases to treat dogs with inflammatory or immune-mediated dermatoses such as subcorneal pustular dermatosis, dermatitis herpetiformis and pemphigus complex [46, 584].

Owing to adverse effects, dapsone is generally not recommended for use in cats and is used cautiously in dogs. Adverse effects include hepatotoxicity, blood dyscrasias, gastrointestinal effects, neuropathies and cutaneous drug eruptions. Dapsone is also potentially carcinogenic.

Development and selection of resistance

Resistance to dapsone has not yet been fully characterized; however, mutations in the *folP1* gene-encoded dihydropteroate synthase (DHPS) and genomic reduction has been associated with decreased activity of dapsone in *M. leprae* [585]. High-level resistance is acquired by several sequential mutations. Dapsone resistance became widespread in the 1980s with patients on longterm monotherapy. To slow development of drug resistance, the treatment of leprosy has been standardised with multidrug therapy (dapsone, clofazamine, rifampicin). WHO conducts global surveillance for antimicrobial resistance in leprosy [586, 587].

There is no established mandatory European-wide surveillance of AMR in mycobacterial infections in either companion or food-producing animals.

No information could be found on the occurrence of resistance to sulfones in mycobacteria from animals.

Transmission of resistance

In companion animals, leprosy has been associated with different mycobacterial species (including *Mycobacterium lepraemurium*) from that causing the disease in humans. There is no evidence that

food-producing animals or companion animals could act as reservoirs of *M. leprae* or *M. lepromatosis* that cause disease in humans. Potential wildlife reservoirs have been proposed (outside the EU), but the evidence for transmission from animals to humans is unclear [574].

Pneumocystis is an opportunist fungal pathogen and is not regarded as zoonotic [588].

Sulfones are not authorised in VMPs in the EU and there is scant evidence for their use in animals. Potential selection of resistance in *Mycobacterium* spp. could occur if sulfones were authorised for the treatment of rare cases of lepromatosis/leprae infections in companion animals (other treatments are currently recommended, [244]).

In conclusion, although there would be the potential for emergence of resistance to sulfones in isolates from companion animals if their use became established, the mycobacterial species causing leprosy in companion animals are not regarded as zoonotic and there is no likely significant pathway for transmission of resistance to relevant human pathogens.

Leprosy due to *M. lepraemurium* in young cats and is thought to be acquired from rodent bites or contamination of wounds by saprophytic mycobacteria in soil or on plants [255]. There is no suggestion of direct transmission between companion animals.

In conclusion,

- Sulfones (dapsone) are critically important in human medicine as part of multidrug therapy for leprosy (*M. leprae*). Dapsone is authorised for this indication in the EU, and also for treatment of certain inflammatory and immune-mediated dermatoses and prevention of *Pneumocystis jirovecii* pneumonia in immunodeficient subjects.
- Sulfones are not authorised in veterinary medicines in the EU and cannot be used if food-producing animals. There are scant reports of the use of dapsone in companion animals, mainly to treat feline leprosy (*M. lepraemurium*), other non-zoonotic mycobacterial infections and certain rare dermatoses in dogs.
- Resistance to dapsone has been reported in human leprosy patients on longterm treatment. Leprosy in animals is caused by different species of mycobacteria and there is no evidence that food-producing or companion animals could act as reservoirs of *M. leprae* or *M. lepromatosis* that cause disease in humans. There is also no suggestion of direct transmission of *M. lepreamurium* between companion animals. Hence there does not appear to be a significant pathway for transmission of dapsone resistance from treated companion animals to humans or other animals.

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to Sulfones.

Criterion (c) – availability of other treatments for animals

Clofazimine in combination with rifampicin and clarithromycin is currently recommended to treat feline leprosy and other mycobacterial granulomas in companion animals [517, 566]. In addition, there are a number of immunosuppressive drugs available to treat a wide spectrum of immune-mediated/autoimmune skin diseases in companion animals.

Criterion (d) – availability of other antimicrobial treatments for humans

Second-line drugs for treatment of leprosy include fluoroquinolones, minocycline and clarithromycin. The fluoroquinolones perfloxacin and ofloxacin have high bactericidal activity against *M. leprae*. Patients that have adverse effects with the WHO MDT regimen may alternatively be treated with the triple drug combination of rifampin, ofloxacin and minocycline (ROM) [589].

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

- Sulfones (dapsone) are critically important in human medicine as part of multidrug therapy for leprosy (*M. leprae*); although second-line drugs are available. Dapsone is also used for treatment of certain dermatoses and prevention of *Pneumocystis jirovecii* pneumonia in immunodeficient subjects.
- Sulfones are not authorised in veterinary medicines in the EU and cannot be used if food-producing animals. There are scant reports of the use of dapsone in companion animals, mainly to treat feline leprosy (*M. lepraemurium*), other non-zoonotic mycobacterial infections and certain rare dermatoses in dogs. Alternatives are available and it is likely that dapsone would only be used as last resort owing to its adverse effects.
- Resistance to dapsone has been reported in human leprosy patients on longterm treatment. There is no evidence that animals could act as reservoirs of *M. leprae* or *M. lepromatosis* that cause disease in humans and no significant pathway for transmission of dapsone resistance from treated companion animals to humans or other animals has been identified.

Therefore, considering the points above relevant to criteria (b), (c) and (d), it is recommended that no conditions should be placed on the use of Sulfones outside the terms of the marketing authorisation, although responsible antimicrobial use principles should be applied.

4.27. Pseudomonic acids

Pseudomonic acids are authorised in human medicinal products in the EU. At present they are not authorised in veterinary medicinal products in the EU.

4.27.1. Background information

Examples of substances in the class that are authorised in human medicine in the EU

Examples of substances authorised for human use	Examples of ATC codes
Mupirocin	D06AX09 R01AX06

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

Pseudomonic acids are not included in Table 1 (allowed substances) of the Annex to the MRL Regulation (EU) 37/2010 and cannot be used in food-producing animals in the EU.

Examples of EU-authorised HMP formulations, from Article 57 database

Substance	Route of administration		
	Injection	Oral e.g. tablet, liquid	Topical/local
Mupirocin			x

Existing recommendations

WOAH recommendations

Pseudomonic acids are not classified by WOAH (formerly OIE).

WHO classifications

WHO: HIA

- (C1: No) In certain geographic settings, Criterion 1 may be met: the class may be one of limited therapies for topical *Staphylococcus aureus* infections.
- (C2: Yes) May result from transmission of MRSA from nonhuman sources.

WHO AWaRe: -

AMEG recommendations

Pseudomonic acids are included in the AMEG Category A: these classes are not authorised in veterinary medicine but are authorised in human medicine in the EU. These antibiotic classes may only be used exceptionally in individual companion animals in compliance with the prescribing "cascade". Substances in these classes cannot be used for food-producing animals in the absence of established maximum residue limits.

Mupirocin is a first-line antibiotic for the treatment and decolonisation of MDR staphylococci (e.g. MRSA).

Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Disclaimer: The information in this section reflects reported use of antimicrobials outside the terms of a marketing authorisation. No evaluation is made in this section by the working group on the efficacy or safety of the reported uses, or on their potential impact on development and dissemination of AMR.

Information from published sources

An ointment containing mupirocin is authorised by FDA for the topical treatment of canine bacterial infections of the skin, including superficial pyoderma, caused by susceptible strains of *Staphylococcus aureus* and *Staphylococcus intermedius*. In North America, mupirocin has also been described for the treatment of feline acne and localized lesions of equine pyoderma [590, 591]. Other reported uses are mentioned in the Evaluation section, below.

Information from the open call for data on use of antimicrobials in animals

The information below is summarised from the open call for data. Inclusion in the table does not endorse use or imply that it is consistent with use according to legislative provisions in Articles 112 to 114.

Substance	Species	Indication	Alternatives	Consequences of unavailability
Mupirocin	dog, cat, horse	cutaneous infection e.g. furunculosis	systemic antimicrobial, local antiseptic	increase use of systemic antimicrobial
	dog	furunculosis	no	animal welfare
	dog	furunculosis		
	dog	furunculosis	no	chronic evolution
	dog, cat	MRSA, MRSP	no VMP with mupirocin available in CZ	both health and welfare concerns

4.27.2. Evaluation

Scope of permitted use according to the MRL Regulation

Pseudomonic acids are not included in Table 1 (allowed substances) of the Annex to Regulation (EU) 37/2010 and hence cannot be used in food-producing species in accordance with Articles 113 and 114 of Regulation (EU) 2019/6.

Pseudomonic acids can be used in non-food-producing animals in accordance with Article 112.

The scope of this evaluation is limited to the use of mupirocin in non-food-producing animals.

Examples of veterinary-authorised formulations/species

There are no veterinary-authorised formulations of pseudomonic acids. In human medicine, mupirocin is authorised in formulations intended for cutaneous and nasal use.

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Importance for human health

Mupirocin is the only antibiotic authorised for the treatment of carriage (decolonisation therapy) of *Staphylococcus aureus*, including MRSA. Decolonisation therapy of patients screened positive is recommended to decrease the risk of subsequent *S. aureus* infection, including MRSA surgical site infection, particularly in patients undergoing cardiothoracic and orthopaedic surgery and those receiving implantable devices or undergoing organ or stem-cell transplants. Topical mupirocin is a cornerstone in decolonisation therapy and thereby an essential component of the public health response against MRSA in many EU/EEA countries [592].

Importance for animal health

Mupirocin is used outside the terms of the marketing authorisation in companion animals as topical treatment of serious skin infections caused by MRSP or MRSA that have been shown to be resistant to antimicrobials of lower importance [593-595]. Based on international guidelines, the frequency of such use is low and mupirocin is not used for decolonisation therapy in companion animals [334]. Whilst MRSA is more commonly associated with prosthetic implants or wounds, MRSP is most commonly implicated in canine skin infections, but may also be involved with surgical wounds, urinary and respiratory tract infections, which can be life-threatening. Where possible, early topical treatment is preferred and susceptibility testing may show that the only effective alternatives are systemically administered antibiotics of higher importance in human medicine (e.g. vancomycin, linezolid, rifamycin) [146, 334, 375, 596-599]. It should be noted that certain last resort antimicrobials (e.g. glycopeptides and oxazolidinones) are now reserved for use in humans only under Regulation (EU) 2022/1255 [2].

The WAVD recommendations on treatment of MRS [334] conclude that there is currently insufficient evidence to recommend antibiotic use for routine decolonization of MRS carrier animals that pose a risk to susceptible in-contact people and animals.

The Open call for data also received reports of use of mupirocin for treatment of furunculosis in dogs, cats and horses.

Selection, development and transmission of resistance

Acquired resistance to mupirocin can be chromosomal and plasmid-mediated. In staphylococci, mupirocin resistance can be either low level (LL) or high level (HL). LL resistance is the result of point mutations in the chromosomally located *ile*S gene. High level resistance in staphylococci is mediated by the *mup*A gene which is located on a conjugative plasmid and can spread clonally and horizontally, even between different staphylococcal species and MRSA. The *mup*B gene is located on non-conjugative plasmids [265, 600, 601]. There is no cross-resistance between mupirocin and other antimicrobial agents. Co-resistance of mupirocin with clindamycin, tetracycline, erythromycin and levofloxacin has been reported in MRSA isolates from humans in the USA [602]. Mupirocin is not authorised for use in animals and presently mupirocin resistance in staphylococci of animal origin is not reported from food-producing animals and is rare in companion animals [334, 603-605].

Staphylococcus spp. (including MRSA/MRSP) may be transmitted between livestock, companion animals and humans [44, 90, 153, 605].

The actual prevalence of mupirocin resistance in staphylococci isolates from companion animals in the EU is unknown but there are occasional reports, with one study in Poland detecting high-level mupirocin resistance in 2.6% of MRS strains [603, 604, 606]. There is evidence for potential selection and transmission of resistance to pseudomonic acids between animals and humans through zoonotic and target *Staphylococcus* spp. if use of pseudomonic acids in animals became well established.

In conclusion,

- Mupirocin is highly important in human medicine, used for MRSA-decolonisation prior to surgical and other critical interventions to prevent serious MRSA infections.
- In veterinary medicine mupirocin is used outside the terms of the marketing authorisation in companion animals as topical treatment of serious skin infections caused by methicillin-resistant staphylococci that have been shown to be resistant to antimicrobials of lower importance in terms of the AMR risk.

- There is evidence for potential for transfer of resistance to mupirocin from companion animals to humans and other animals.

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to Pseudomonic acids.

Criterion (c) – availability of other treatments for animals

Where possible, early topical treatment is preferred for localised staphylococcal skin infections, including those due to MRS. For superficial bacterial folliculitis, fusidic acid or antibacterial agents e.g. chlorhexidine or benzoyl peroxide are possible alternatives to mupirocin. However, susceptibility testing may show that the only effective alternatives to mupirocin for MRS are systemically administered antibiotics of higher importance in human medicine and mostly to be prohibited from use in animals (e.g. vancomycin, linezolid, rifamycin) [2, 146, 334, 375, 596-599]. MRSA/P infections in companion animals are potentially zoonotic and considering the close and sustained contact between pet and owner, such infections should be treated effectively and efficiently [607].

Criterion (d) – availability of other antimicrobial treatments for humans

Mupirocin is of essential importance for patient management in hospitals for eradication of MRSA carriage. For topical treatment of SSTIs, fusidic acid is an alternative [608]. A further alternative is retapamulin ointment for topical treatment of cutaneous bacterial infections, particularly those caused by *S. aureus*; retapamulin is, however, not indicated for treatment of MRSA infections [609, 610].

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

- Mupirocin is highly important in human medicine, used for MRSA-decolonisation prior to surgical and other critical interventions to prevent serious MRSA infections. Although limited systemic antibiotic alternatives are available for treatment of clinical MRSA infections in humans, mupirocin is the cornerstone of decolonisation therapy for MRSA.
- In veterinary medicine mupirocin is used only outside the terms of the marketing authorisation in companion animals for topical treatment of serious skin infections caused by methicillin-resistant staphylococci. Susceptibility testing may show that the only effective alternatives are systemically administered antibiotics of higher importance in human medicine and mostly prohibited from use in animals [2].
- Prevalence of resistance to mupirocin in isolates from companion animals is unknown; although likely to be low at present. High level resistance in staphylococci is plasmid-borne and can spread clonally and horizontally, even between different staphylococcal species and MRSA. hence there is evidence for potential transfer of resistance to mupirocin from companion animals to humans and other animals which could become significant if use of mupirocin became well established.
- In the absence of MRLs, pseudomonic acids cannot be used in food-producing animals in the EU. The extent of use of mupirocin in companion animals is unknown; although reports relate to topical use only.

Therefore, considering the points above relevant to criteria (b), (c) and (d), it should be considered if conditions or a prohibition should be placed on the use of Pseudomonic acids outside the terms of the marketing authorisation.

Step 2. Considerations of conditions to be placed on use outside the terms of a marketing authorisation

Please refer to Section 3.1.2. of the main report for the general rationale behind the proposed conditions.

In the absence of authorised VMPs containing Pseudomonic acids, (i) and (ii) are not addressed.

(iii) Administration by an unauthorised route or use of extemporaneous formulation

Due to high protein binding and rapid elimination of mupirocin [611], authorised human medicines are currently available for topical use only.

Condition:

- For topical administration only

Rationale: See Section 3.1.2(iii) of this advice.

(iv) Use of a human medicinal product

Mupirocin is authorised in HMPs in the EU for topical use and is available as creams, ointments, nasal ointments.

Mupirocin-containing human medicinal products are authorised for nasal decolonisation of staphylococci, including MRSA, and for treatment of primary bacterial skin infections and secondarily infected traumatic lesions such as small lacerations, sutured wounds or abrasions, due to susceptible strains of *Staphylococcus aureus* and *Streptococcus pyogenes*.

Conditions:

- Use must be based on target pathogen identification and antimicrobial susceptibility testing that demonstrates that Pseudomonic acids are likely to be effective and that antimicrobials from a lower AMEG category would not be effective, unless it can be justified that this is not possible. See *'Special note on the use of AST for pathogens treated topically or locally'* in Annex 1.
- To be used only for treatment of MRSA and MRSP infections. Authorised topical treatments for staphylococcal infections should not have been effective.
- Not to be used for routine decolonisation of MRSA/P.
- To be used in individual animals only.

Rationale: See Section 3.2.1(iv) of this advice.

(v) Use of a third country veterinary medicinal product

An ointment containing mupirocin is authorised by FDA for the topical treatment of canine bacterial infections of the skin, including superficial pyoderma, caused by susceptible strains of *Staphylococcus aureus* and *Staphylococcus intermedius*. In North America, mupirocin has also been described for the treatment of feline acne and localized lesions of equine pyoderma.

According to Articles 112(2), 113(2) and 114(4), third country VMPs may only be used in the same species and for the same indication.

Conditions under (iv) are also applicable. No further conditions are proposed.

Rationale: See Section 3.2.1(v) of this advice.

Step 3. Consideration of Criteria (a) and (e) in view of proposed conditions to be placed on use outside the terms of a marketing authorisation

Criterion (a) – risk to animal health or public health if the antimicrobial is used in accordance with Articles 112, 113 and 114

Mupirocin appears to be well tolerated in animals, with contact reactions occurring rarely [119]. Administration is likely to be to individual animals. Sensitisation and local irritation may occur. Attention should be paid to special warnings and precautions for use included in the SPC for human medicines.

Criterion (e) Impact on aquaculture and farming if the animal affected by the condition receives no treatment

No MRLs exist for pseudomonic acids. The class can be used in non-food-producing equines in accordance with Article 112; the proposed conditions do not prevent treatment of such animals.

Step 4. Final conclusion - recommendations made for conditions to be placed on use outside the terms of a marketing authorisation

Based on the discussion above, the following conditions are proposed:

- Use must be based on target pathogen identification and antimicrobial susceptibility testing that demonstrates that Pseudomonic acids are likely to be effective and that antimicrobials from a lower AMEG category would not be effective, unless it can be justified that this is not possible. See *'Special note on the use of AST for pathogens treated topically or locally'* in Annex 1.
- To be used only for treatment of MRSA and MRSP infections. Authorised topical treatments for staphylococcal infections should not have been effective.
- Not to be used for routine decolonisation of MRSA/P.
- To be used in individual animals only.
- For topical administration only.

4.28. Steroid antibacterials

4.28.1. Background information

Examples of substances in the class that are authorised in veterinary and human medicine in the EU

Examples of substances authorised for veterinary use	**Examples of ATCvet codes**
Fusidic acid	QS01AA13
Examples of substances authorised for human use	**Examples of ATC codes**
Fusidic acid	J01XC01

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

Steroid antibacterials are not included in Table 1 (allowed substances) of the Annex to the MRL Regulation (EU) 37/2010 and **cannot be used in food-producing animals in the EU**.

EU-authorised VMP formulations, based on sales reported to ESVAC

Species		**Route of administration**					
		Group		**Individual**			
		In-feed	**In-water**	**Injection**	**Oral e.g. tablet, paste, powder**	**Topical/local (incl. intrauterine)**	**Intra-mammary**
Major	**Cattle**						
	Sheep (for meat)						
	Pigs						
	Chickens						
	Dogs					FA	
	Cats					FA	
Limited market species As listed in SPCs	**Rabbits**					FA	

FA: Fusidic acid

Summary of main indications and contra-indications for EU-authorised VMPs, based on selected SPCs

Main indications	Fusidic acid is available in topical products (combined with corticosteroids) for treatment of localised acute moist (pyotraumatic) dermatitis and intertrigo and for bacterial otitis externa, in particular caused by staphylococcal infections (including MRSA), in cats and dogs. It is also available as a topical ophthalmic product for treatment of bacterial conjunctivitis in dogs, cats and rabbits.
Contraindications	Do not use for conjunctivitis due to *Pseudomonas aeruginosa*.

Examples of EU-authorised HMP formulations, from Article 57 database

Substance	**Route of administration**		
	Injection	**Oral e.g. tablet, liquid**	**Topical/local**
Fusidic acid	x	x	x

Existing recommendations

WOAH recommendations

Fusidic acid is categorised VIA by WOAH (formerly OIE). *Specific comments*: Fusidic acid is used in the treatment of ophthalmic diseases in cattle and horses.

WHO classifications

WHO: HIA

- (C1: No) In certain geographic settings, Criterion 1 may be met: the class may be one of limited therapies for infections with MRSA.
- (C2: Yes) May result from transmission of MRSA from non-human sources.

WHO AWaRe: Watch: Fusidic acid

AMEG and CVMP recommendations

Steroid antibacterials are included in the AMEG Category D. There are alternative treatments in human and veterinary medicine for their indications and that do not select for resistance to Category A substances through specific multiresistance genes.

These antibiotics are not devoid of negative impact on resistance development and spread. To keep the risk from use of these antibiotic classes as low as possible it is important that responsible use principles are complied with in everyday practice. Unnecessary use and unnecessarily long treatment periods should be avoided and group treatment restricted to situations where individual treatment is not feasible.

Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Disclaimer: The information in this section reflects reported use of antimicrobials outside the terms of a marketing authorisation. No evaluation is made in this section by the working group on the efficacy or safety of the reported uses, or on their potential impact on development and dissemination of AMR.

Information from published sources

In addition to treatment of surface pyoderma, fusidic acid may also be used for targeted topical therapy for localised skin infections including superficial pyoderma and decubital (pressure-point) pyoderma [103]. Alternatives include mupirocin and antibacterials e.g. benzoyl peroxide, chlorhexidine [612].

Information from the open call for data on use of antimicrobials in animals

The information below is summarised from the open call for data. Inclusion in the table does not endorse use or imply that it is consistent with use according to legislative provisions in Articles 112 to 114.

Substance	Species	Indication	Alternatives	Consequences of unavailability
Fusidic acid	Horse	Local topical treatment of infections in skin and wounds		
Fusidic acid	Various species including horses, guinea pigs, reptiles, birds	Eye infections		
Fusidic acid (human formulation – eye drops)	Horse	Bacterial eye infections	Chloramphenicol	
Fusidic acid – tablets	Dogs	Osteomyelitis		

4.28.2. Evaluation

Scope of permitted use according to the MRL Regulation

Steroid antibacterials are not included in Table 1 (allowed substances) of the Annex to Regulation (EU) 37/2010 and hence cannot be used in food-producing species in accordance with Articles 113 and 114 of Regulation (EU) 2019/6.

Steroid antibacterials can be used in non-food-producing animals in accordance with Article 112.

Examples of veterinary-authorised formulations/species

Steroid antibacterials are available only in topical formulations, as ear drops and topical gel for treatment of dogs and cats and as eye drops for dogs, cats and rabbits.

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Importance for human health

Fusidic acid has a narrow spectrum, bacteriostatic activity. It inhibits protein synthesis by interfering with ribosome translation. Fusidic acid is active against Gram-positive cocci and bacilli such as *Staphylococcus aureus* (including MRSA), most coagulase-positive staphylococci, beta-haemolytic streptococci, *Corynebacterium* spp., and most *Clostridioides* spp. Fusidic acid has only limited activity against Gram-negative bacteria [613].

Fusidic acid is mainly used for combination therapy in humans (systemic treatment) of staphylococcal infections or topically for treatment of skin or eye infections. Although effective, it is not recommended for initial monotherapy of severe staphylococcal infections owing to its bacteriostatic activity and the high risk of development of resistance. There are several alternative treatment options including penicillinase-resistant penicillins. Staphylococcal infections, especially *S. aureus*, cause a wide range of diseases – from minor skin infections to life-threatening sepsis. Fusidic acid may also be used topically for decolonisation of MSSA and MRSA carriers, as an alternative to mupirocin.

Fusidic acid is approved nationally in several EU countries for systemic use. The approved indications are the treatment of primary and secondary skin infections caused by sensitive strains of *Staphylococcus aureus, Streptococcus* spp. and *Corynebacterium minutissimum*. Primary skin infections that may be expected to respond to treatment with fusidic acid applied topically include impetigo contagiosa, superficial folliculitis, sycosis barbae, paronychia and erythrasma; also such secondary skin infections as infected eczematoid dermatitis, infected contact dermatitis and infected cuts/abrasions.

Importance for animal health

Fusidic acid (combined with a corticosteroid) is authorised in the EU for use in cats and dogs as a topical gel for treatment of localised acute moist (pyotraumatic) dermatitis and intertrigo and as ear drops for bacterial otitis externa, in particular caused by staphylococci. It is also authorised as a topical ophthalmic product for treatment of bacterial conjunctivitis in dogs, cats and rabbits. Fusidic acid generally shows good activity against MRSA/P and may be used as an alternative to CIAs for the treatment of these infections when topical treatment is appropriate [334].

According to guidelines, fusidic acid is also used (outside the terms of the marketing authorisation) for targeted topical therapy of localised skin infections including superficial pyoderma and decubital

(pressure-point) pyoderma in dogs [103]. There were reports to the Open call for data of use of fusidic acid in unauthorised species including horses, small pets and reptiles, and use of human tablet formulation to treat osteomyelitis in dogs.

Development, selection and transmission of resistance

Fusidic acid inhibits bacterial protein synthesis by binding to elongation factor G (EF-G) on the ribosome, preventing elongation of the peptide chain. There are several mechanisms of resistance to fusidic acid. In staphylococci, high-level resistance is due to mutation in the *fus*A gene that encodes EF-G; this resistance emerges during therapy. Low-level resistance is generally caused by plasmid-borne *fus*B and *fus*C that encode an inducible protein that protects the ribosomal target. *Fus*B and the homologue *fus*C are the most common fusidic acid resistance determinants in MRSA in Europe. Development of resistance has been associated with increased use of fusidic acid especially as a topical monotherapy and epidemic clones associated with chromosomal *fus*B have emerged in European *S. aureus* strains causing impetigo. Other resistance mechanisms, found in Enterobacterales, include binding by chloramphenicol acetyl transferase type I and overexpression of endogenous efflux pumps [285, 614, 615].

A meta-analysis of studies published from 2000 to 2020 showed an overall prevalence of fusidic acid (FA) resistance in human clinical strains of *S. aureus* from Europe of 4.7% (95% CI 4.3 – 5.2%), with the prevalence of FA-resistance in MRSA being 1.9%. The incidence of FA-resistant *S. aureus* showed an increasing trend over the period of the study [616].

The FA resistance genes *fus*A, *fus*B and *fus*C have been found in canine *S. aureus* and *S. pseudintermedius* from Finland, UK and Germany, although resistance appears to occur at low prevalence. The clinical significance of this resistance is unclear as topical applications may achieve high concentrations at the infection site [334, 612, 617, 618]. A study on canine *S. pseudintermedius* isolates in Korea reported a higher prevalence of resistance (27% of 52 isolates from dogs with chronic or recurrent infections) [619].

Zoonotic and reverse zoonotic transmission of staphylococci (including MRSA and MRSP) between pet dogs and owners has been reported to occur infrequently and noted as a public health concern [62, 620-622]. Likewise, transmission of staphylococci including MRSP also occurs between pets in households and veterinary clinics [61, 623, 624].

In conclusion,

- In human medicine fusidic acid is mostly used as topical formulations for treatment of skin infections caused by *S. aureus* (including MRSA) and other Gram-positive bacteria and for decolonisation of MSSA and MRSA carriers. It has more limited use for systemic treatment of staphylococcal infections but is not recommended as a monotherapy.
- In veterinary medicine fusidic acid is an important topical therapy in companion animals for eye, ear and localised skin infections, especially those caused by staphylococci.
- Resistance to fusidic acid has increased in Europe in the last 20 years, associated with its increased use. Epidemic clones associated with chromosomal *fus*B have emerged in European *S. aureus* strains causing impetigo in humans. The same *fus* resistance genes have been identified in *S. aureus* and *S. pseudintermedius* isolates from dogs in the EU. *Staphylococcus* spp., including MRSA/P, can be transmitted between humans and animals.

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to Steroid antibacterials.

Criterion (c) – availability of other treatments for animals

For surface and other focal pyodermas, alternative topical treatments could include antiseptics e.g. chlorhexidine, benzoyl peroxide; however, where localised topical antibiotic treatment is required, fusidic acid is preferable to mupirocin (AMEG Category A) due to the importance of the latter for MRSA decolonisation in humans. For MRSA/P skin infections in companion animals, if topical treatment is likely to be effective, fusidic acid could also be used instead of resort to systemic administration of antibiotics potentially from a higher AMEG category.

Alternatives are available for treatment of otitis externa involving Gram-positive cocci [96], but are from a higher AMEG category e.g. aminoglycosides (Category C) and fluoroquinolones (Category B). Likewise, for ocular infections, alternatives could include cloxacillin, aminoglycosides or chloramphenicol.

Criterion (d) – availability of other antimicrobial treatments for humans

Fusidic acid was not considered to fulfil criterion A for the Article 37(5) List as other treatment alternatives exist to treat staphylococcal infections in humans, including penicillinase-resistant penicillins.

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

- In human medicine, fusidic acid is mostly used as topical formulations for treatment of skin infections caused by *S. aureus* (including MRSA) and decolonisation of MSSA/MRSA carriers and has more limited use for systemic treatment of staphylococcal infections. Its usefulness is limited by its bacteriostatic activity and the high risk of development of resistance during treatment. Several alternative treatment options are available.
- In veterinary medicine, fusidic acid is an important topical therapy in companion animals for eye, ear infections, pyotraumatic dermatitis and intertrigo caused by Gram-positive bacteria, especially staphylococci. It is also used outside the marketing authorisation for treatment of other focal or localised pyodermas and is used in unauthorised companion animal species. Fusidic acid is also recommended for treatment of MRSA/P where topical treatment is indicated.
- Resistance to fusidic acid has increased in Europe in the last 20 years, associated with its increased use. Epidemic clones associated with chromosomal *fus*B have emerged in European *S. aureus* strains causing impetigo in humans. The same *fus* resistance genes have been identified in *S. aureus* and *S. pseudintermedius* isolates from humans and dogs in the EU and since these bacteria (including MRSA/P) can be transmitted between humans and animals there is a pathway for transmission of resistance.
- There is a risk to animal and public health due to development of resistance to steroid antibacterials. However, steroid antibacterials are in the AMEG Category D - alternatives are available for the indications for fusidic acid in companion animals but are likely to be from a higher AMEG category.
- In addition, although the extent of use of fusidic acid in animals is unknown, it can only be used in non-food-producing animals and is mainly administered by the topical route of administration.

Therefore, considering the points above relevant to criteria (b), (c) and (d), it is recommended that no conditions should be placed on the use of Steroid antibacterials outside the terms of the marketing authorisation, although responsible antimicrobial use principles should be applied.

4.29. Bicyclomycin (bicozamycin)

4.29.1. Background information

ATC codes: None found

ATCvet codes: None found

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

Bicyclomycin is not included in Table 1 (allowed substances) of the Annex to the MRL Regulation (EU) 37/2010 and **cannot be used in food-producing animals in the EU**.

Bicyclomycin can be used in non-food-producing species in accordance with Article 112.

Existing recommendations

WOAH recommendations

Bicyclomycin is categorised VIA by WOAH (formerly OIE). *Specific comments*: Bicyclomycin is listed for digestive and respiratory diseases in cattle and septicaemias in fish.

WHO classifications

Not included in the WHO classifications.

AMEG recommendations

Bicyclomycin / Bicozamycin is not included in the AMEG categorisation.

Authorisation in Third countries

Identification of authorised products outside the EU is based on web searches and may not be exhaustive. Veterinary products can be identified, but it is difficult to determine the current authorisation status of these products.

A product was identified containing bicozamycin, intended for treatment of *Photobacterium damsela* sp*. piscicida* and Gram-negative bacteria [625].

Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Information from published sources

None found.

Information from the open call for data on use of antimicrobials in animals

No information on use outside the terms of a marketing authorisation was provided in the open call for data.

4.29.2. Evaluation

Scope of permitted use according to the MRL Regulation

Bicyclomycin is not included in Table 1 (allowed substances) of the Annex to Regulation (EU) 37/2010 and hence cannot be used in food-producing species in accordance with Articles 113 and 114 of Regulation (EU) 2019/6.

Bicyclomycin can be used in non-food-producing species in accordance with Article 112.

Examples of veterinary-authorised formulations/species

None identified.

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Bicyclomycin has activity against Gram-negative bacteria including *E. coli* by inhibiting the *rho* transcription termination factor [626]. It has little activity against Gram-positive bacteria (except *Micrococcus luteus*), anaerobes, *Proteus* or *Pseudomonas* spp. Resistance in *E. coli* has been identified due to mutations in or near *rho* and *rpoB* loci [627], but cross-resistance is not expected due to the mode of action [628]. In vitro studies have shown that bicyclomycin exhibits lethal synergy when combined with bacteriostatic concentrations of protein synthesis inhibitors (tetracyclines, chloramphenicol, rifampicin) [629]. It has low oral bioavailability and has been used (historically) for treatment of acute and traveller's diarrhoea associated with enteric infections in humans and for enteric infections livestock [628, 630].

No evidence could be found that bicyclomycin is authorised as a human or veterinary medicine in the EU.

Although WOAH has listed bicyclomycin for digestive and respiratory diseases in cattle and septicaemias in fish, and one product was identified for treatment of *Photobacterium damselae* subsp. *piscicida* and Gram-negative bacteria in fish, no evidence could be found for use of bicyclomycin in animals in the EU.

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to Bicyclomycin.

Criterion (c) – availability of other treatments for animals

Bicyclomycins cannot be used in food-producing species in the EU and no potential uses were identified for use in non-food-producing species in the EU.

Criterion (d) – availability of other antimicrobial treatments for humans

No clinical uses were identified for bicyclomycins in human medicine the EU.

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

No antimicrobials from this class are authorised in human or veterinary medicines in the EU and no evidence was found for the use of bicyclomycins in humans or animals in the region. In the absence of MRL status, VMPs from third countries could only be used in non-food-producing species in the EU and no such products could be identified. The AMR risk to animal and public health is low.

Therefore, considering the points above relevant to criteria (b), (c) and (d), it is recommended that no conditions should be placed on the use of Bicyclomycin outside the terms of the marketing authorisation, although responsible antimicrobial use principles should be applied.

4.30. Orthosomycins/oligosaccharides

4.30.1. Background information

ATC codes: None found

ATCvet codes: Avilamycin (QA07AA95)

ATC codes were not found for other substances in the class: e.g. evernimicin, flambamycin, hygromycin

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

Avilamycin is included in Table 1 (allowed substances) of the Annex to Regulation (EU) 37/2010 and can be used accordingly in food-producing species in compliance with Articles 113 and 114 of Regulation (EU) 2019/6. 'Other provisions' state that Avilamycin cannot be used in animals from which eggs are produced for human consumption.

Avilamycin can be used in non-food-producing animals in accordance with Article 112.

Existing recommendations

WOAH recommendations

Orthosomycins are categorised VIA by WOAH (formerly OIE). Avilamycin is used for enteric diseases of poultry, swine and rabbit. This class is currently only used in animals.

WHO classifications

Included in Annex 2: Antimicrobial classes not used in humans

AMEG recommendations

Orthosomycins are not included in the AMEG categorisation.

Authorisation in Third countries

Avilamycin is authorised for use as a veterinary medicine in chickens, turkeys, pigs and rabbits to control bacterial enteric infections

In the US, it is authorised as a premix in Swine for the reduction in incidence and overall severity of diarrhea in the presence of pathogenic *Escherichia coli* in groups of weaned pigs [631].

In Canada, it is authorised for the prevention of necrotic enteritis due to *Clostridium perfringens* in growing broiler chickens and in pigs for the reduction in incidence and severity of post-weaning diarrhoea associated with *Escherichia coli* in pigs [632]. Avilamycin is to be used in pigs that are at risk of developing, but not yet showing clinical signs of, diarrhoea in the presence of pathogenic *E. coli*.

In Australia**,** an avilamycin premix is used to increase weight gain and improve feed efficiency in broiler chickens [633].

Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Information from published sources

None found.

Information from the open call for data on use of antimicrobials in animals

No information on use outside the terms of a marketing authorisation was provided in the open call for data.

4.30.2. Evaluation

Scope of permitted use according to the MRL Regulation

Avilamycin is included in Table 1 (allowed substances) of the Annex to Regulation (EU) 37/2010 and can be used accordingly in food-producing species in compliance with Articles 113 and 114 of Regulation (EU) 2019/6. 'Other provisions' state that Avilamycin cannot be used in animals from which eggs are produced for human consumption

Avilamycin can be used in non-food-producing animals in accordance with Article 112.

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Orthosomycins are active against Gram-positive bacteria including enterococci, staphylococci, and streptococci, inhibiting protein synthesis by binding to the 50S ribosomal subunit. Evernimicin was investigated in human medicine as a treatment for Gram-positive infections including penicillin-resistant pneumococci but development was suspended due to poor efficacy [630]. In vitro, evernimicin has shown higher activity than vancomycin against Gram-positive cocci, including MRSA [634, 635].

Orthosomycins are not authorised as human medicines in the EU.

Avilamycin was banned as a growth promoter (AGP) in the EU in 2006; however, it is included in the Annex to Regulation (EU) 37/2010 where it has MRLs for pigs, rabbits and poultry. MRLs have also been recommended by JECFA. The intended veterinary medicinal use in the EU is treatment of bacterial enteric infections, but no current EU marketing authorisation can be found. Outside the EU, avilamycin is authorised for reduction of diarrhoea due to *E. coli* in weaned pigs and for prevention of necrotic enteritis (*Clostridium perfringens*) in chickens, and is used as growth promoter in poultry, rabbits and pigs [636]. According to Article 113(2), products from third countries can only be used for the same animal species and same indication. Under Article 107, the Regulation includes stringent provisions in relation to the use of antimicrobials as growth promoters or for prophylaxis and additionally administration of antimicrobial VMPs in medicated feed as prophylaxis is prohibited according to the Medicated Feed Regulation (EU) 2019/4 (Article 17(3)).

No evidence was identified in the literature or the open call for data relating to use of Orthosomycins in animals in the EU.

Resistance is mediated by methyltransferase enzymes that modify rRNA [637] an ABC transporter and variations in ribosomal protein L16. According to Arenz et al. [638] the binding site and mode of action of orthosomycins are distinct from other ribosome-targeting antibiotics and they do not display cross-resistance with other classes, suggesting possible scope for development of new agents.

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to Orthosomycins.

Criterion (c) – availability of other treatments for animals

In pigs, vaccinations (sows or piglets) can be an effective way to reduce the occurrence of neonatal and post-weaning diarrhoea caused by *E. coli*; however, it is necessary to use the appropriate vaccine

for the most prevalent ETEC pathotype on the farm and to ensure that the vaccine is administered at the optimal time, consequently vaccination may not be consistently effective. Other measures can be introduced to reduce the need for antibiotics to treat infections such as ETEC (e.g. later weaning, improved genetics, changes in nutrition, improved housing and biosecurity) (De Busser et al., 2013; EIP-AGRI, 2014; Rhouma et al., 2017). Alternative antibiotics are authorised in the EU for treatment and metaphylaxis of post-weaning diarrhoea caused by *E. coli* e.g. aminoglycosides, aminopenicillins; however, in case of resistance to these classes, AMEG Category B substances may be the only option.

Necrotic enteritis in poultry is associated with predisposing factors including coccidiosis, poor feed quality and immunosuppressive diseases, hence prevention is focused on the reduction of these factors e.g. use of coccidiostats, improvement in nutrition and use of vaccination [36]. In the EU, alternative antibiotics for the treatment of necrotic enteritis include penicillin, lincomycin and macrolides.

Criterion (d) – availability of other antimicrobial treatments for humans

No uses were identified for orthosomycins in human medicine the EU.

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

- Orthosomycins are not authorised in human medicinal products and no clinical uses were identified in human medicine the EU.
- Although there are MRLs for avilamycin in the EU, there are no authorised veterinary medicines containing this substance. Alternative antibiotic VMPs are available for the indications of veterinary medicines containing avilamycins that are authorised in third countries, and in the case of antibiotics for treatment of post-weaning *E. coli* infections, these are of higher importance to animal and public health. No evidence was found for use of orthosomycins in any animal species in the EU.
- There is little evidence relating to resistance to orthosomycins in animal isolates and the extent of use outside the terms of the marketing authorisation is expected to be very limited, hence the AMR risk to animal and public health is considered very low.

Therefore, considering the points above relevant to criteria (b), (c) and (d), it is recommended that no conditions should be placed on the use of Orthosomycins outside the terms of the marketing authorisation, although responsible antimicrobial use principles should be applied.

4.31. Quinoxalines

4.31.1. Background information

ATC codes: None found

ATCvet codes: olaquindox QJ01MQ01

Other substances in the class are carbadox, quinocetol, mequindox, quinocetone, cyadox

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

No quinoxalines are included in the Annex to the MRL Regulation (EU) 37/2010 and they cannot be used in food-producing animals in the EU.

Quinoxalines can be used in non-food-producing animals in accordance with Article 112.

Existing recommendations

WOAH recommendations

Quinoxalines are categorised VIA by WOAH (formerly OIE). *Specific comments:* Quinoxalines (carbadox) is used for digestive disease of pigs (e.g. swine dysentery). This class is currently only used in animals.

WHO classifications

Included in Annex 2: Antimicrobial classes not used in humans

AMEG recommendations

Quinoxalines are not included in the AMEG categorisation.

Use of carbadox as a growth promotor was stopped in the EU in 1999 (Commission Regulation (EC) No 2788/98), due to its carcinogenic properties and teratogenic effects.

Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Information from published sources

No evidence could be found for use of quinoxalines in animals in the EU.

Information from the open call for data on use of antimicrobials in animals

No information on use outside the terms of a marketing authorisation was provided in the open call for data.

Authorisation in Third countries

Carbadox is authorised in the US as growth promotor for pigs and for therapeutic purposes to control swine dysentery and bacterial swine enteritis [639].

4.31.2. Evaluation

Scope of permitted use according to the MRL Regulation

Quinoxalines are not included in Table 1 (allowed substances) of the Annex to Regulation (EU) 37/2010 and hence cannot be used in food-producing species in accordance with Articles 113 and 114 of Regulation (EU) 2019/6.

Quinoxalines can be used in non-food-producing animals in accordance with Article 112.

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

The mode of action of quinoxalines is not fully understood although they demonstrate bioreductive effects [640]. They are active against Gram-positive, Gram-negative bacteria, including *E. coli* and *Salmonella* spp., and anaerobes.

No evidence could be found for current use of quinoxalines in human medicine.

Quinoxalines have been shown to be genotoxic carcinogens [641] and carbadox has been banned from use in food-producing animals in Canada, Australia and the EU. In the US it is used as an AGP in pigs and as a veterinary medicine for control of swine dysentery and bacterial swine enteritis [642].

No VMP authorised outside the EU has been identified for use in companion animals.

No evidence was identified in the literature or the open call for data relating to use of quinoxalines in non-food-producing animals in the EU.

Both chromosomal and plasmid-borne resistance to carbadox has been identified in Enterobacterales isolates from pigs. In addition to quinoxalines, the OqxAB efflux pump conveys resistance to multiple antimicrobials including those of importance to animal and public health [643, 644].

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to Quinoxalines.

Criterion (c) – availability of other treatments for animals

Quinoxalines cannot be used in food-producing animals in the EU and no potential uses were identified for use in non-food-producing animals in the EU.

Criterion (d) – availability of other antimicrobial treatments for humans

No uses were identified for quinoxalines in human medicine the EU.

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

No antimicrobials from this class are authorised in human or veterinary medicines in the EU and no evidence was found for the use of quinoxalines in humans or animals in the region. In the absence of MRL status, VMPs from third countries could only be used in non-food-producing animals in the EU and no such products could be identified. The AMR risk to animal and public health is low.

Therefore, considering the points above relevant to criteria (b), (c) and (d), it is recommended that no conditions should be placed on the use of Quinoxalines outside the terms of the marketing authorisation, although responsible antimicrobial use principles should be applied.

4.32. Thiopeptides

4.32.1. Background information

Substances in the class: thiostrepton, cyclothiazomycin, nosiheptide, lactocillin

Examples of substances in the class that are authorised in veterinary and human medicine in the EU

Examples of substances authorised for veterinary use	**Examples of ATCvet codes**
thiostrepton	None found
Examples of substances authorised for human use	**Examples of ATC codes**
None found	None found

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

No thiopeptides are included in Table 1 (allowed substances) of the Annex to the MRL Regulation (EU) 37/2010 and they cannot be used in food-producing animals in the EU.

EU-authorised VMP formulations, based on sales reported to ESVAC

Species		**Route of administration**					
		Group		**Individual**			
		In-feed	**In-water**	**Injection**	**Oral e.g. tablet, paste, powder**	**Topical/local (incl. intrauterine)**	**Intra-mammary**
Major	**Cattle**						
	Sheep (for meat)						
	Pigs						
	Chickens						
	Dogs					Thiostrepton	
	Cats					Thiostrepton	
Limited market species							

Summary of main indications and contra-indications for EU-authorised VMPs, based on selected SPCs

Main indications	Thiostrepton in combination with neomycin and nystatin is authorised for treatment of mixed bacterial and fungal otitis externa in dogs and cats.
Contraindications	Do not use in cases of perforated tympanum.

Examples of EU-authorised HMP formulations, from Article 57 database

None found.

Existing recommendations

WOAH recommendations

Thiopeptides are categorised VIA by WOAH (formerly OIE). *Specific comments:* This class is currently used in the treatment of some dermatological conditions.

WHO classifications

Included in Annex 2: Antimicrobial classes not used in humans

AMEG recommendations

Thiopeptides are not included in the AMEG categorisation.

Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Information from published sources

No evidence could be found for use of thiopeptides outside the terms of the marketing authorisation in animals in the EU.

Information from the open call for data on use of antimicrobials in animals

No information on use outside the terms of a marketing authorisation was provided in the open call for data.

4.32.2. Evaluation

Scope of permitted use according to the MRL Regulation

No Thiopeptides are included in the Annex to the MRL Regulation (EU) 37/2010 and therefore they cannot be used in food-producing animals in the EU.

Thiopeptides can be used in non-food-producing animals in accordance with Article 112.

Examples of veterinary-authorised formulations/species

Thiostrepton has limited availability, but one topical combination product was found for treatment of otitis externa in dogs and cats [645].

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

The thiopeptides class contains several substances such as thiostrepton, cyclothiazomycin, nosiheptide, lactocillin. Thiopeptides possess a common mode of action, inhibiting bacterial protein synthesis by binding to the 23S rRNA and the N-terminal domain of ribosomal protein uL11 in Gram-positive bacteria.

Thiostrepton is a cyclic peptide, produced by *Streptomyces aureus*, that is active predominantly against Gram-positive bacteria [646]. Thiopeptides have been investigated in in vitro studies for effectiveness against MRSA, methicillin resistant *Enterococcus faecium*, VRE, penicillin-resistant *Streptococcus pneumoniae* and mycobacteria, and in a human clinical study for treatment of *Clostridioides difficile*. Thiopeptides also have antimalarial, antifungal and anticancer properties [647-650].However, thiopeptides are not approved in the EU as a human medicine and do not appear to have clinical use in humans.

In dogs and cats, thiostrepton is available in a combination product for topical treatment of otitis externa in dogs and cats, where it is used for its activity against Gram-positive bacteria in mixed infections.

Outside the EU, ointments are approved for local therapy of infectious kerato-conjunctivitis (pinkeye). A similar ointment is also approved for the treatment of otitis, cysts, and anal gland infections in cats and dogs.

A search in Google identified premix products containing nosiheptide for use as a growth promotor in pigs, poultry and livestock in China.

No evidence was identified in the literature or the open call for data relating to use of thiopeptides in animals in the EU.

There is no evidence for the selection and potential transmission of resistance to thiostrepton from animals to humans and other animals.

In conclusion,

- Thiostrepton is authorised for local treatment in cats and dogs and can only be used in non-food-producing species in the EU. No evidence was found relating to use outside the terms of a marketing authorisation.
- Very little information is available on resistance mechanisms and occurrence of resistance to thiopeptides.

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to Thiopeptides.

Criterion (c) – availability of other treatments for animals

Considering the non-specific indication, various alternative VMPs are available for topical treatment of bacterial otitis externa in companion animals.

Criterion (d) – availability of other antimicrobial treatments for humans

No clinical uses were identified for thiopeptides in human medicine the EU.

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

- Thiopeptides are not authorised in human medicinal products and no clinical uses were identified for thiopeptides in human medicine the EU.
- Thiopeptides (thiostrepton) are authorised in cats and dogs for the topical treatment of otitis externa.
- In the absence of MRL status, thiopeptides cannot be used in food- producing animals in the EU and no evidence was found relating to use outside the terms of a marketing authorisation in non-food-producing species.
- Little information is available on resistance to thiopeptides; however, considering the low importance to human and animal health and limited extent of use of thiopeptides in animals, the AMR risk to animal and public health is considered to be low.

Therefore, considering the points above relevant to criteria (b), (c) and (d), it is recommended that no conditions should be placed on the use of Thiopeptides outside the

terms of the marketing authorisation, although responsible antimicrobial use principles should be applied.

4.33. Phosphoglycolipids / moenomycins

4.33.1. Background information

ATC codes: None found

ATCvet codes: Bambermycin (QA07AA96)

Examples of substances in the class that are authorised in veterinary and human medicine in the EU

None found.

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

Substance	MRL tissues	MRL milk	MRL eggs	Other provisions
Bambermycin	Rabbit – No MRL required	-	-	For oral use only
	Poultry	-	-	Not for use in animals from which eggs are produced for human consumption

Authorisation of veterinary medicines in Third countries

Identification of authorised products outside the EU is based on web searches and may not be exhaustive.

No veterinary medicines containing moenomycins have been identified as used for disease prevention or treatment.

Bambermycin products have been identified in US and other countries for use as growth promotor in poultry, swine and cattle.

Existing recommendations

WOAH recommendations

Phosphoglycolipids / moenomycins are not classified by WOAH (formerly OIE).

WHO classifications

Included in Annex 2 of the WHO CIA list Antimicrobial classes not currently used in humans

AMEG recommendations

Phosphoglycolipids/moenomycins are not included in the AMEG categorisation.

Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Information from published sources

No information found.

Information from the open call for data on use of antimicrobials in animals

No information on use outside the terms of a marketing authorisation was provided in the open call for data.

4.33.2. Evaluation

Scope of permitted use according to the MRL Regulation

Bambermycin is included in Table 1 (allowed substances) of the Annex to Regulation (EU) 37/2010 and can be used accordingly in food-producing species in compliance with Articles 113 and 114 of Regulation (EU) 2019/6. 'Other provisions' state that Bambermycin can only be used by the oral route in rabbits and should not be used in animals from which eggs are produced for human consumption.

Bambermycins can be used in non-food-producing animals in accordance with Article 112.

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Moenomycins are phosphoglycolipid antibiotics. They have a distinct mode of action, competing as substrates for peptidoglycan glycosyltransferase enzymes involved with bacterial cell wall formation. They are mostly active against Gram-positive bacteria, including methicillin- and vancomycin-resistant cocci, and have activity against some Gram-negative bacteria. Intrinsic resistance to bambermycin is reported for Gram-negative bacilli (e.g., *Pseudomonas* spp. and Enterobacterales) and *Campylobacter* spp. Bacteria of the *Enterococcus gallinarum* group (*E. gallinarum* and *E. casseliflavus*), and most species from the *E. faecium* group (*E. faecium*, *E. mundtii*, and *E. hirae*) show natural resistance to bambermycin.

Moenomycins have not been used in human medicine due to poor pharmacokinetics - they are poorly absorbed from the gastrointestinal tract and have a very long half-life - although there is some renewed interest in their development [651].

Although no moenomycins are authorised in VMPs in the EU at present, bambermycin is included in the Annex to Regulation (EU) 37/2010 (Maximum Residue Limits), with MRL status for use in rabbits and poultry. The intended medicinal use is for treatment of enzootic rabbit enteropathy (ERE) and necrotic enteritis in poultry. Outside the EU, bambermycin/flavomycin is used as a growth promoter (AGP) in cattle, pigs, chickens and turkeys.

No evidence was identified in the literature or the open call for data relating to use of phosphoglycolipids/moenomycins in animals in the EU.

No specific mechanisms for resistance to moenomycins have been described, but mutations have been induced in *Staphylococcus aureus* in in vitro studies [652]. Interest has been paid to the potential anti-conjugative properties of flavophosphinol (bambermycin) in livestock [653, 654].

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to Phosphoglycolipids.

Criterion (c) – availability of other treatments for animals

Alternative veterinary medicines are available in the EU for the treatment of ERE in rabbits (e.g. pleuromutlins) and necrotic enteritis in poultry (e.g. penicillin, macrolides, lincosamides).

Criterion (d) – availability of other antimicrobial treatments for humans

No clinical uses were identified for moenomycins in human medicine the EU.

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

- Moenomycins are not authorised in human medicinal products and no clinical uses were identified in human medicine the EU.

- Although there are MRLs for bambermycin in the EU, there are no authorised veterinary medicines containing this substance in the EU. Alternative antibiotic VMPs are available for the proposed indications in food-producing species. No evidence was found for use of moenomycins in non-food-producing species.
- No authorised veterinary medicines containing moenomycins were identified in third countries.
- There is little evidence relating to resistance to moenomycins in animal isolates and the extent of use outside the terms of the marketing authorisation is expected to be very limited, hence the AMR risk to animal and public health is considered very low.

Therefore, considering the points above relevant to criteria (b), (c) and (d), it is recommended that no conditions should be placed on the use of Phosphoglycolipids outside the terms of the marketing authorisation, although responsible antimicrobial use principles should be applied.

4.34. Elfamycins

4.34.1. Background information

ATC codes: None found

ATCvet codes: None found

Substances in the elfamycin class include efrotomycin, kirromycin, enacycloxin, pulvomycin and aurodox.

Examples of substances in the class that are authorised in veterinary and human medicine in the EU

None found.

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

No elfamycins are included in the Annex to the MRL Regulation (EU) 37/2010 and hence they cannot be used in food-producing animals in the EU.

Existing recommendations

WOAH recommendations

Elfamycins are not classified by WOAH (formerly OIE).

WHO classifications

Not included in the WHO classifications

AMEG recommendations

Elfamycins are not included in the AMEG categorisation.

Authorisation in Third countries

Identification of authorised products outside the EU is based on web searches and may not be exhaustive.

Efrotomycin products have been identified in US for use as growth promotor in swine.

No veterinary medicinal product containing efrotomycin has been identified as used for disease treatment.

Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Information from published sources

No information found.

Information from the open call for data on use of antimicrobials in animals

No information on use outside the terms of a marketing authorisation was provided in the open call for data.

4.34.2. Evaluation

Scope of permitted use according to the MRL Regulation

Elfamycins are not included in Table 1 (allowed substances) of the Annex to Regulation (EU) 37/2010 and hence cannot be used in food-producing species in accordance with Articles 113 and 114 of Regulation (EU) 2019/6.

Elfamycins can be used in non-food-producing animals in accordance with Article 112.

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Efrotomycin is produced by *Nocardia lactamdurans*. Elfamycins act by interfering with bacterial protein synthesis, binding to the elongation factor EF-Tu. Efrotomycin is effective against a narrow spectrum of Gram-positive bacteria, *Moraxella, Pasteurella, Yersinia, Haemophilus*, *Arcanobacterium* spp. and *Clostridoides difficile* [655, 656]. *Enterococcus faecium* and closely related spp. are susceptible, whereas other enterococcal spp. including *E. faecalis* are resistant [657]. Elfamycins have been understudied due to poor pharmacokinetics, but there is some renewed interest in their potential for clinical use [658].

No current therapeutic uses were found in human or veterinary medicine.

No cross-resistance between Efrotomycin and other antibacterials were found [659].

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to Elfamycins.

Criterion (c) – availability of other treatments for animals

Elfamycins cannot be used in food-producing species in the EU and no potential uses were identified for use in non-food-producing species in the EU.

Criterion (d) – availability of other antimicrobial treatments for humans

No clinical uses were identified for elfamycins in human medicine the EU.

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

No antimicrobials from this class are authorised in human or veterinary medicines in the EU and no evidence was found for the use of elfamycins in humans or animals in the region. In the absence of MRL status, VMPs from third countries could only be used in non-food-producing species in the EU and no such products could be identified. The AMR risk to animal and public health is low.

Therefore, considering the points above relevant to criteria (b), (c) and (d), it is recommended that no conditions should be placed on the use of Elfamycins outside the terms of the marketing authorisation, although responsible antimicrobial use principles should be applied.

4.35. Aminocoumarins

4.35.1. Background information

Examples of substances in the class that are authorised in veterinary and human medicine in the EU

Examples of substances authorised for veterinary use	**Examples of ATCvet codes**
Novobiocin	None found
Examples of substances authorised for human use	**Examples of ATC codes**
None found	None found

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

Novobiocin is included in Annex I of the MRL Regulation (EU) 37/2010 and can be used in food-producing animals in the EU.

Substance	**Species**	**MRL tissues**	**MRL milk**	**MRL eggs**	**Other provisions**
Novobiocin	Bovine		Yes	-	For intramammary use only.
	Bovine	No MRL required for all tissues except milk			

EU-authorised VMP formulations, based on sales reported to ESVAC

Species		**Route of administration**					
		Group		**Individual**			
		In-feed	**In-water**	**Injection**	**Oral e.g. tablet, paste, powder**	**Topical/local (incl. intrauterine)**	**Intra-mammary**
Major	**Cattle**						Novobiocin
	Sheep (for meat)						
	Pigs						
	Chickens						
	Dogs						
	Cats						
Limited market species							

Summary of main indications and contra-indications for EU-authorised VMPs, based on selected SPCs

Main indications	Novobiocin is authorised in antimicrobial combination intramammary preparations for treatment of mastitis in lactating and dry cows.

	Specifically, it is included for its activity against beta-lactamase-producing Staphylococci.
Contraindications	

Examples of EU-authorised HMP formulations, from Article 57 database

None found.

Existing recommendations

WOAH recommendations

Aminocoumarins are categorised VIA by WOAH (formerly OIE). *Specific comments:* Novobiocin is used in the local treatment of mastitis and in septicaemias in fish. This class is currently only used in animals.

WHO classifications

Included in Annex 2: Antimicrobial classes not used in humans.

AMEG recommendations

Aminocoumarins are not included in the AMEG categorisation.

Use outside the terms of a marketing authorisation reported in literature or in the open call for data

Information from published sources

No evidence could be found for use of novobiocin outside the terms of a marketing authorisation in animals in the EU.

Information from the open call for data on use of antimicrobials in animals

No information on use outside the terms of a marketing authorisation was provided in the open call for data.

4.35.2. Evaluation

Scope of permitted use according to the MRL Regulation

Novobiocin is included in Table 1 (allowed substances) of the Annex to the MRL Regulation (EU) 37/2010 and can be used accordingly in food-producing species in compliance with Articles 113 and 114 of Regulation (EU) 2019/6. 'Other provisions' state that it is for intramammary use only.

Novobiocin can be used in non-food-producing species in accordance with Article 112 of the Regulation (EU) 2019/6.

Examples of veterinary-authorised formulations/species

Intramammary products are available in the EU to treat mastitis in lactating and dry cows.

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Novobiocin is an aminocoumarin antibiotic which inhibits bacterial DNA synthesis by targeting at the bacteria DNA gyrase and the related enzyme DNA topoisomerase IV [660].

Novobiocin is primarily active against Gram-positive microorganisms such as *Staphylococcus aureus* (including beta-lactamase-producing strains) and the pneumococci. *Enterococcus faecalis* is usually moderately resistant, but *Enterococcus faecium*, including MDR strains, is susceptible. It also has an activity against some Gram-negative bacteria (e.g., *Haemophilus influenzae*, pathogenic *Neisseria* spp.). Other Gram-negative bacilli, such as *Escherichia coli*, *Enterobacter*, *Klebsiella*, *Salmonella* and *Shigella* spp., and *Pseudomonas aeruginosa*, are resistant [660].

Novobiocin formerly had a role in the treatment of staphylococcal infections [660]. With the advent of the penicillinase-resistant penicillins and other antistaphylococcal agents, novobiocin is no longer used for this indication in humans [660].

No human medicinal products containing novobiocin are authorised in the EU.

In veterinary medicine, novobiocin is used only by the intramammary route, in combination with benzylpenicillin (and sometimes other antimicrobials) to treat bovine mastitis. In particular it is included for its activity against beta-lactamase-producing *Staphylococcus aureus*.

Novobiocin is initially active against *Staphylococcus* spp. infections (excluding *S. saprophticus*), but resistance to this antibiotic develops quickly [661, 662]. Acquired resistance to novobiocin in staphylococci and bacteria of other genera is predominantly due to the accumulation of point mutations in the gene *gyr*B, encoding the DNA gyrase B subunit (GyrB), the target of novobiocin. Use of Novobiocin is likely to lead to selection of resistant bacteria.

Considering the characterisation of criterion (b) above, there is a risk for animal and public health due to the development of resistance to Aminocoumarins.

Criterion (c) – availability of other treatments for animals

Alternative intramammary products are available to treat bovine mastitis; in particular, antistaphylococcal penicillins e.g. cloxacillin or 1st-generation cephalosporins.

Criterion (d) – availability of other antimicrobial treatments for humans

No current clinical uses were identified for aminocoumarins in human medicine the EU.

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

- Aminocoumarins are not authorised in human medicinal products and no current clinical uses were identified in human medicine the EU.
- Novobiocin is authorised in the EU in intramammary preparations for treatment of mastitis in cattle, specifically involving beta-lactamase-producing staphylococci. No other aminocoumarins are authorised in VMPs in the EU, and no specific evidence could be found relating to use outside the marketing authorisation.
- Resistance to novobiocin may develop rapidly in staphylococci during treatment; alternatives are available but are likely to be of higher importance to animal and public health. Use of aminocoumarins outside the marketing authorisation is expected to be uncommon and the AMR risk to animal and public health is considered to be low.

Therefore, considering the points above relevant to criteria (b), (c) and (d), it is recommended that no conditions should be placed on the use of Aminocoumarins outside

the terms of the marketing authorisation, although responsible antimicrobial use principles should be applied.

5. Evaluation of antivirals

Please refer to Section 3.4. of this advice for the methodology used to evaluate antiviral substances.

Recommendations for certain antivirals to be designated as reserved for human use only were established in a previous EMA advice [3]. In addition to considering their importance to human health and need for animal health, antivirals/antiviral classes were evaluated for the potential that use in animals could lead to the selection and dissemination of a resistant zoonotic virus and that there would be a likely significant risk of transmission to humans.

According to CIR (EU) 1255/2022 [2], the following antivirals are restricted to use in humans, only:

Amantadine, Baloxavir marboxil, Celgosivir, Favipiravir, Galidesivir, Lactimidomycin, Laninamivir, Methisazone/metisazone, Molnupiravir, Nitazoxanide, Oseltamivir, Peramivir, Ribavirin, Rimantadine, Tizoxanide, Triazavirin, Umifenovir, Zanamivir

These antivirals cannot be used in animals at all, including outside the terms of a marketing authorisation.

Other antivirals that are not listed as reserved for human used may be used in accordance with Article 112 in non-food-producing animals.

Regulation (EC) 1950/2006 (amended by Commission Regulation (EU) 122/2013) (substances essential for the treatment of equidae) lists acyclovir and idoxuridine for topical treatment of ocular ulcers in equines, and hence these substances/indications are out of scope of this advice.

5.1. Review of the literature relating to the potential therapeutic use in non-food-producing animals of antivirals not included in the Article 37(5) list

Antivirals shown to have potential therapeutic use in non-food-producing animals were identified mainly through textbooks and bibliographic data. Owing to the nature of the use, some reports are not from peer-reviewed journals, but are cited as they provide evidence for use of the antivirals in veterinary practice. It cannot be excluded that some antiviral substances have been overlooked.

There was one report to the Open Call for Data relating to the use of famciclovir to treat viral infections in pinnipeds. Otherwise, although there are experimental studies on the use of antivirals in laboratory animals, very little published evidence was found to support their therapeutic use in species other than horses, cats and dogs. The focus of the review is therefore on the use of antivirals in the latter animal species, with the understanding that use of AVs in equines is restricted to designated non-food-producing animals.

For each antiviral, the findings are presented including any veterinary disease indications referred to in the publication and information on reported treatment outcomes, when available.

A conclusion on the potential use of the AV in animals to treat specific diseases is provided.

Antivirals potentially used in companion animals in the EU

Antiviral	Available data		Reported outcomes
Brincidofovir/cidofovir	Used against	Conjunctivitis and keratitis in cats, due to feline herpesvirus-1	Positive outcome reported.
		Respiratory disease in horses, due to equine herpesvirus-1	minimal outcome in already affected horses

		Ulcerative keratitis in dogs, due to canine herpesvirus-1	No (successful in only one reported case)
	Reference(s)	[663-668]	
	Conclusion	Cidofovir is currently used in cats against ocular herpesvirus infections. No other evidence of widespread use of cidofovir was identified in veterinary practice for companion animals.	
Brivudin* (against HSV, EBV, HHV, CMV, VZV, …)	Used against	Disease due to equine herpesvirus-1	None
	Reference(s)	[669]	
	Conclusion	No evidence of widespread use of brivudin was identified in veterinary practice for companion animals	
Camostat mesylate	Used against	canine pancreatitis	Not recorded
	Reference(s)	[670]	
	Conclusion	No evidence of widespread use of camostat mesylate was identified in veterinary practice for companion animals	
Famciclovir/Penciclovir	Used against	Disease due to feline herpesvirus-1 (conjunctivitis, rhinosinusitis, keratitis, and FHV-1 associated dermatitis).	Positive outcomes reported
	Reference(s)	[178, 666, 671, 672]	
	Conclusion	Famciclovir/penciclovir is currently used in cats against herpesvirus-1 infections. No other evidence of widespread use of famciclovir/penciclovir was identified in veterinary practice for companion animals.	
Idoxuridine* (against HSV, VZV)	Used against	Eye disease due to feline herpesvirus-1	Some positive outcomes but not consistent in all reports.
	Reference(s)	[178, 673-675]	
	Conclusion	Idoxuridine is currently used in cats against FHV-1 ocular infections. No other evidence of widespread use of idoxuridine was identified in veterinary practice for companion animals.	
Indinavir	Used against	stage III splenic hemangiosarcoma in dogs	Unconvincing
	Reference(s)	[676]	
	Conclusion	No evidence of widespread use of indinavir was identified in veterinary practice for companion animals	
Raltegravir	Used against	Disease due to feline leukemia virus infection in cats	Reported outcome: weak
		Disease due to feline immunodeficiency virus infections in cats	Weak
		Disease due to feline herpesvirus-1 infections in cats	Weak
	Reference(s)	[677-680]	
	Conclusion	No evidence of widespread use of raltegravir was identified in veterinary practice for companion animals	
Remdesivir	Used against	Feline infectious peritonitis (FIP) in cats	Positive outcomes reported.
	Reference(s)	[681-687]	
	Conclusion	Remdesivir is currently used in cats against Feline infectious peritonitis. No other evidence of widespread use of remdesivir was identified in veterinary practice for companion animals.	
Tipranavir	Used against	Disease due to feline immunodeficiency virus in cats	show anti-FIV properties
	Reference(s)	[688]	
	Conclusion	No evidence of widespread use of tipranavir was identified in veterinary practice for companion animals	
Trifluridine* (Trifluorothymidine) (against HSV, VZV, HCMV, some adenoviruses)	Used against	Eye disease due to feline herpesvirus-1	Yes, but serious side effects
	Reference(s)	[675]	
	Conclusion	No evidence of widespread use of tipranavir was identified in veterinary practice for companion animals	
Valacyclovir/acyclovir **(Valaciclovir/aciclovir)**	Used against	Oral treatment against feline herpesvirus-1	Use discouraged due to poor efficacy and toxicity.

		Disease due to canine herpesvirus-1	May be beneficial, but often ineffective
		Disease due to canine parvovirus	Control of the infection, but significant side effects
	Reference(s)	[119, 666, 675, 689-692]	
	Conclusion	No evidence of widespread use of valacyclovir/acyclovir was identified in veterinary practice for companion animals (available results against animal herpesviruses are disappointing), except for topical treatment of feline herpesvirus-1 ocular disease. Based on one publication only, acyclovir could be active against canine parvovirus, but adverse effects are considered significant.	
Vidarabine* (against HSV, HCMV, Rous sarcoma virus, some adenoviruses, HBV)	Used against	Disease due to feline herpesvirus-1	Modest outcomes reported
	Reference(s)	[675]	
	Conclusion	No evidence of widespread use of vidarabine was identified in veterinary practice for companion animals	
Zalcitabine* (against HIV)	Used against	Disease due to feline leukemia virus infections in cats	Modest outcomes reported
		Disease due to feline immunodeficiency virus infections in cats	Not recorded
	Reference(s)	[693, 694]	
	Conclusion	No evidence of widespread use of zalcitabine was identified in veterinary practice for companion animals	
Zidovudine (AZT)	Used against	Disease due to feline immunodeficiency virus infections in cats	Mitigated (helps against stomatitis or neurologic symptoms; does not prolong survival and can have serious side effects)
		Disease due to feline leukemia virus in cats	Weak (helps against stomatitis); serious side effects
	Reference(s)	[178, 695-697]	
	Conclusion	No widespread use of zidovudine was identified in veterinary practice for companion animals	

The following antiviral substances were also investigated but no pertinent references or evidence for their widespread use in companion animals in the EU was identified:

Abacavir
Adefovir dipivoxil
Amdoxovir* (against HIV)
Apricitabine* (against HIV)
Atazanavir* (against HIV)
Bictegravir
Clevudine* (against HBV)
Daclatasvir* (against HCV)
Darunavir* (against HIV)
Dasabuvir* (against HCV)
Delavirdine* (against HIV)
Didanosine* (against HIV)
Dolutegravir
Efavirenz
Elbasvir
Elvitegravir
Emtricitabine
Enfuvirtide
Entecavir* (against HBV)
Etravirine* (against HIV)
Fosamprenavir
Foscarnet
Glecaprevir
Grazoprevir
Lamivudine
Ledipasvir
Letermovir* (against HCMV)
Lopinavir
Maravidoc
Maribavir* (against HCMV, EBV)
Nelfinavir* (against HIV)
Nevirapine
Nirmatrelvir
Ombitasvir* (against HCV)
Paritaprevir* (against HCV)
PF-07304814
Pibrentasvir
Rilpivirine
Ritonavir
Rupintrivir (rupinavir)
Saquinavir* (against HIV)
Stavudine* (against HIV)
Sofosbuvir
Tecovirimat
Telbivudine* (against HBV)
Tenofovir alafenamide
Tenofovir disoproxil
Torcitabine/ valtorcitabine* (against HBV)
Valganciclovir/ ganciclovir
Velpatasvir
Voxilaprevir

* Newly identified antivirals with regard to document EMA/CVMP/678496/2021 – "Advice on the designation of antimicrobials or groups of antimicrobials reserved for treatment of certain infections in humans - in relation to implementing measures under Article 37(5) of Regulation (EU) 2019/6 on veterinary medicinal products" [3]

It can be determined from the table above that, in most cases, widespread use of antivirals was not identified in veterinary practice for companion animals in the EU, except for:

- **Cidofovir** for cats, as a topical agent to treat infections of the eye (conjunctivitis and keratitis) associated with feline herpes virus (FHV-1).
- **Famciclovir** for cats, administered orally, against clinical diseases due to FHV-1 (rhinotracheitis).
- **Idoxuridine** for cats, as a topical agent to treat cats with corneal or conjunctival diseases attributed to FHV-1.
- **Remdesivir** for cats, as a liquid or powder for infusion, against feline infectious peritonitis (FIP). To be noted that the mechanism of action of remdesivir is not virus-specific: it could potentially be used against RNA viruses belonging to table 6 ("zoonoses that are frequent or endemic in humans in the EU") in document EMA/CVMP/678496/2021.
- **Valacyclovir/acyclovir** (valaciclovir/aciclovir) as a topical agent to treat cats with ocular diseases attributed to FHV-1.

It should be noted that there is currently limited evidence (safety, efficacy) supporting use of these antivirals in companion animals: controlled experiments relying on tested procedures and recognized statistical methods are currently lacking.

In the Open Call for data, the following antivirals were quoted:

famciclovir (against animal herpesviruses), acyclovir (against feline rhinotracheitis caused by FHV-1), valacyclovir (against infections in horses caused by equine herpesvirus type 1).

5.2. Evaluation of Cidofovir, Idoxuridine, Famciclovir, Acyclovir, Valacyclovir

Scope of permitted use according to the MRL Regulation

Antiviral_substances are not authorised as veterinary medicinal products in the EU and none of these substances is 'allowed' in accordance with the MRL Regulation (EC) 470/2009.

Hence antivirals, can only be used in non-food-producing animals, including non-food-producing equines, under Article 112 of the Regulation.

Use of an antiviral in veterinary practice is therefore based on personal initiative and responsibility of the veterinarian only, and limited to treatment of individual animals in most cases. Accordingly, use of antivirals is presently very limited quantitatively (no mass treatment, even in animal groups, such as dog kennels/catteries, or at owner level with several pet animals at home).

Substances/indications in equines out of scope of evaluation for conditions due to listing in Regulation (EC) 1950/2006, as amended by Regulation (EU) 122/2013

Acyclovir and idoxuridine for topical treatment of ocular ulcers in equines,.

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Importance for human health

Brincidofovir/cidofovir is an experimental antiviral for the treatment of humans against cytomegalovirus, adenovirus and poxvirus infections [698]. Its activity against BK virus (polyomavirus) and Herpes simplex virus is also quoted. In laboratory tests, cidofovir and brincidofovir have been shown to be effective against the variola virus (smallpox) [699]. Cidofovir was formerly approved in the EU for treatment of cytomegalovirus retinitis, but the authorisation is now withdrawn.

Idoxuridine has very limited use as an HMP in the EU, for cutaneous treatment of herpes simplex and herpes zoster (Virexen SPC).

Famciclovir is currently authorised against herpesvirus simplex types 1 and 2, varicella-zoster virus (human alphaherpesvirus type 3), Epstein-Barr virus (human gammaherpesvirus type 4) and cytomegalovirus (family *Herpesviridae*, subfamily *Betaherpesvirinae*).

Acyclovir (aciclovir) is currently authorised against varicella-zoster virus (human alphaherpesvirus type 3) and herpesvirus simplex types 1 and 2. **Valacyclovir** (valaciclovir) is authorised against herpesvirus simplex types 1 and 2, varicella-zoster virus (human alphaherpesvirus type 3), Epstein-Barr virus (human gammaherpesvirus type 4), cytomegalovirus (family Herpesviridae, sub-family Betaherpesvirinae) and human herpesvirus type 6.

Importance for animal health

Cidofovir, idoxuridine and famciclovir are all used for treatment of disease associated with FHV-1 in cats. In a study in cats experimentally infected with FHV-1, topical cidofovir led to improved clinical scores in the treated compared to a controlled group [663]. Studies investigating oral administration of famciclovir have shown improvements in ocular disease, rhinosinusitis and other clinicopathological outcomes [671, 672, 700]. Use of acyclovir to treat FHV-1 has been quoted by stakeholders.

Under natural conditions, viruses undergo an infinitely long series of replication cycles as they are transmitted from host to host. During this process, spontaneous mutants are continually generated, because of copying mistakes of the viral nucleic acid that are continuously occurring during the replication process. Because these copy errors occur randomly, most of them are either lethal or neutral, in the sense that they provide disadvantages or no particular benefits to the virus from an evolutionary perspective. However, copy errors may sometimes be beneficial, improving survival and evolutionary progression of the mutated virus.

Thus, antivirals do not generate or facilitate mutations by themselves - mutations will occur at the same rate with or without their use. Antiviral use may create an environmental selection pressure beneficial to some mutated viruses - the antiviral will block the replication of the antiviral-sensitive viruses without having any effect on those that are resistant. However, there are no mass antiviral treatments in animals in the EU at present and the number of viable viral mutants selected by use of antivirals in individual animals is negligible compared to the occurrence of resistant mutants generated during natural productive viral infections.

Many data are currently available relating to antiviral drug resistance in the human field; whilst on the contrary, such data are scarce in the veterinary field, most likely because antivirals are not regularly nor extensively used in veterinary practice. It has, however, to be assumed that antiviral drug resistance (and maybe cross-resistance) would occur at a similar level in the veterinary domain if antiviral use became established/extensive. The veterinary viruses which could be affected are

currently not identifiable because the activity (if any) of the antivirals used in the human domain cannot be directly transposed to veterinary viruses.

In conclusion,

(Brin)cidofovir, famciclovir, idoxuridine, acyclovir and valacyclovir and have important uses in human medicine; however, the viruses they are used to treat are not zoonotic and therefore there is no risk for human health in case of the use of these AVs in animals.

Considering that, in the veterinary domain:

- The use of antivirals is currently very limited quantitatively,
- The current (unauthorised) therapeutic indications for these substances are limited to FHV-1 and EHV-1,
- Viable viral mutations (including antiviral drug resistance) are continually generated, with or without the use of antivirals,
- The number of viable viral mutants promoted through the use of antivirals in animals is quantitatively negligible so far,

then, although some relevant uses of (brin)cidofovir, idoxuridine, valacyclovir/acyclovir and famciclovir have been identified in veterinary medicine, it follows that the risk for animal health in case of resistance cannot be adequately appreciated yet, but it is doubtless negligible at present time.

Criterion (c) – availability of other treatments for animals

Given that, in the veterinary domain:

- No antiviral is presently authorised for use in companion animals,
- There is currently no proven relevance (safety, efficacy) of using antivirals in companion animals,
- The current documented (unauthorised) therapeutic indications are limited to the FHV-1, EHV-1,
- The use of antivirals is currently very limited quantitatively,

it follows that the notion of 'availability of alternative treatments' does not apply here; in the absence of specific antivirals, treatment of viral diseases remains largely supportive and symptomatic.

To be noted that in some instances, veterinary vaccines do exist, e.g. against FHV or EHV. However, in individual animals, vaccination may not be effective e.g. due to lack of immunocompetence. In addition, vaccination is not applicable for sick animals at the point when treatment is required.

Criterion (d) – availability of other antimicrobial treatments for humans

This criterion does not apply as these antiviral diseases (FHV or EHV) are animal specific and are not zoonotic diseases; therefore, there is no risk to human health from AV-resistance due to the use to treat these infections in animals.

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

According to Article 112, cidofovir, idoxuridine, famciclovir, acyclovir, valacyclovir can only be used in exceptional circumstances to treat serious diseases in companion animals on an individual basis.

These diseases are animal specific (not zoonotic).

Taking account of the diseases under consideration for treatment in animals, the risk of transfer of antiviral resistant viruses from animals to humans and other animals is considered as negligible, in particular based on current patterns of use in animals.

There are no alternative treatments for animals specifically directed at the viral diseases cited.

Therefore, it is considered that no legislative conditions should be placed on the use of these antivirals under Article 112 of the Regulation.

5.3. Evaluation of Remdesivir

Scope of permitted use according to the MRL Regulation

Antiviral substances are not authorised as veterinary medicinal products in the EU and none of these substances is 'allowed' in accordance with the MRL Regulation (EC) 470/2009.

Hence antivirals, can only be used in non-food-producing animals, including non-food-producing equines, under Article 112 of Regulation (EU) 2019/6.

Step 1. Assessment against the criteria (b), (c) and (d) of Article 107(6)

Criterion (b) – risk for animal or public health in case of development of antimicrobial resistance

Importance for human health

In human medicine, remdesivir showed activity against Ebola and SARS-CoV-2; and in vitro against multiple filoviruses, pneumoviruses, paramyxoviruses, and coronaviruses. Some of these viruses represent major threats to humans. For instance:

- Filoviruses (e.g. Ebola, Marburg disease) cause severe haemorrhagic fever, frequently lethal. The mortality rate of Ebola is about 50%.

- SARS-CoV-2 (Covid-19 coronavirus) has already caused more than 6 million deaths worldwide [701].

- Paramyxoviruses are an important class of viruses which are associated with respiratory ailments, and common childhood diseases such as measles and mumps. Paramyxoviruses are a significant cause of morbidity and mortality globally, especially in children and the elderly.

- Respiratory syncytial virus (pneumovirus) can be serious for infants where it is a common cause of bronchiolitis and pneumonia. Hospitalization is required each winter for 0.5%–2% of infections of children less than 6 months old.

Remdesivir received a conditional authorisation in the EU to treat Covid-19 in July 2020; the FDA officially granted EUA for remdesivir to treat Covid-19 in severe hospitalized patients in May 2020.

In animals, Remdesivir has been investigated to treat feline infectious peritonitis (caused by a coronavirus). FIP is a serious disease in cats for which no adequate and efficacious treatment has been established yet and that ultimately leads to death. In that context, remdesivir could be seen as a potential future effective treatment. This interest in the substance is supported by literature and also byreports of use in clinical practice. For example in UK, remdesivir is legally available to veterinarians where the product for human use is reformulated to facilitate its use in cats.

GS-5734 (Remdesivir; Gilead Sciences) is the phosphoramidate prodrug of GS-441524. Nucleoside analogue GS-441524, has been tested for its efficacy against natural and experimental feline infectious peritonitis in a series of recent field trials and/or case studies (proof of concept). Notably, Pedersen et

al. reported safety and efficacy of GS-441524 for treatment of cats with naturally occurring FIP (25 of 31 cats successfully treated with subcutaneous application of 2.0 - 4.0 mg/kg GS-441524). Dickinson et al. reported on clear clinical improvements of four naturally occurring FIP cases and described clearance and long-term resolution of neurological FIP following treatment with GS-441524. A retrospective study by Yin et al. reported a 67% mortality rate in FIP-suspected cats, but described survival in the majority of cats treated with GS-441524. Murphy et al. described an experimental FIP infection of cats and reported on a rapid reversal of disease signs and return to normality with as little as two weeks of GS-441524 treatment in 10/10 cats and with no apparent toxicity. The multi-component drug Xraphconn® (GS-441524 identified as an active component) was recently shown by Krentz et al. to be highly effective as an oral treatment for FIP [685].

The Royal Veterinary College (University of London) reported on the successful treatment of FIP in a 24-week-old male neutered Bengal cat with 15mg/kg remdesivir intravenously [702]. Hughes et al describe the treatment of FIP in cats with remdesivir, citing as the main advantage of remdesivir therapy that the product is subject to quality assurance (unlike unlicensed GS-441524 formulations). Richard Malik of the Centre for Veterinary Education (University of Sydney) describes a two-stage approach for antiviral therapy wherein intravenous/subcutaneous remdesivir injections are followed by a consolidating oral treatment with GS-441524 tablets (80% of cats successfully treated). The International Society of Feline Medicine has issued dose recommendations for remdesivir (based on experiences of colleagues in Australia where remdesivir is legally available to vets).

Resistance to this antiviral is strongly suspected [703].

Paramyxoviruses (e.g. Newcastle disease), coronaviruses (e.g. feline infectious peritonitis) and pneumoviruses (RSV) represent major threats to domestic animals. They are however distinct from the human pneumoviruses, paramyxoviruses and coronaviruses.

In conclusion, remdesivir is an important antiviral in human medicine to treat life-threatening infections. Amongst the quoted genera or families of viruses above, none represents a zoonotic threat to humans. No transfer of remdesivir-resistant viruses, from domestic animals to humans, was identified so far. However, the mode of action of remdesivir, by interference with viral RNA-dependent RNA polymerase) is not virus-specific, hence it could potentially also be used to treat zoonotic viruses which could act as a route for transmission of antiviral resistance between animals and humans.

There is a potential risk of transfer of resistant viruses from animals to humans, but considering companion animals, this is likely to be negligible.

Criterion (c) – availability of other treatments for animals

No treatment is currently authorised for FIP. Feline interferon-omega, although not authorised for this indication in the EU, has been investigated but showed no effect on survival time or quality of life in a clinical trial of affected cats [704].

Criterion (d) – availability of other antimicrobial treatments for humans

Specific direct-acting antiviral treatment options, with proven efficacy, for measles, mumps and Ebola are not available. This criterion does not apply for EHV and FHV-1, which are animal specific.

Conclusion to consideration of criteria (b), (c) and (d) of Article 107(6)

Hence, considering the above, **there is a potential significant risk for animal and public health due to the development of resistance to remdesivir.**

It is proposed that conditions should be considered for use of remdesivir outside the terms of the marketing authorisation to reduce the AMR risk to public and animal health.

Step 2. Considerations of conditions to be placed on use outside the terms of a marketing authorisation

Use of a human medicinal product

A human product is available as a solution for IV infusion.

Condition proposed:

For treatment of feline infectious peritonitis only.

Rationale: Due to the importance of this antiviral in humans, and the potential risk of transfer of resistant viruses, it is proposed to limit the use to only the specific diseases where remdesivir has the potential to treat a serious disease in animals and for which there is no zoonotic risk.

Step 3. Consideration of Criteria (a) and (e) in view of proposed conditions to be placed on use outside the terms of a marketing authorisation

Criterion (a) – risk to animal health or public health if the antimicrobial is used in accordance with Articles 112, 113 and 114

Injection site reaction and pain on injection have been reported in clinical studies in cats [681]. In the absence of MRL status, remdesivir cannot be used in food-producing animals.

Criterion (e) Impact on aquaculture and farming if the animal affected by the condition receives no treatment

Remdesivir can be used in non-food-producing equines in accordance with Article 112; however, no evidence was identified for its use in these species and therefore no impact of the proposed condition is expected on farming.

Step 4. Final conclusion - recommendations made for conditions to be placed on use outside the terms of a marketing authorisation

- For the treatment of feline infectious peritonitis (FIP), only.

6. Evaluation of antifungals

Antifungal substances, potential uses outside the terms of a marketing authorisation, assessment against the Article 107(6) criteria and recommendations for conditions

Please refer to the EMA *Advice on the designation of antimicrobials reserved for treatment of certain infections in humans* [3] for supporting references.

Class/ substance	**Human use** –Indications for authorised human medicines in the EU and other reported antifungal uses	**Veterinary use** - Antifungal indications for authorised veterinary medicines in the EU MRL status according to Regulation (EU) 37/2010	**Limitations on the scope of the evaluation** **Reported potential uses outside the terms of a marketing authorisation for fungal infections**
Azoles e.g. miconazole, itraconazole, ketoconazole, voriconazole	Important as first-line treatment for life-threatening invasive aspergillosis and chronic aspergillosis; and as a second-line and follow up treatment for invasive candidiasis. May be used prophylactically in high risk immunosuppressed patients. Voriconazole is used for the treatment of *Fusarium* spp. and *Scedosporium* spp. that cause severe infections in immunocompromised patients. Other indications include histoplasmosis, cryptococcal meningitis and coccidioidomycosis. By oral administration, azoles are authorised for oral and oesophageal candidiasis, for vulvovaginal candidiasis, pityriasis and dermatophytoses.	Enilconazole has been authorised to treat dermatophytosis in cattle and horses (by topical application). Itraconazole and ketoconazole are authorised for oral treatment of dermatophytoses in cats and dogs. Itraconazole is authorised for the treatment of respiratory infections caused by *Aspergillus* and *Candida* spp. in ornamental birds. Clotrimazole, miconazole and posaconazole are authorised in topical preparations to treat otitis due to *Malassezia* infections in cats and dogs. According to Reg (EU) 37/2010, enilconazole has 'no MRL required' status for topical use only, in bovines and *Equidae*. Parconazole has 'no MRL required' for guineafowl, with no 'other provisions'.	Use in food-producing species is limited, according to availability of MRL status, to parconazole and topical administration of enilconazole. The following substances/indications in equines are out of scope of evaluation for conditions due to listing in Regulation (EU) 122/2013: Ketoconazole as a systemic treatment for fungal pneumonia and guttural pouch mycosis; Miconazole for topical treatment of fungal eye infections in equines. In companion and zoo animals, use outside an SPC of azoles is important for treatment of a wide range of mostly sporadic but serious fungal infections e.g. aspergillosis, histoplasmosis, zygomycosis, sporotrichosis and cryptococcosis. Systemic use of azoles has also been reported for *Malassezia* infection in dogs and dermatophytosis in rabbits.
Consideration of Article 107(6) criteria (a) See general comments in Section 3.1. of the advice. In animals, adverse effects relate to liver dysfunction. Caution is needed for pregnant animals. Target animal safety warnings in the SPCs of authorised VMPs should be followed. Some of the reported uses outside the marketing authorisation have zoonotic potential (below). (b) Azole-resistance in *Candida* spp. and *Aspergillus fumigatus* is a serious threat to human health, but these diseases are not considered to be direct zoonoses and the contribution to drug-resistance relating to azole use in animals is not likely to be significant. There is limited evidence for azole-resistance in animal isolates of dermatophytes; due to zoonotic potential of these infections, there is a potential risk for transmission of resistance from animals to humans but this is unlikely to differ compared with any risk from authorised use. Of the identified uses outside the SPC, sporotrichosis (*S. brasiliensis*) is the only other infection with significant zoonotic potential and hence possibility for direct transfer of azole resistance; however, this disease does not occur naturally in the EU and treatment is likely to be sporadic in individual animals. Resistance due to use under Articles 112 and 113 is unlikely to impact significantly on authorised use in animals. (c) Limited information is available and there are very few alternative treatments (e.g. amphotericin B, flucytosine) for the uses outside the SPC identified for serious infections in companion and zoo animals. Azoles are preferred owing to their safety profile.			

(d) Availability of antimicrobial treatment options for fungal infections in humans is generally limited. Echinocandins are the first-line treatment for invasive candidiasis and may be used for refractory aspergillosis. Amphotericin B is mostly used as second-line or salvage treatment.
(e) No conditions proposed on use outside the terms of the marketing authorisation; therefore, no impact is expected on aquaculture and farming.

Recommendation: No conditions are proposed on use outside the terms of the marketing authorisation.

Class/ substance	Human use	Veterinary use	Limitations on the scope of the evaluation Reported potential uses outside the terms of a marketing authorisation for fungal infections
Polyenes e.g. nystatin, natamycin, amphotericin B	Amphotericin B (AmB) is recommended as a second-line (salvage) choice for treatment of severe invasive candidiasis or aspergillosis, or for strains resistant to first-line drugs. AmB is also recommended as first-line treatment for mucormycosis, cryptococcal meningitis (combined with flucytosine) and some less common mycoses (blastomycosis, histoplasmosis, mucormycosis and sporotrichosis) when infections are severe and disseminated. Nystatin and natamycin are authorised as oral and vaginal tablets for treatment of *Candida* infections and for fungal dermatoses.	Amphotericin B is not authorised for use in animals in the EU. Nystatin is authorised in topical ear products for pets. Natamycin is included in Reg (EU) 37/2010 for topical use only in bovines and equines.	Use in food-producing animals is limited by the availability of MRL status for natamycin only, for topical use. The following substances/indications in equines are out of scope of this evaluation for conditions due to listing in Regulation (EU) 122/2013: Nystatin for treatment of yeast infections of the eye and genital tract. According to textbooks, nystatin has been used to treat yeast mastitis in cattle and *Candida* metritis in horses. In exotic birds and pets, including reptiles, nystatin has been used to treat intestinal candidiasis and other mycoses. Textbooks advise that amphotericin B has been used to treat systemic fungal infections in companion animals (*Candida, Blastomyces, Coccidoides, Histoplasma, Cryptococcus* spp). In cats, it has been used in combination with azoles to treat sporotrichosis. In horses it has been used to treat pulmonary cryptococcosis, by regional limb perfusion to treat pythiosis and by subconjunctival injection to treat ocular fungal disease. Use has also been reported in cetaceans. Amphotericin B is included in the *WSAVA List of Essential Medicines for Cats and Dogs (Complementary list)* for treatment of fungal infections [178].

Consideration of Art 107(6) criteria
(a) See general comments in Section 3.1. of the advice. Use outside a marketing authorisation/SPC in animals is sporadic. Administration of AmB is intravenous and therefore will be undertaken by professionals. The most important adverse reaction to AmB in animals is dose-related nephrotoxicity.
(b) Resistance to polyenes remains very rare but has been reported in human *Candida* and *Aspergillus* spp. AmB is important for treatment of severe candidiasis and aspergillosis in humans and various systemic fungal infections in humans and animals, including as second-line treatments. There is little convincing evidence of resistance to polyenes in animal isolates. Other than *Sporothrix brasiliensis* (which does not occur naturally in the EU) the fungal infections that are treated with polyenes in humans are not considered zoonotic. Transmission of polyene-resistant fungal infections from animals to humans is not likely to be significant in the EU.

Resistance due to use of polyenes outside an SPC is unlikely to impact significantly on authorised use in animals.
(c) Availability of other treatments for identified uses use outside an SPC in animals is very limited – in the case of nystatin and natamycin, azoles are a possible alternative. AmB is usually used in cases of previous treatment failure or in combination with other antifungals for refractory cases; therefore, it is last resort when there are no alternatives.
(d) Availability of antimicrobial treatment options for fungal infections in humans is generally limited. AmB is often a salvage-treatment, as noted above, but is a first-line treatment for certain less common but serious fungal infections. Azoles may be an alternative to nystatin and natamycin, according to circumstances.
(e) No conditions proposed on use outside the terms of the marketing authorisation; therefore, no impact is expected on aquaculture and farming.

Recommendation: No conditions are proposed on use of nystatin and natamycin outside the terms of the marketing authorisation. Please refer to conditions for use of amphotericin B outside of a marketing authorisation in regard to treatment of Leishmania and use in countries where the disease is endemic (Ref. Antiprotozoals table).

Class/ substance	**Human use**	**Veterinary use**	**Limitations on the scope of the evaluation** **Reported potential uses** outside the terms of the marketing authorisation **for fungal infections**
<u>Pyrimidine analogues</u> - flucytosine	Flucytosine, in combination with amphotericin B (AmB), is important as first line for the treatment of cryptococcal meningitis, a serious disease in immunosuppressed humans, with few available alternatives. It is also authorised for candidiasis and chromomycosis, and as an oral treatment, including additionally for certain *Aspergillus* spp.	No EU-authorised veterinary medicines were found. Flucytosine is not included in Reg (EU) 37/2010, therefore it cannot be used in food-producing animals in the EU.	Use only allowed in non-food-producing species due to lack of MRL status. Cryptococcosis occurs rarely or sporadically in domestic animals in Europe; flucytosine is part of the first-line treatment (combined with AmB) for serious CNS or systemic cryptococcosis in cats (European Advisory Board on Cat Diseases).

Consideration of Art 107(6) criteria
(a) See general comments in Section 3.1. of the advice. Flucytosine is not recommended for use in dogs due to cutaneous eruptions. Anaemia and thrombocytopenia are the most common adverse effects in animals. May be teratogenic. Use in animals is likely to be sporadic and limited to individual animals.
(b) Resistance to flucytosine develops rapidly during treatment and is likely to have an impact on human health considering use of the substance as a first-line treatment for cryptococcal meningitis. No reports were found that specifically identified resistance to flucytosine in fungal isolates from domestic animals. It could be speculated that resistance would develop rapidly under treatment. Although bird droppings have been implicated as a source of *Cryptococcus* for human infections, evidence of transmission from animals is weak. In addition, disease, and therefore treatment is rare in (pet) animals suggesting that the risk of transfer of drug-resistance in *Cryptococcus* spp. from animals to humans is not likely to be significant at population level.
(c) Availability of other treatments for cryptococcosis in animals is very limited – azoles are an alternative for mild-moderate disease only.

(d) Availability of antimicrobial treatment options for fungal infections, particularly cryptococcal meningitis, in humans is generally limited.
(e) No conditions proposed on use outside the terms of the marketing authorisation; therefore, no impact is expected on aquaculture and farming.

Recommendation: No conditions are proposed on use outside the terms of the marketing authorisation.

Class/ substance	Human use	Veterinary use	Limitations on the scope of the evaluation **Reported potential uses** outside the terms of the marketing authorisation **for fungal infections**
<u>Griseofulvin</u>	Griseofulvin is used for the topical or systemic treatment of dermatophyte infections in humans, as a second-line alternative to more modern antifungals.	Griseofulvin is authorised as a VMP in the EU for oral administration to non-food equines, only, for the treatment of dermatophytes. Griseofulvin is not included in the MRL Regulation (EU) 37/2010.	Use is only allowed in non-food-producing species due to the lack of MRL status. The following substances/indications in equines are out of scope of this evaluation for conditions due to listing in Regulation (EU) 122/2013: Griseofulvin for the treatment of ringworm. Griseofulvin is recommended in treatment guidelines for dermatophytosis in dogs and cats where systemic treatment is needed, but it is noted that it has more potential side effects compared with itraconazole and terbinafine. Use is also reported in rabbits and rodents.

Consideration of Art 107(6) criteria
(a) See general comments in Section 3.1. of the advice. Griseofulvin is teratogenic. Adverse effects include anaemia and leucopenia, (hepatotoxicity). Target animal safety warnings in the SPCs of authorised VMPs should be followed. Use outside the SPC in animals is likely to be uncommon. Dermatophytosis is a potential zoonosis.
(b) There are limited reports of resistance to griseofulvin in human dermatophyte isolates. Dermatophytosis is a zoonosis and hence there is a potential pathway for transmission of griseofulvin-resistance from animals to humans, but no reports were found. Although dermatophytosis is a common infection and an important public health issue, disease is rarely serious in humans or animals.
Resistance due to use outside the SPC is unlikely to impact on authorised use in animals.
(c) Azoles are the only authorised alternative systemic treatment for cats and dogs for dermatophytosis, and have a more favourable safety profile compared with griseofulvin. Terbinafine could also be an alternative.
(d) Availability of antimicrobial treatment options for fungal infections in humans is generally limited, but alternatives for dermatophytosis include azoles or terbinafine.
(e) No conditions are proposed on use outside the terms of the marketing authorisation; therefore, no impact is expected on aquaculture and farming.

Recommendation: No conditions are proposed on use outside the terms of the marketing authorisation.

Class/ substance	Human use	Veterinary use	Limitations on the scope of the evaluation **Reported potential uses** outside the terms of the marketing authorisation **for fungal infections**
<u>Allylamines</u> e.g. terbinafine, naftifine	Terbinafine is important for the topical and systemic treatment of dermatophytosis in humans, which although a common infection in immunocompromised and non-immunocompromised people, is treatable and rarely has serious consequences.	Terbinafine is authorised in topical ear products for pets in the EU, for treatment of *Malassezia pachydermatis*. Allylamines are not included in the MRL Regulation (EU) 37/2010.	Use only allowed in non-food-producing species due to lack of MRL status. Studies have investigated the use of oral terbinafine to treat dermatophytosis and *Malassezia* spp. infections in dogs and cats and it is recommended in treatment guidelines for dermatophytosis where systemic treatment is needed. Use in zoo animals was reported to the 'open call for data'.

			Terbinafine (oral) is included in the *WSAVA List of Essential Medicines for Cats and Dogs (Complementary list)* for treatment of fungal infections.

Consideration of Art 107(6) criteria
(a) See general comments in Section 3.1. of the advice. Adverse effects in animals include skin reactions and gastrointestinal effects. Allergic reactions and liver dysfunction have been reported in humans. Use outside the SPC in animals is likely to be uncommon. Dermatophytosis is a potential zoonosis.
(b) There are rare but increasing reports of resistance to terbinafine in dermatophytes. Dermatophytosis is a zoonosis and hence there is a potential pathway for transmission of allylamine-resistant organisms from animals to humans, but no reports were found. Although dermatophytosis is a common infection and an important public health issue, disease is rarely serious in humans or animals. Although humans can act as carriers of *Malassezia pachydermatis*, it considered of low public health significance [705]and there is currently limited reliable information on resistance development in this organism [706]. Resistance due to use outside the SPC is unlikely to impact significantly on authorised use in animals.
(c) Azoles may be an alternative systemic treatment in dogs and cats for the identified uses outside the SPC. For dermatophytosis, griseofulvin is also an alternative. For *Malassezia* infections, alternative topical treatments are available e.g. azoles, chlorhexidine.
(d) Availability of antimicrobial treatment options for fungal infections in humans is generally limited; alternatives are available for dermatophytosis e.g. azoles, griseofulvin, but may not be suitable for all cases.
(e) Allylamines are not used in food-producing animals and no evidence was found for use in fur animals, so no impact is expected on aquaculture and farming.

Recommendations: No conditions are proposed for use outside the terms of the marketing authorisation.

Class/ substance	**Human use**	**Veterinary use**	**Limitations on the scope of the evaluation** **Reported potential uses** outside the terms of the marketing authorisation **for fungal infections**
<u>Echinocandins</u> e.g. Caspofungin, micafungin, anidulafungin	Echinocandins are the first-line choice for treatment of invasive candidiasis, an important cause of mortality and morbidity in immunosuppressed patients. They are also authorised for prophylaxis of *Candida* infections in immnunocompromised patients undergoing HSCT[12], and as a rescue treatment in invasive aspergillosis.	No EU-authorised veterinary medicines were found. No echinocandins are included in Reg (EU)37/2010, therefore they cannot be used in food-producing animals in the EU.	Use only allowed in non-food-producing species due to lack of MRL status. No specific evidence was found for use of echinocandins.

Consideration of Art 107(6) criteria
(a) See general comments in Section 3.1. of the advice. No information is available on clinical safety in animals. In humans, adverse effects include gastrointestinal and allergic reactions, haemolysis, hepatic and renal dysfunction. Use in animals is likely to be sporadic and limited to individuals.

(b) Resistance to echinocandins is rare but has been reported in human *Candida* spp. isolates and is a serious threat to human health. Aspergillosis and candidiasis are not regarded a zoonoses and there is no direct pathway for transmission of echinocandin-resistant infections from animals to humans.
(c) As no specific uses have been identified, it is difficult to propose alternatives, but azoles (or polyenes) are most likely to be used for treatment of systemic fungal infections in animals.
(d) Availability of antimicrobial treatment options for fungal infections in humans is generally limited. Alternatives for candidiasis include azoles, but multidrug-resistant species have emerged and are a serious threat to human health. Amphotericin B is another alternative, but with less favourable safety.
(e) Echinocandins cannot be used in food-producing animals and there is no reported use in other farmed animals; therefore, no impact is expected on aquaculture and farming.

[12] HSCT: Haematopoietic Stem Cell Transplant

Recommendation: Although there is currently no evidence for potential transfer of echinocandin-resistant fungal infections from animals to humans, considering the high importance of echinocandins in human medicine for the treatment of invasive candidiasis, for which there are few treatment options, and lack of evidence for use or critical need of echinocandins in veterinary medicine, it is proposed to place conditions on use outside the terms of the marketing authorisation.

Proposed conditions: Echinocandins should only be used outside the terms of a marketing authorisation as a last resort treatment for individual animals, where alternative treatments have been shown not to be, or unlikely to be, effective and preferably after target pathogen idientification and susceptibility testing.

7. Evaluation of antiprotozoals

Antiprotozoal substances, potential uses outside a marketing authorisation, assessment against the Article 107(6) and recommendations for conditions

Please refer to the EMA *Advice on the designation of antimicrobials reserved for treatment of certain infections in humans* [3], Table 65, for the supporting references.

Note that the antiprotozoal activity of substances that are primarily used as antibiotics is addressed in Section 4.

Class/Substance	**Human use –** Indications for authorised human medicines in the EU and other reported antiprotozoal uses	**Veterinary use** - Antiprotozoal indications for authorised veterinary medicines in the EU MRL status according to Regulation (EU) 37/2010	**Limitations on the scope of the evaluation** **Reported potential use for protozoal infections in EU outside the terms of a marketing authorisation**
<u>Nitroimidazole derivatives</u> (see Section 4.22. for evaluation of antibacterial use)			
Metronidazole, dimetridazole	Authorised in the EU for various bacterial infections and protozoa: *Trichomonas, Entamoeba histolytica, Giardia lamblia* and *(Neo)balantidium coli* Nitroimidazoles are the main treatments for these infections.	VMPs authorised in the EU for cats and dogs: • Anaerobic bacterial infections • *Giardia* spp. Metronidazole and dimetridazole are prohibited substances according to Reg (EU) 37/2010.	Use prohibited in food-producing species according to Reg (EU) 37/2010. Included in the *WSAVA List of Essential Medicines for Cats and Dogs* for 'enteric protozoal infections in cats and dogs'. Use reported for intestinal protozoa including *Giardia, Trichomonas* and *Entamoeba* spp. in species including dogs, cats, horses. Use reported in 'open call for data' also for treatment of histomoniasis in ornamental birds; giardiasis in horses; protozoal diseases in teleosts (metronidazole); *Trichomonas* spp. in pigeons (dimetridazole).
Tinidazole	Authorised in the EU for trichomoniasis, giardiasis, amoebiasis	No EU authorisation found. Not included in Reg (EU) 37/2010	Use only allowed in non-food-producing species due to absence of MRL status. Reported use for *Giardia* spp. in dogs and cats and *Tritrichomonas foetus* in cats.
Ronidazole	No EU authorisation found. Antiprotozoal activity: *Trichomonas*	No EU authorisation found. Ronidazole is a prohibited substance according to Reg (EU) 37/2010.	Use prohibited in food-producing species according to Reg (EU) 37/2010. Use reported, including in 'open call for data', for *T. foetus* in cats
Secnidazole	Authorised in the EU for trichomoniasis, giardiasis, amoebiasis	No EU authorisation found. Not included in Reg (EU) 37/2010	Use only allowed in non-food-producing species due to absence of MRL status. Reported use for *Trypansoma cruzi* in dogs (not endemic in EU).

			Use reported in 'open call for data' for *Entamoeba* spp. infection in *Macaca fascicularis*
Benzinidazole	No EU authorisation found. Antiprotozoal activity: *Trypansoma cruzi*	No EU authorisation found. Not included in Reg (EU) 37/2010	Use only allowed in non-food-producing species due to absence of MRL status. Reported use for *Trypansoma cruzi* in dogs (not endemic in EU).
Nitroimidazoles - Consideration of Art 107(6) criteria (a) See general comments in Section 3.1. of the advice. Nitroimidazoles (NI) are suspected mutagens and carcinogens. They should not be administered to pregnant animals. Adverse effects reported in animals include neurotoxicity/CNS signs. Target animal safety warnings in the SPCs of authorised VMPs should be followed. Considering the identified uses and prohibition from use of certain NI in food-producing animals, use outside the SPC is likely to be restricted to single or restricted numbers of animals. (b) There is evidence of resistance to nitroimidazoles in G*iardia* spp., and limited evidence of resistance in *Entamoeba* and *Trichomonas* spp. There is theoretically a low risk that nitroimidazole-resistant giardia infections could be transmitted from pets to people anyhow in relation to authorised use. The potential risk for transfer of drug-resistant *Entamoeba histolytica* from animals to humans is extremely low, and there is no evidence of zoonotic transfer of *Trichomonas* spp. and hence transfer of drug resistance. Although pigs are a reservoir for zoonotic (*Neo)balantidium coli*, they cannot be treated with NI in the EU. Considering the anticipated extent and indications for use of nitroimidazoles outside the SPC to treat protozoal infections, it is unlikely that related resistance development will impact on authorised use in animals. (c) Availability of other treatments for the identified uses outside an SPC in animals is very limited. For giardia infections in cats and dogs, benzimidazoles are an (authorised) alternative. For *T. cruzi* in dogs, nifurtimox is an option if available. Insufficient evidence for effective alternatives was identified for *T. foetus* in cats. (d) Availability of antimicrobial treatment options for protozoal infections in humans is generally limited. Nitazoxanide (not authorised in EU) is an alternative for amoebiasis and albendazole is an alternative for giardiasis. (e) No conditions are proposed on use outside the terms of the marketing authorisation and this class cannot be used in food-producing animals; therefore no impact is expected on aquaculture and farming. **Recommendation**: No conditions proposed on use outside the terms of the marketing authorisation.			
<u>Triazines</u>			
Diclazuril, clazuril	No EU authorisation found.	VMPs authorised in the EU for: • Coccidiosis (*Eimeria* spp) in calves and lambs According to Reg (EU) 37/2010, Diclazuril has MRLs for edible tissues in rabbits and poultry. Other provisions state not for use in animals from which eggs are produced for human consumption. Clazuril has 'No MRL required' status for use in pigeons, with no provisions.	Diclazuril is restricted from use in in food-producing animals from which eggs are produced for human consumption. Use has been reported for treatment of equine protozoal myeloencephalitis – EPM (*Neospora hughesi* and, in Americas, *Sarcocystis neurona*). Use of diclazuril and toltrazuril has been reported as important to treat coccidiosis (*Eimeria* spp.) in goats. Use reported in 'open call for data' for treatment of coccidiosis in rabbits.
Toltrazuril (parent drug) ('**ponazuril**' = metabolite toltrazuril sulfone)	No EU authorisation found.	VMPs containing toltrazuril authorised in the EU for: • Coccidiosis (*Eimeria* spp) in poultry, calves and lambs, *Isospora* spp. in piglets and dogs According to Reg (EU) 37/2010, toltrazuril has	Toltrazuril cannot be used in animals producing milk or eggs for human consumption. The following substances/indications in equines are out of scope of this evaluation for conditions due to listing in Regulation (EU) 122/2013:

		MRLs for all mammalian food-producing species and in poultry for edible tissues. The marker residue is toltrazuril sulfone. 'Other provisions' state not for use in animals producing milk or eggs for human consumption. Although toltrazuril sulfone is the residue marker for toltrazuril, in itself it is not listed as an active substance in the regulation.	Ponazuril for treatment of equine protozoal myeloencephalitis (Sarcocystis neurona). Use of toltrazuril has been reported in cats and exotic animals to treat coccidiosis and toxoplasmosis; in horses to treat equine protozoal myeloencephalitis (EPM); in dogs to treat hepatozoonosis (*Hepatozoon canis)*. Use reported in 'open call for data' for treatment of coccidiosis in reptiles and rabbits. Ponazuril has been used to treat *Isospora suis* in pigs; *Cytoisospora* in cats. Also *Neospora caninum* in calves – experimentally [707]; in adult cattle the disease is usually sub-clinical but is an important cause of abortions.

Triazines - Consideration of Art 107(6) criteria

(a) See general comments in Section 3.1. of the advice. No serious safety issues identified in animal species. Target animal safety warnings in the SPCs of authorised VMPs should be followed. Use outside the SPC may involve group medications, as for authorised use.
(b) No relevant evidence was found for use of this class as an antiprotozoal in human medicine in the EU. Resistance to triazines has been identified in coccidia from broilers, pigs and lambs [707, 708]. Considering the anticipated extent and indications for use outside an SPC, it is unlikely that related resistance development will impact on authorised use in animals.
(c) Availability of other treatments for the identified uses outside an SPC in animals is limited. Pyrimethamine combined with sulfadiazine is an alternative to toltrazuril to treat EPM in non-food-producing equines, although ponazuril is out of scope of Art 107(6) for this indication. For *Hepatozoon canis*, imidocarb or doxycycline have been recommended. No alternatives were found for neosporosis in calves. Decoquinate or sulfonamides may be an alternative for treatment of coccidiosis (*Eimeria* spp.) in goats. Sulfonamides may be an alternative for the treatment of coccidiosis in dogs, cats, rabbits and exotic species.
(d) Not applicable as no evidence was found for use of this class to treat protozoal infections in humans.
(e) No conditions proposed on use outside the terms of the marketing authorisation; therefore no impact is expected on aquaculture and farming.

Recommendation: No conditions proposed on use outside the terms of the marketing authorisation.

Benzimidazoles with Antiprotozoal activity

Albendazole	Authorised in EU as an anthelmintic. Reports of use to treat giardiasis.	VMPs authorised in the EU as: • anthelmintics for ruminants. According to Reg (EU) 37/2010, MRLs for all ruminants, edible tissues and milk. No 'other provisions'.	Reported as used to treat *Giardia* spp. in calves.
Fenbendazole	No EU authorisation found.	VMPs authorised in the EU as anthelmintic for food-producing and companion animals, and for: • giardiasis in cats and dogs According to Reg (EU) 37/2010, MRLs for all food-	Reported as used to treat *Giardia* spp. in calves, reptiles and birds and *Encephalitozoon cuniculi* (microsporidian) in rabbits [709].

		producing species except fin fish, for edible tissues, milk and eggs. No 'other provisions'.	
Febantel (metabolised to fenbendazole + oxfendazole)	No EU authorisation found.	Combination VMPs have been authorised in the EU for: • giardiasis in cats and dogs. According to Reg (EU) 37/2010, MRLs are established in edible tissues for all ruminants, porcine and *Equidae*, and for ruminants in milk. No 'other provisions'.	No specific use identified outside the terms of the marketing authorisation.
(a) See general comments in Section 3.1. of the advice. Albendazole has been associated with bone marrow toxicity in small animals, hence other agents are used. Target animal safety warnings in the SPCs of authorised VMPs should be followed. Use outside the SPC may involve group medications, as for authorised use. (b) Resistance to albendazole has been shown in *Giardia* spp. and there is a theoretical risk that this resistance could be transmitted from animals to humans. *E. cuniculi* is a rare zoonosis in immunosuppressed humans; therefore, there is a theoretical transmission pathway for benzimidazole-resistant organisms from treated rabbits, although it is important to control any reservoir of infection. Considering the anticipated extent and indications for use outside the SPC, it is unlikely that related resistance development will impact on authorised use in animals. (c) Availability of other treatments for identified uses outside the SPC in animals is limited. Use of paromomycin to treat giardiasis in calves has been reported. No alternatives were identified for treatment of *Encephalitozoon* in rabbits. (d) Nitroimidazoles are the treatment of choice for giardiasis in humans, although treatment failures have been reported. No alternatives to albendazole were found for treatment of *E.cuniculi* in humans. (e) No conditions are proposed on use outside the terms of the marketing authorisation; therefore no impact is expected on aquaculture and farming. **Recommendations**: No conditions proposed on use outside the terms of the marketing authorisation.			
Other Antiprotozoals			
Pyrimethamine (usually in combination with sulfonamide)	Authorised in HMPs in the EU for Toxoplasmosis. Pyrimethamine + sulfadiazine are important for treatment of toxoplasmosis in immunosuppressed patients. Also used to treat malaria.	No EU VMP authorisation found. Not included in the MRL Reg (EU) 37/2010.	Use only allowed in non-food-producing species due to absence of MRL status. The following substances/indications in equines are out of scope of this evaluation for conditions due to listing in Regulation (EU) 122/2013: Pyrimethamine for treatment of Equine protozoal myeloencephalitis (EPM). Reported to be used to treat *Neospora (caninum)* in puppies, dogs being the definitive host. Infections in adult dogs and cattle are mostly sub-clinical but in cattle may manifest as abortions. (*Toxoplasma gondii* in cats).
(a) See general comments in Section 3.1. of the advice. Adverse effects in animals, particularly cats, include folate deficiency anaemia in longer treatments, allergic reactions. Based on reported use outside the SPC, administration is likely to be to single or restricted numbers of animals. (b) Resistance to pyrimethamine has been shown to *T. gondii* in vitro, but clinical resistance does not appear to be significant in humans at present. There is a theoretical risk that this resistance could be transmitted from treated cats to humans but pyrimethamine is not a preferred treatment option in cats.			

(c) Availability of other treatments for identified uses outside the SPC in animals is limited. Clindamycin is the preferred treatment for toxoplasmosis in cats. Puppies affected by *N. caninum* may alternatively be treated with clindamycin or trimethoprim+sulfonamides. (d) Clindamycin, spiramycin or atovaquone are alternatives for treatment of toxoplasmosis in humans (off-label). (e) No conditions are proposed on use outside the terms of the marketing authorisation and pyrimethamine cannot be used in food-producing animals; therefore no impact is expected on aquaculture and farming. **Recommendation**: No conditions proposed on use outside the terms of the marketing authorisation.			
(Hydroxy)Chloroquine	Authorised in HMPs the EU for malaria and amoebiasis. Chloroquine is important for prophylaxis and treatment of susceptible strains of malaria.	No EU VMP authorisation found. Not included in Reg (EU) 37/2010.	Use only allowed in non-food-producing species due to absence of MRL status. Reported to be used (in combination with primaquine) for treatment of avian malaria in penguins. Use reported in 'open call for data' for cryptocariosis (ciliate parasite) in teleosts.
(a) See general comments in Section 3.1. of the advice. No information could be found regarding safety relating to use outside the SPC in animals. In humans, the main toxic effects relate to cardiovascular (quinidine-like effects), respiratory and gastrointestinal effects. Based on reported identified use outside the SPC, administration is likely to be to single or restricted numbers of animals. (b) Although resistance to chloroquine is problematic in malaria in humans, no reports of resistance were found relating to potential uses outside the SPC in animals and the cited diseases are not zoonotic; therefore no clear pathway for transmission of resistance from animals to humans. (c) Availability of other treatments for identified uses outside the SPC reported in animals could not be found. (d) Not applicable as resistance to chloroquine in human malaria is not related to veterinary use. (e) No conditions are proposed on use outside the terms of the marketing authorisation and (hydroxy)chloroquine cannot be used in food-producing animals; therefore no impact is expected on aquaculture and farming. **Recommendations**: No conditions proposed on use outside the terms of the marketing authorisation.			
Fumagillin	Authorised in HMPs the EU for microsporidiosis (*Enterocytozoon bieneusi*)	No EU VMP authorisation found. Not included in Reg (EU) 37/2010	According to Regulation (EU) 37/2010, MRLs are not available; therefore, fumagillin can only be used under in non-food-producing animals. Has been used historically to treat *Nosema* spp. in honeybees.
Evidence was found relating to use in honeybees, only, in veterinary medicine. In the absence of MRL status, fumagillin cannot be used in food-producing species; therefore, this substance is not considered further [710].			
Halofuginone (quinazolinone derivative)	No EU authorisation found.	VMPs authorised in the EU for: • control of *Cryptosporidium* in calves According To Reg (EU) 37/2010, MRLs for bovines, all edible tissues. 'Other provisions' state not to be used in animals from which milk is produced for human consumption.	Cannot be used in animals from which milk is produced for human consumption. Halofuginone may be used to treat cryptosporidiosis in ruminant species. Halofuginone has also been proposed as a treatment for *Theileria* spp. in goats [711].
(a) See general comments in Section 3.1. of the advice. Target animal safety warnings in the SPCs of authorised VMPs should be followed. Use outside the SPC may involve group medications, as for authorised use. Ruminants are an important reservoir for *Cryptosporidium* spp of zoonotic relevance. (b) No evidence was found for use of halofuginone to treat protozoal infections in humans in the EU. Resistance has been reported to halofuginone in coccidia from poultry, but no reports were found relating to ruminants.			

Considering the anticipated extent and identified indications for use outside the SPC, it is unlikely that related resistance development will impact on authorised use in animals. (c) Paromomycin is an authorised alternative for treatment of cryptosporidiosis in sheep, goats and calves. Sulfonamides/TMPs may also be an alternative. (d) Not applicable. (e) No conditions are proposed on use outside the terms of the marketing authorisation; therefore, no impact is expected on aquaculture and farming. **Recommendation**: No conditions proposed on use outside the terms of the marketing authorisation.			
Decoquinate (hydroxyquinolone)	No EU authorisation found.	VMPs authorised in the EU for: • Toxoplasmosis in sheep • Coccidiosis in lambs and calves (*Eimeria* spp.) According to Reg (EU) 37/2010, no MRLs are required in bovine and ovines. 'Other provisions' state for oral use only; not to be used in animals from which milk is produced for human consumption	Decoquinate may only be administered orally in food-producing species and cannot be used in animals from which milk is produced for human consumption. Use also reported as important to treat coccidiosis (*Eimeria* spp.) in goats [712].
(a) See general comments in Section 3.1. of the advice. Target animal safety warnings in the SPCs of authorised VMPs should be followed. Use outside the SPC may involve group medications, as for authorised use. (b) No evidence was found for use of decoquinate to treat protozoal infections in humans in the EU. Resistance has been reported to decoquinate in coccidia from poultry, but no reports were found relating to ruminants. Considering the anticipated extent and identified indications for use outside the SPC, it is unlikely that related resistance development will impact on authorised use in animals. (c) Triazines (or amprolium) may be an alternative for the treatment of coccidosis (*Eimeria* spp) in goats. (d) Not applicable. (e) No conditions are proposed on use outside the terms of the marketing authorisation; therefore no impact is expected on aquaculture and farming. **Recommendations**: No conditions proposed on use outside the terms of the marketing authorisation.			
Amprolium (thiamine analogue)	No EU authorisation found.	VMPs authorised in the EU for: • Coccidiosis (*Eimeria* spp) in poultry 'No MRL required' for poultry. Other provisions state for oral use only.	Amprolium may only be administered orally in food-producing species. Use reported to treat coccidosis (*Eimeria, Isospora* spp) in young ruminants, dogs, cats and birds including pheasant.
(a) See general comments in Section 3.1. of the advice. Adverse effects in animals include thiamine deficiency, neurological signs including polioencephalomalacia in ruminants (de Sant'Ana 2009). Target animal safety warnings in the SPCs of authorised VMPs should be followed. Use outside the SPC may involve group medications, as for authorised use. (b) No evidence was found for use of amprolium to treat protozoal infections in humans in the EU. Resistance to amprolium has been reported in *Eimeria* spp. from poultry and goats. Considering the anticipated extent and indications for use outside the SPC, it is unlikely that related resistance development will impact on authorised use in animals. (c) Alternatives authorised for treatment of coccidiosis in ruminants include decoquinate (calves, lambs), halofuginone (calves), paromomycin (calves, lambs, goats) and triazines (calves, lambs). In dogs, cats and birds, sulphonamides and triazines may be alternatives. (d) Not applicable. (e) No conditions are proposed on use outside the terms of the marketing authorisation; therefore, no impact is expected on aquaculture and farming. **Recommendation**: No conditions proposed on use outside the terms of the marketing authorisation.			
Amphotericin B (polyene) (see section 6. for use as antifungal)	Amphotericin B is authorised in HMPs in the EU for fungal infections and Leishmaniasis.	No EU VMP authorisation found. Not included in Reg (EU) 37/2010.	Use only allowed in non-food-producing species due to absence of MRL status.

	AmB is the first-line treatment for visceral leishmaniasis.		Amphotericin B is primarily used to treat fungal infections but has also been used to treat leishmaniasis in dogs.
(a) See general comments in Section 3.1. of the advice. Administration of amphotericin B (AmB) is intravenous and therefore will be undertaken by professionals and is likely to be to individual animals. AmB causes dose-related nephrotoxicity. Leishmaniasis is potentially zoonotic. (b) Dogs are the main reservoir for *L infantum* and resistance to AmB has been shown in leishmania. Leishmaniasis is zoonotic hence drug resistant parasites could pass from animals to humans. For this reason, use of AmB in dogs has been discouraged in veterinary practice; however, treatment of sick dogs reduces their infectiveness and euthanasia is of questionable value as a means to control the leishmania reservoir (see Human Reserved List advice [3]). (c) Availability of other treatments for leishmaniasis in animals is limited. Preferred treatments in dogs are meglumine antimonate + allopurinol as first-line, or miltefosine, dependent on disease stage; although resistance may develop. Vaccination in dogs and vector control may be used to prevent infections. AmB is also used to treat certain rare but serious fungal infections in companion animals. (d) Availability of other treatments for leishmaniasis in humans is limited. In humans, sodium stibolgluconate, meglumine antimonate and miltefosine are alternatives and preferred for cutaneous leishmaniasis, but resistance may develop. (e) This substance cannot be used in food-producing animals; therefore, no impact is expected on aquaculture and farming. **Recommendation:** it is proposed that **conditions should be placed on the use of amphotericin B outside the terms of the marketing authorisation** so that in cases where used for treatment of leishmaniasis, or for treatment of other diseases in animals in regions where leishmaniasis is endemic, it is to be **used only as last resort when other treatments have failed**, or can be expected to fail. To date it appears that standardised, clinically-approved drug susceptibility testing platforms for *Leishmania* spp. have not yet been established [713, 714].			
Meglumine antimonate (Pentavalent antimonial)	Authorised in the EU for Leishmania	VMPs authorised in EU for: • Leishmania in dogs Not included in the MRL Reg (EU) 37/2010.	Use only allowed in non-food-producing species due to absence of MRL status. Meglumine antimonate and allopurinol are recommended for sporadic cases of clinical leishmaniasis in cats. Other canids and horses could also be candidates for treatment.
(a) See general comments in Section 3.1. of the advice. Administration is by injection and therefore will be undertaken by trained personnel under veterinary supervision and is likely to be to individual animals. No information is available on toxicity relating to use outside the SPC. Target animal safety warnings in the SPCs of authorised VMPs should be followed. Leishmaniasis is potentially zoonotic. (b) Resistance to meglumine antimonate has been shown in leishmania; however, dogs (authorised) are the main reservoir and there is unlikely to be a significantly increased risk of transfer of resistant parasites to humans resulting from sporadic use in unauthorised species. Considering the anticipated extent of use outside the SPC, it is unlikely that related resistance development will impact substantially on authorised use in animals. (c) Availability of other treatments for leishmaniasis in animals is limited. Alternatives include miltefosine + allopurinol and amphotericin B, dependent on disease stage; although resistance may also develop to these and AmB should be reserved as a last resort treatment due to its importance in human medicine. Vector control may be used to prevent infections. (d) Availability of other treatments for leishmaniasis in humans is limited. In humans, AmB is the first-line treatment for visceral leishmaniasis. Sodium stibolgluconate and miltefosine are alternatives, but resistance may also develop to these. (e) This class cannot be used in food-producing animals; no conditions are proposed on use outside the terms of the marketing authorisation therefore no impact is expected on aquaculture and farming. **Recommendation**: No conditions proposed on use outside the terms of the marketing authorisation.			
Miltefosine	Authorised in the EU for Leishmania	VMPs authorised in EU for: • Leishmania in dogs Not included in the MRL Reg (EU) 37/2010.	Use only allowed in non-food-producing spp. due to absence of MRL status.

			Miltefosine is recommended for sporadic cases of clinical leishmaniasis in cats. Other canids and horses could also be candidates for treatment.
(a) See general comments in Section 3.1. of the advice. No information was found on toxicity relating to use outside the SPC. Animal safety warnings in the SPCs of authorised VMPs should be followed. Administration is likely to be to individual animals. Leishmaniasis is potentially zoonotic. (b) Resistance to miltefosine has been shown in leishmania; however, dogs (authorised) are the main reservoir and there is unlikely to be a significantly increased risk of transfer of resistant parasites to humans resulting from sporadic use in unauthorised species. Considering the anticipated extent of use outside the SPC, it is unlikely that related resistance development will impact substantially on authorised use in animals. (c) Availability of other treatments for leishmaniasis in animals is limited. Alternatives include meglumine antimonate + allopurinol and amphotericin B, dependent on disease stage; although resistance may develop and AmB should be reserved as a last resort treatment due to its importance in human medicine. Vector control may be used to prevent infections. (d) Availability of other treatments for leishmanisis in humans is limited. In humans, AmB is the first-line treatment for visceral leishmaniasis. Sodium stibolgluconate and meglumine antimonate are alternatives, but resistance may develop. (e) This class cannot be used in food-producing animals; no conditions are proposed on use outside the terms of the marketing authorisation therefore no impact is expected on aquaculture and farming. **Recommendation**: No conditions proposed on use outside the terms of the marketing authorisation.			
Allopurinol (xanthine oxidase inhibitors)	Authorised in the EU for gout and hyperuricaemia	No EU VMP authorisation found Not included in the MRL Reg (EU) 37/2010	Use only allowed in non-food-producing species due to absence of MRL status. Use of allopurinol in combination with meglumine antimonate has been reported to treat leishmaniasis in dogs and cats as it may decrease the effects of the parasite in the kidneys and improve skin lesions. Allopurinol may also be used for dogs and other species to treat non-infectious diseases (e.g. uric acid urolithiasis). Use reported in 'open call for data' for leishmania in dogs.
(a) See general comments in Section 3.1. of the advice. Allopurinol may cause dermal hypersensitivity reactions in animals. Administration is likely to be to individual animals. Leishmaniasis is potentially zoonotic. (b) Allopurinol is not authorised or generally recommended to treat protozoal disease in humans in the EU. Resistance to allopurinol has been shown in leishmania; however, it is part of the preferred treatment regimen for this disease in pet animals. (c) Availability of other treatments for leishmaniasis in animals is limited. Alternatives include meglumine antimonate (less effective when used without allopurinol combination), miltefosine and amphotericin B, dependent on disease stage; although resistance may develop to these and the latter should be reserved as a last resort treatment due to its importance in human medicine. Vaccination in dogs and vector control may be used to prevent infections. (d) Not applicable as allopurinol is not authorised or generally recommended to treat protozoal disease in humans in the EU. (e) This class cannot be used in food-producing animals; no conditions are proposed on use outside the terms of the marketing authorisation therefore no impact is expected on aquaculture and farming. **Recommendation**: No conditions proposed on use outside the terms of the marketing authorisation.			
Imidocarb (carbinalide)	No EU authorisation found.	VMPs authorised in the EU for: • Named *Babesia* spp. in horses, cattle and dogs,	Imidocarb cannot be used in sheep producing milk for human consumption.

		• Anaplasmosis (*A. marginale*)(rickettsia) in cattle Regulation (EU) 37/2020 includes MRLs for bovine and ovine edible tissues and bovine milk. 'Other provisions' state not to be used in ovines producing milk for human consumption.	Use reported, including in 'open call for data', for: *Babesia* spp. infections in small ruminants [711] cats, dogs (large form *Babesia* spp.) *Theileria equi* in horses (piroplasmosis) *Hepatozoon canis* in dogs. Use has also been reported for: Treatment of *Theileria* in cattle [715]. Treatment of *Cytauxzoon felis* in cats.
(a) See general comments in Section 3.1. of the advice. Imidocarb is administered by injection and therefore will be handled by trained professionals. Administration is likely to be to single animals. SPCs warnings relating to authorised use indicate cholinergic adverse effects. Injection pain, kidney and hepatic damage are also reported in cats. Animal safety warnings in the SPCs of authorised VMPs should be followed. (b) No evidence was found for use of imidocarb to treat protozoal infections in humans in the EU. Resistance to imidocarb has been reported in *Theileria* [716] and my occur in other protozoal species. Considering the anticipated extent and indications for use outside the SPC, it is unlikely that related resistance development will impact on authorised use in animals. (c) For babesiosis, tetracyclines may be alternatives in horses and cattle; in dogs, diminazene (if available in the EU) and pentamidine are also cited as treatments for large form babesia. Efficacy of different treatments may vary according to the *Babesia* species. Doxycycline or triazenes have been recommended to *Hepatozoon canis* in dogs. In cats, atovaquone in combination with azithromycin has been used to treat *Cytauxzoon felis.* Buparvaquone is proposed as a more effective alternative to treat *Theileria* in cattle, but does not have MRLs; oxytetracycline is reported as less effective. (d) Not applicable. (e) No conditions are proposed on use outside the terms of the marketing authorisation; therefore no impact is expected on aquaculture and farming. **Recommendations**: No conditions proposed on use outside the terms of the marketing authorisation.			
Diminazen(e) (diamidine derivative)	No EU authorisation found.	No EU VMP authorisation found Not included in the MRL Reg (EU) 37/2010.	Use only allowed in non-food-producing species due to absence of MRL status. Has been used to treat (large form) *Babesia* spp. and *Hepatozoon canis* in dogs. In horses it has been used to treat piroplasmosis (*Theileria equi, Babesia caballi*).
(a) See general comments in Section 3.1. of the advice. Diminazene is administered by injection and therefore will be handled by trained professionals. It can cause injection site reactions, neurological and gastrointestinal disturbances and, due to adverse effects, use is not recommended in cats. Administration is likely to be to single animals. (b) No evidence was found for use of diminazene to treat protozoal infections in humans in the EU. Diminazene is mainly used in animals in treatment of African trypanosomiasis and to treat *T.evansi* in dogs. Livestock and companion animals can act as a reservoir for human infections but these diseases are not endemic in the EU. Resistance to diminazene has been reported in trypanosomes (with likely cross-resistance to pentamidine) and babesia but in the EU there is no transmission pathway between animals and humans. (c) For babesiosis, tetracyclines may be an alternative in horses; in dogs, imidocarb and pentamidine are also cited as treatments for large form babesia and are authorised as V/HMPs in the EU. Imidocarb is authorised for use in (non-food) horses in the EU and is therefore more likely to be used to treat piroplasmosis. Doxycycline has been recommended to treat *Hepatozoon canis* in dogs, and triazines may also be used. Imidocarb is preferred over diminazene in dogs as it is less toxic. (d) Not applicable. (e) No conditions are proposed on use outside the terms of the marketing authorisation and diminazene cannot be used in food-producing animals; therefore no impact is expected on aquaculture and farming. **Recommendations**: No conditions proposed on use outside the terms of the marketing authorisation.			
Pentamidine (diamidine derivative)	Authorised in the EU for leishmaniasis (cutaneous) and	No EU VMP authorisation found.	Use only allowed in non-food-producing species due to absence of MRL status.

	African trypanosomiasis (*T. gambiense*) Pentamidine is a second-line treatment for visceral leishmaniasis and for pneumonia caused by *Pneumocystis jirovecii*.	Not included in the MRL Reg (EU) 37/2010	Use reported for babesiosis and leishmaniasis in dogs.
(a) See general comments in Section 3.1. of the advice. Pentamidine is administered by injection and therefore will be handled by trained professionals. Pain and muscle necrosis at injection site and toxicity limit use in dogs. Administration is likely to be to single animals. Leishmaniasis is potentially zoonotic. (b) Resistance to pentamidine has been reported in *Trypanosoma* spp. and in *Leishmania* spp. Animals do not act as a reservoir for trypanosomes in the EU. Although dogs act as a reservoir for *Leishmania* in the EU and could be a source of pentamidine-resistant organisms, pentamidine is a second-line treatment in humans and use in dogs is likely to be infrequent. (c) For large form babesiosis in dogs, imidocarb (and diminazene) are also cited as treatments. Efficacy of different treatments may vary according to the parasite species. Alternatives and preferred treatments for leishmaniasis in dogs include miltefosine and meglumine antimonate + allopurinol; although resistance may develop. Amphotericin B may also be used although it should be reserved as a last resort treatment due to its importance in human medicine. Vaccination and vector control may be used to prevent leishmania infections. (d) Availability of treatments for leishmanisis in humans is limited. Amphotericin B is the first-line treatment for visceral leishmaniasis. Sodium stibolgluconate, miltefosine and meglumine antimonate are alternatives, but resistance may develop. (e) This class cannot be used in food-producing animals; no conditions are proposed on use outside the terms of the marketing authorisation therefore no impact is expected on aquaculture and farming. **Recommendations**: No conditions proposed on use outside the terms of the marketing authorisation.			
Atovaquone (hydroxyquinolone)	Authorised in the EU for malaria (and pneumocystis pneumonia). Atovaquone is used for prophylaxis and treatment of falciparum malaria. It is also an alternative treatment for toxoplasmosis.	No EU VMP authorisation found Not included in MRL Reg (EU) 37/2010.	Use only allowed in non-food-producing species due to absence of MRL status. Combined with azithromycin, atovaquone has been used to treat *Cytauxzoon felis* in cats and *Babesia gibsoni* in dogs, and other refractory protozoal infections.
(a) See general comments in Section 3.1. of the advice. Administration is likely to be to single animals. (b) Resistance to atovaquone has been reported in human malaria parasites, *Cytauxzoon felis* and *Babesia gibsoni* [717, 718]. As these organisms are not zoonotic, there is no risk that this resistance will transmit from treated animals to humans. (c) Limited alternatives. Small form *Babesia* spp. such as *B. gibsoni* are poorly susceptible to alternatives such as imidocarb and diminazene, but these may be effective used in combination with clindamycin [719]. Imidocarb may be an alternative for treatment of *Cytauxzoon felis* in cats. (d) Not applicable as resistance to atovoquone in human malaria is not related to veterinary use. (e) This class cannot be used in food-producing animals; no conditions are proposed on use outside the terms of the marketing authorisation therefore no impact is expected on aquaculture and farming. **Recommendations**: No conditions proposed on use outside the terms of the marketing authorisation.			
Nitrofurans			
Nifurtimox	No EU authorisation found. Nifurtimox is used to treat American trypanosomiasis (*Trypanosoma cruzi*).	No EU VMP authorisation found. Nitrofurans are prohibited substances according to the MRL Reg (EU) 37/2010.	Prohibited from use in food-producing species. Use reported for treatment of *T. cruzi* in dogs (imported to EU).

Furazolidone	Is or has been authorised in the EU for gastrointestinal infections including giardiasis.	Authorised in the EU for dogs, cats and other minor companion animals e.g. pigeons, often in combination with antibiotics, to treat protozoa (*Eimeria, Histomonas* and *Trichomonas* spp.) Prohibited substance according to Reg (EU) 37/2010.	Prohibited from use in food-producing species (Regulation (EU) 37/2010).
(a) See general comments in Section 3.1. of the advice. Administration is likely to be to single animals. (b) Nifurtimox is used to treat American trypanosomiasis (*Trypanosoma cruzi*). Resistance to nifurtimox has been reported in *T. cruzi*; but animals do not act as a reservoir for human infection in the EU as infection is not endemic; therefore, there is no transmission pathway between animals and humans. Use outside the SPC is likely to be very rare. Resistance to furazolidone has been shown in *giardia* spp. There is theoretically a low risk furazolidone-resistant giardia infections could be transmitted from pets to people (c) Limited alternatives for treatment of *T. cruzi*. Benzinidazole (or secnidazole) is an alternative treatment for *T. cruzi* in dogs, also under use outside an SPC. For giardia infections in cats and dogs, benzimidazoles are an (authorised) alternative. (d) Limited alternatives for treatment of *T. cruzi*. Benzinidazole is an alternative for treatment of *T. cruzi* in humans. Availability of antimicrobial treatment options for protozoal infections in humans is generally limited. Albendazole is an alternative for giardiasis. (e) This class cannot be used in food-producing animals; no conditions are proposed on use outside the terms of the marketing authorisation therefore no impact is expected on aquaculture and farming. **Recommendations**: No conditions proposed on use outside the terms of the marketing authorisation.			

8. Conclusion

Summary table of the recommended conditions for antimicrobial classes

Recommended condition	Aminopenicillin-BLI combinations	3rd- and 4th-gen. cephalosporins	Polymyxins	Amphenicols	(Fluoro)quinolones	Rifamycins	TB drugs	Riminofenazines	Pseudomonic acids	Remdesivir	Echinocandins	Amphotericin B
Target pathogen identification and AST	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	
Restricted around certain indication (e.g. Salmonella)		✓	✓		✓	✓			✓			✓
Restricted to use for certain indications only						✓			✓	✓		
Restricted from use in certain species e.g. poultry, aquaculture	✓	✓	✓									
Use in individual animals only		✓				✓	✓	✓	✓			
Restriction on route of administration			✓		✓				✓			
HMPs only for use in individual animals*			✓		✓							

*Conditions on use of VMPs also apply to other formulations, as relevant

For a more detailed overview of the recommended conditions, please refer to the table (b) in the Summary/Recommendations, page 8 of the advice.

Annex

1. Considerations on target pathogen identification and antimicrobial susceptibility testing

For several antimicrobial classes, the following condition is recommended for use outside the terms of the marketing authorisation:

'Use must be based on the results of target pathogen identification and antimicrobial susceptibility testing that demonstrates that <*Class X*> is likely to be effective and that antimicrobials from a lower (AMEG) category would not be effective, unless it can be justified that this is not possible'.

Further comments and exemptions to this condition are noted below.

In addition to knowledge of the animal's medical history and clinical examination, laboratory tests including target pathogen isolation and antimicrobial susceptibility testing (AST) are important for correct diagnosis and decision-making in relation to use of antimicrobials. Hence AST is recommended as a condition, unless it can be justified by the attending veterinarian that it is not possible. It is acknowledged that there should be standardisation of the diagnostic process from sample collection, processing, pathogen identification, selection of isolates and interpretation of AST in veterinary medicine [720]. In the context of the proposed conditions and exemptions, certain aspects are highlighted, below.

- Sampling

The veterinary clinician has the responsibility to collect samples that limit normal microbiota/contaminant growth and that are representative of the disease process in the individual animal or group of animals [721]. In respiratory diseases, for instance, swab samples taken from the trachea or a bronchoalveolar lavage will give more valid results than those from the pharynx, while nasal swabs provide limited diagnostic value [722]. The materials intended for diagnosis should be collected as soon as possible after the onset of clinical disease and preferably before the administration of antimicrobials. Adequate amounts of sample should be collected (in transport medium, if appropriate), stored appropriately for the sample type and transported to the laboratory as soon as possible to maintain sample integrity.

- Target pathogen identification

Identification (ID) of the micro-organism is an important prerequisite to AST to distinguish between potentially pathogenic micro-organisms (there may be more than one causative pathogen in a mixed infection) and possible contaminants from the commensal microbiota on nonsterile body sites [723]. Currently, culture-based isolation and identification are the "gold standard" methods for laboratory detection of veterinary pathogens [724]. However, some pathogens are less or un-amenable to culture (e.g. *Lawsonia* spp., *Chlamydia* spp.) and alternative specialist methods for isolation and/or identification may be required [725, 726]. In addition, molecular tools such as point of care tests (POCTs), gene-based resistance detection platforms, single or multiplex PCR assays, and whole genome sequencing (WGS) are used to detect pathogens or resistance mechanisms.

- Standards for Antimicrobial Susceptibility Testing

AST should be conducted on pure cultures of the isolate(s) considered to have greatest pathogenic potential/clinical significance. The laboratory must test and report the antimicrobial agents that are most appropriate for the organism isolated, for the site of the infection and the animal species involved. Methods used for AST in veterinary medicine include disk diffusion tests, gradient diffusion tests, micro broth dilution or automated systems. Laboratory methods for bacterial antimicrobial susceptibility testing should preferably be validated to international standards (CLSI, EUCAST, ISO 20776-1:2019 [727]). Broth microdilution (or agar dilution for a few antibiotics and anaerobes) is the gold standard for AST. The result of a microdilution test is given as the minimum inhibitory concentration (MIC), a quantitative result that indicates the susceptibility of the tested bacterial strain. MICs should be interpreted according to available criteria.

- Interpretative criteria

Preferably, susceptibility should be determined based on veterinary clinical breakpoints from EUCAST/VetCAST or CLSI; however, it is acknowledged that at present there are few validated veterinary breakpoints available, especially for less common pathogens and minor animal species. Although Veterinary Antimicrobial Susceptibility Testing subcommittees have been established within both the CLSI (-VAST) and EUCAST (VetCAST), there is still a shortage of animal-, infection- and pathogen-specific clinical breakpoints (CBPs) for antimicrobial drugs used in veterinary medicine.

In the absence of veterinary clinical breakpoints for the antimicrobial(s) concerned, then PK/PD breakpoints may be available, or the choice could consider the likelihood of efficacy based on the presence of a wild-type MIC (determined by the ECOFF) and use supported by relevant clinical studies.

- Quality of diagnostic laboratories services

To date, veterinarians may do pathogen isolation and AST themselves or use laboratory services provided by public sector and commercial laboratories, universities or even human hospitals. Individual Member States may have mandatory accreditation requirements in place. The WOAH Terrestrial Manual [728] sets the key considerations for the design and maintenance of a quality management system necessary for effective delivery of a veterinary laboratory diagnostic service.

Further comments and exemptions regarding the recommended condition

For certain antimicrobial classes, it has been recommended that a condition should be applied requiring use outside the terms of the marketing authorisation to be based on the results of target pathogen identification and antimicrobial susceptibility testing. The following points and exemptions should be noted in relation to this condition:

- Identification of target pathogens and AST should be conducted in accredited laboratories, where available.
- A justification for exemption from the condition may be made by the attending veterinarian under certain circumstances, e.g.
  - If the sampling procedure would involve an unacceptable safety risk to the animal (or group of animals), for example, if it would require administration of sedation/anaesthesia that would carry significant risk to the animal's life;
  - If there is no identification/culture system for the pathogen or no reliable means to determine susceptibility. In this case, the presence of the target pathogen should be suspected based on other diagnostic methods e.g. specific microbiology staining, serology;

  - limited market species and sheep should be exempted if the VMP is already authorised for use in a major animal species (see Section 3.1.2.)

- If it is necessary to start treatment whilst waiting for the results of target pathogen identification and/or AST, or there is an exemption as identified above, then use of the antimicrobial should be based on epidemiological information and knowledge of susceptibility of the target bacteria at farm level or at local/regional level.
- If there is a lack of facility at veterinary laboratories to test susceptibility to a specific antimicrobial, then this substance may be used provided that its spectrum of activity is relevant for the identified target pathogen and a lack of susceptibility to less critical alternatives has been demonstrated.

Special note regarding the diagnosis of mycobacterial infections in companion animals and antimicrobial susceptibility testing

- Diagnosis of mycobacterial disease is based initially on cytology/histology, with culture being undertaken to identify the species involved. Culture can only be done in a specialist laboratory, growth may take 2-3 months and culture fails in a high proportion of cases. PCR testing followed by DNA sequencing allows more rapid speciation. Interferon gamma and other immunoassays may also be used. However, it is noted that there is limited and regional variability in access to diagnostic laboratories with the required testing facilities available [551, 552, 554, 556].
- Owing to differences in susceptibility patterns, treatment is most successful when the causative mycobacterium has been speciated so that the drug regimen can be tailored to known inherent resistance and susceptibility patterns. Drug susceptibility testing can be based on culture-based phenotypic methods, which are the gold standard but are time-consuming and require specialist laboratories. Alternatively, rapid genotypic tests can be used [553].
- Considering the potential zoonotic risk of some mycobacterial species, the long treatment courses required and associated risk of development of drug resistance, it is proposed that all reasonable attempts should be made to achieve speciation of the mycobacterial infection; however, it is acknowledged that treatment may need to be started before test results are available.

Special note on the use of AST for pathogens treated topically or locally

Considering the differences in formulations and administration schedules for topically applied antimicrobials and the variation between infection sites in how long concentrations are maintained, EUCAST has proposed that ECOFFs should be used to exclude acquired resistance rather than using breakpoints [729]. It is generally considered that AST may underestimate the activity of topical treatments since they may reach concentrations significantly higher at the site of action than those that can be achieved through systemic treatment; however, it should also be considered that AST cannot account for local factors that can affect antimicrobial activity e.g. presence of pus or low oxygen tension [103, 730]. It has also been suggested that AST may have poor predictive values for intramammary treatment of mastitis in cattle [731]. The testing laboratory should be advised of the intention to treat an infection topically or locally so that the appropriate antibiotics are used in the testing panel and test results are interpreted correctly.

2. Abbreviations

AGP	antibiotic growth promoter
AIDS	Acquired immunodeficiency syndrome
AMEG	Antimicrobial Advice ad hoc Expert Group (EMA)
AMR	antimicrobial resistance
APEC	Avian pathogenic Escherichia coli
AST	antimicrobial susceptibility testing
ATC	anatomical therapeutic chemical classification system
BLI	beta-lactamase inhibitor
CHMP	Committee for Medicinal Products for Human Use (EMA)
CIA	Critically Important Antimicrobials for Human Medicine (WHO)
CoV	coronavirus
CVMP	Committee for Veterinary Medicinal Products (EMA)
DHFR	dihydrofolate reductase
DHPS	dihydropteroate synthase
EARS-Net	European Antimicrobial Resistance Surveillance Network
EC	European Commission
ECDC	European Centre for Disease Prevention and Control
EEA	European Economic Area
EFSA	European Food Safety Authority
EMA	European Medicines Agency
EML	Essential Medicines List (WHO)
EPAR	European Public Assessment Report
ESBL	extended-spectrum beta-lactamase
ESCMID	European Society of Clinical Microbiology and Infectious Diseases
ESVAC	European Surveillance of Veterinary Antimicrobial Consumption
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FDA	The United States Food and Drug Administration
FVE	Federation of Veterinarians of Europe
HBV	hepatitis B virus
HCV	hepatitis C virus
HIA	Highly Important Antimicrobials for Human Medicine (WHO)
HIV	human immunodeficiency virus
HMP	human medicinal product
HPCIA	Highest Priority Critically Important Antibiotics for Human Medicine (WHO)
ICU	intensive care unit
IDWP	Infectious Diseases Working Party
IMI	intramammary infection
ISCAID	International Society for Companion Animal Infectious Diseases
IV	intravenous
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JIACRA	Joint inter-agency antimicrobial consumption and resistance analysis
KPC	*Klebsiella pneumoniae* carbapenemase
LA(MRSA)	livestock-associated MRSA

AGP	antibiotic growth promoter
MDR	multidrug resistant
MERS	Middle East Respiratory Syndrome
MGE	mobile genetic element
MIC	minimum inhibitory concentration
MRL	maximum residue limit
MRSA	methicillin-resistant *Staphylococcus aureus*
MRSP	methicillin-resistant *Staphylococcus pseudintermedius*
MSSA	methicillin-susceptible *Staphylococcus aureus*
PBP	penicillin-binding protein
PCP	pneumocystis pneumonia
PMQR	plasmid-mediated quinolone resistance
RTI	respiratory tract infection
SCC	Staphylococcal cassette chromosome
SPC	Summary of Product Characteristics
spp.	species (plural)
SSTI	skin and soft tissue infection
TB	tuberculosis
TMP	trimethoprim
TMPS	Trimethoprim/Sulfamethoxazole
UTI	urinary tract infection
VCIA	Veterinary Critically Important Antimicrobial Agent (WOAH)
VHIA	Veterinary Highly important Antimicrobial Agent (WOAH)
VIA	Veterinary Important Antimicrobial Agent (WOAH)
VMP	veterinary medicinal product
VRE	vancomycin-resistant enterococci
VRSA	vancomycin-resistant *Staphylococcus aureus*
WHO	World Health Organization
WOAH	World Organization for Animal Health (formerly OIE)
WSAVA	World Small Animal Veterinary Association
XDR	extensively drug-resistant

3. Data sources used for the preparation of the monographs

During the preparation of the monographs, the following data sources were used in addition to the published references listed in Annex 4.

3.1. Background information

EMA/CVMP: Advice on the designation of antimicrobials or groups of antimicrobials reserved for treatment of certain infections in humans - in relation to implementing measures under Article 37(5) of Regulation (EU) 2019/6 on veterinary medicinal products [3]

Examples of substances in the class that are authorised in veterinary and human medicine in the EU

- ATC and ATC(vet) codes: https://www.whocc.no/

Maximum Residue Limit status in the EU according to Regulation (EU) 37/2010

- Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin [29]

Substances/indications in equines out of scope of evaluation for conditions due to listing in Regulation (EC) 1950/2006, as amended by Regulation (EU) 122/2013

- Commission Regulation (EU) No 122/2013 of 12 February 2013 amending Regulation (EC) No 1950/2006 establishing, in accordance with Directive 2001/82/EC of the European Parliament and of the Council on the Community code relating to veterinary medicinal products, a list of substances essential for the treatment of equidae [30, 732]

EU-authorised VMP formulations, based on sales reported to ESVAC; Summary of the main indications, contra-indications

- European Surveillance of Veterinary Antimicrobial Consumption [733]
- Union Product Database: https://www.ema.europa.eu/en/veterinary-regulatory/overview/veterinary-medicines-regulation/union-product-database

Examples of EU-authorised HMP formulations, from Article 57 database

- Public data from Article 57 database: https://www.ema.europa.eu/en/human-regulatory/post-authorisation/data-medicines-iso-idmp-standards/public-data-article-57-database

Existing recommendations

- WHO: Critically important antimicrobials for human medicine : 6th revision [6]
- WHO, AWaRe classification. [7]
- WHO, WHO Model List of Essential Medicines, 22nd List [364]
- WOAH: OIE List of antimicrobial agents of veterinary importance [5]
- EMA: Categorisation of antibiotics in the European Union (EMA/CVMP/CHMP/682198/2017) [8]

3.2. Evaluation

Textbooks

- Giguère, Steeve, John F. Prescott, and Patricia M. Dowling. Antimicrobial therapy in veterinary medicine. John Wiley & Sons, 2013. [33]
- Grayson, M. Lindsay, et al. Kucers' The Use of Antibiotics: A Clinical Review of Antibacterial, Antifungal, Antiparasitic, and Antiviral Drugs, -Three Volume Set. CRC Press, 2018. [285]
- Ettinger, Stephen J., Edward C. Feldman, and Etienne Cote. Textbook of Veterinary Internal Medicine. Elsevier health sciences, 2018. [255]
- Papich, Mark G. Papich Handbook of Veterinary Drugs. Elsevier Health Sciences, 2020. [46]
- Plumb, Donald C. Plumb's veterinary drug handbook: Desk. John Wiley & Sons, 2018. [119]
- Riviere, Jim E., and Mark G. Papich. Veterinary pharmacology and therapeutics. John Wiley & Sons, 2018. [125]
- Schwarz, Stefan, Cavaco, Lina Maria and Shen, Jianzhong. Antimicrobial Resistance in Bacteria from Livestock and Companion Animals, John Wiley & Sons, 2018. [294]
- Smith, Bradford P. Large animal internal medicine. Mosby, 2020. [53]
- Swayne, David E. Diseases of poultry. John Wiley & Sons., 2020 [36]
- Zimmerman, Jeffrey J., et al., eds. Diseases of swine. John Wiley & Sons, 2019. [34]

The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food [28, 59, 734]

EFSA Animal Health Law Scientific opinions: In the context of the Animal Health Law, Regulation (EU) 2016/429, EFSA has assessed AMR bacteria responsible for animal transmissible diseases, with a view to such pathogens being listed for EU action. For this assessment, EFSA conducted an extensive review of literature studies published since 2010 to determine the global state of play of selected resistant bacteria that constitute a threat to animal health. This was used by EFSA experts to identify those bacteria most relevant to the EU. Scientific opinions were developed separately for dogs and cats, horses, pigs, poultry cattle, small ruminants, rabbits and aquatic species. Information on resistance in target pathogens pooled from the European studies has been extracted from EFSA's reports for the purpose of this advice.

- Assessment of animal diseases caused by bacteria resistant to antimicrobials: cattle [55]
- Assessment of animal diseases caused by bacteria resistant to antimicrobials: swine [108]
- Assessment of animal diseases caused by bacteria resistant to antimicrobials: poultry [295]
- Assessment of animal diseases caused by bacteria resistant to antimicrobials: sheep and goats [204]
- Assessment of animal diseases caused by bacteria resistant to antimicrobials: horses [154]
- Assessment of animal diseases caused by bacteria resistant to antimicrobials: dogs and cats [136]
- Assessment of animal diseases caused by bacteria resistant to antimicrobials: rabbits [735]
- Assessment of animal diseases caused by bacteria resistant to antimicrobials: kept fish species [446]

Published literature references, as listed in **Annex 4**, below.

4. References

1. Official Journal of the European Union, *Regulation (EU) 2019/6 of the European Parliament and of the Council of 11 December 2018 on veterinary medicinal products and repealing Directive 2001/82/EC*. 2019: https://eur-lex.europa.eu/eli/reg/2019/6/oj.
2. Official Journal of the European Union, *Commission Implementing Regulation (EU) 2022/1255 of 19 July 2022 designating antimicrobials or groups of antimicrobials reserved for treatment of certain infections in humans, in accordance with Regulation (EU) 2019/6 of the European Parliament and of the Council*. 2022: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32022R1255.
3. EMA/CVMP, *Advice on the designation of antimicrobials or groups of antimicrobials reserved for treatment of certain infections in humans - in relation to implementing measures under Article 37(5) of Regulation (EU) 2019/6 on veterinary medicinal products (EMA/CVMP/678496/2021)*. 2022: https://www.ema.europa.eu/en/documents/regulatory-procedural-guideline/advice-designation-antimicrobials-groups-antimicrobials-reserved-treatment-certain-infections-humans/6-veterinary-medicinal-products_en.pdf.
4. Official Journal of the European Union, *Regulation (EC) No 470/2009 of the European Parliament and of the Council of 6 May 2009 laying down Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin, repealing Council Regulation (EEC) No 2377/90 and amending Directive 2001/82/EC of the European Parliament and of the Council and Regulation (EC) No 726/2004 of the European Parliament and of the Council*. 2009: https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32009R0470.
5. WOAH (formerly OIE), *OIE List of antimicrobial agents of veterinary importance*. 2021: https://www.oie.int/app/uploads/2021/06/a-oie-list-antimicrobials-june2021.pdf.
6. WHO, *Critically Important Antimicrobials for Human Medicine (6th revision) 2018 - Ranking of medically important antimicrobials for risk management of antimicrobial resistance due to non-human use*. 2019: https://www.who.int/publications-detail-redirect/9789241515528.
7. WHO, *WHO AWaRe Classification Database of Antibiotics for evaluation and monitoring of use*. 2019: https://www.who.int/publications/i/item/WHOEMPIAU2019.11.
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