16 April 2026
EMA/CVMP/382370/2025
Committee for Veterinary Medicinal Products

Guidance under Article 141(1)(f) of Regulation (EU) 2019/6 on veterinary medicinal products with regards five substances not included in Commission Implementing Regulation (EU) 2025/901.

Request of 24 November 2025 with reference Ares(2025)10199539

Introduction

In a letter dated 25 November 2025, the European Commission requested the EMA's Committee for Veterinary Medicinal Products (CVMP) to provide guidance pursuant to Article 141(1)(f) of Regulation (EU) 2019/6[1].

The request pertained to scientific issues in relation to five substances (i.e., midazolam, rifampicin, griseofulvin, ketoconazole, and sevoflurane) not included in Commission Implementing Regulation (EU) 2025/901[2], which establishes a list of substances which are essential for the treatment of equine species, or which bring added clinical benefit compared to other treatment options available for equine species and for which the withdrawal period for equine species shall be six months and repealing Regulation (EC) No 1950/2006.

To prepare the guidance, the CVMP appointed one member as rapporteur, supported by a multinational assessment team comprising three additional Committee members.

A draft of this guidance was submitted to the CVMP on 24 February 2026 for discussion.

The CVMP adopted the guidance on 16 April 2026.

[1] 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, available from: https://eur-lex.europa.eu/eli/reg/2019/6/oj/eng
[2] Commission Implementing Regulation (EU) 2025/901 of 19 May 2025 establishing a list of substances which are essential for the treatment of equine species, or which bring added clinical benefit compared to other treatment options available for equine species and for which the withdrawal period for equine species shall be six months and repealing Regulation (EC) No 1950/2006, available from: https://eur-lex.europa.eu/eli/reg_impl/2025/901/oj/eng

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1. Terms of reference and scope

1.1. Request from the European Commission

The European Commission is seeking the guidance of the Committee for Veterinary Medicinal Products (CVMP) pursuant to Article 141(1)(f) of Regulation (EU) 2019/6.

In accordance with Article 115(5) of Regulation (EU) 2019/6, the Commission adopted an implementing act, establishing a list of substances which are essential for the treatment of equine species, or which bring added clinical benefit compared to other treatment options available for equine species and for which the withdrawal period for equine species shall be six months[3].

This request arises from new information recently provided by Member States to the Commission in relation to five substances. That information brings to light elements which warrant consideration from the point of view of the applicable provisions in Commission Implementing Regulation (EU) 2025/901. Given the necessity of ensuring that any subsequent decisions are grounded in the most up-to-date scientific evaluation, the Committee's feedback to the above-mentioned questions is essential.

The Committee's replies to those questions may necessitate amendments to the Annex to Commission Implementing Regulation (EU) 2025/901. Such potential amendments would need to be made within the transitional period provided for in that Regulation in order to ensure legal certainty. In view of that, the Commission expects the replies to the questions below no later than 30 April 2026.

1.1.1. Legal framework

In their letter and its annex, the Commission referred to the applicable provisions in Commission Implementing Regulation (EU) 2025/901. To that effect, the request referred to recital (6) and Article 2 of that regulation, as follows (emphasis as presented in EC's request):

> Recital (6)
>
> '*A substance only qualifies as an 'essential substance'* ***where no satisfactory alternative for the treatment or diagnosis of an indication is available*** *and where the condition would, if untreated, create unnecessary suffering for the animal. A substance only qualifies as 'bringing added clinical benefit'* ***where it provides a clinically relevant advantage based on improved efficacy or safety or a major contribution to treatment or diagnosis. This may be the result, inter alia, of different modes of action, different pharmacokinetic or pharmacodynamic profiles, different lengths of treatment or different routes of administration.***'
>
> Article 2:
>
> '*1. Substances which are essential for the treatment of equine species may be used for the indications specified in the Annex to this Regulation,* ***where no veterinary medicinal product authorised for food-producing animals of the equine species or no medicinal product referred to in Article 113 of Regulation (EU) 2019/6 would yield equally satisfactory results*** *in terms of successfully treating the animal or avoiding unnecessary suffering for the animal.*

[3] Scientific advice under Article 115(5) of Regulation (EU) 2019/6 on veterinary medicinal products, regarding the list of substances essential for the treatment of equine species and for which the withdrawal period for equine species shall be six months. Available here: Scientific and technical recommendations: Veterinary Medicines Regulation | European Medicines Agency (EMA)

> *2. Substances which bring added clinical benefit compared to other treatment options may be used for the indications specified in the Annex to this Regulation and taking into account the alternatives listed in that Annex,* ***where they provide a clinically relevant advantage based on improved efficacy or safety or a major contribution to treatment compared to veterinary medicinal products authorised for food-producing animals of the equine species or to medicinal products referred to in Article 113 of Regulation (EU) 2019/6****.'*

The Commission further indicated that:

> The above establishes that to be considered as essential or as bringing added clinical benefits for the purposes of being included in Commission Implementing Regulation (EU) 2025/901, a substance needs to be compared with substances contained in veterinary medicinal products authorised for food-producing animals of the equine species or in medicinal products referred to in Article 113 of Regulation (EU) 2019/6, i.e. substances listed in Table 1 in the Annex to Commission Regulation (EU) No 37/2010, as amended. Below, those substances are referred to as 'eligible alternatives'.
>
> An important distinction needs to be made from the alternatives named in either Commission Regulation (EC) No 1950/2006 or Commission Implementing Regulation (EU) 2025/901 in respect of the substances listed therein. Those alternatives serve a different purpose, namely, to give the veterinarian a clinical choice in the settings of the exceptional use allowed under those two regulations.

1.1.2. Request

The Commission requested the CVMP to consider the questions annexed to their letter (discussed below in section 2) which pertain to five substances and their relevant indications. In doing so, the CVMP was asked to take into consideration the legal framework and eligible alternatives as outlined, as well as the additional elements provided to the Commission as summarised in the annex to their letter and attached to the communication received by the Agency.

1.2. Clarifications

The Commission's request sets out a clear assessment framework grounded in existing legislation. As indicated in the request, new information recently provided by Member States has brought to light elements that require consideration under the applicable provisions of Commission Implementing Regulation (EU) 2025/901. This exercise is therefore not a reassessment of the Agency's previous scientific advice; rather, it is conducted strictly within the parameters of the established framework. This includes the provisions concerning eligible alternatives, defined as substances contained in veterinary medicinal products authorised for food-producing animals of the equine species or in medicinal products referred to in Article 113 of Regulation (EU) 2019/6 (i.e. substances listed in Table 1 of the Annex to Commission Regulation (EU) No 37/2010). It also includes provisions defining substances which are essential, and substances which bring added clinical benefit, as set out in recital (6) and Article 2 of Commission Implementing Regulation (EU) 2025/901.

As requested by the European Commission, the Committee's advice is prepared solely on the basis of the additional elements provided and summarised in the annex to the Commission's letter, which was attached to the communication received by the Agency. As clarified, where new information has emerged after the publication of the initial scientific advice (cut-off date: July 2024) and is relevant to

the questions posed, it is taken into account. All other data fall outside the scope of this exercise and are therefore not considered.

This approach represents a limitation of the guidance, as it is primarily constrained to the material explicitly cited or annexed in the Commission's request.

The supplemental information provided by the European Commission encompassed a diverse range of published evidence with varying degrees of methodological rigor, alongside expert statements derived from uncontrolled data. The CVMP employed the same objective structured appraisal framework to systematically weigh the reliability of each piece of evidence based on its methodological rigor and potential for bias and confounding effects. This evaluation framework prioritised evidence from systematic reviews and meta-analyses, as the most robust indicators for minimising bias of available data. Randomised controlled trials (RCTs) are also considered high-quality evidence due to their ability to isolate causal effects by controlling for confounding variables. Observational studies, such as cohort and case-control studies, are valuable for identifying correlations, though they are recognised as being less robust than RCTs. Additionally, case series and case reports serve to identify emerging trends but cannot demonstrate comparative efficacy; similarly, expert opinions reflect clinical experiences but are based on uncontrolled data/observations. This systematic approach to evaluating evidence is consistent with the methodology applied for the provision of scientific advice under Article 115(5) of Regulation (EU) 2019/6, as well as with other scientific work undertaken by the CVMP.

With regard to the potential risk for consumers when these substances are used in food producing animals of the equine species and a six-month withdrawal period is applied, it is notable that some substances included in Regulation (EC) No 1950/2006 are no longer recommended for inclusion on the list. The original list was established two decades ago. At that time, the accepted position was that a uniform six-month withdrawal period would ensure adequate consumer protection. Since that time, attitudes towards risk tolerance have evolved and, in line with this, Commission Implementing Regulation (EU) 2025/901 emphasises the need to ensure a high level of consumer protection and that substances listed do not pose an unacceptable risk to consumers when used in food-producing animals of the equine species and a six-month withdrawal period is respected. Accordingly, the committee scrutinised the pharmacological and toxicological profile of candidate substances taking, as its starting point, contemporary requirements for drugs used in food producing animals, and applying current approaches to hazard characterisation and management of uncertainty with regard to consumer safety. The evolution of expectations and standards has, for a minority of substances, resulted in different recommendations.

2. Guidance from the CVMP in relation to the Commission's questions on five substances not included in Commission Implementing Regulation (EU) 2025/901.

2.1. Midazolam

Indication: premedication and induction of anaesthesia, mild tranquilisation with minimal cardiovascular and respiratory side effects

2.1.1. Question 1:

The additional information received by the Commission underlined that, as the Agency's scientific advice (EMA/CVMP/159047/2023-Corr.2) established that '[*m*]*idazolam is a benzodiazepine whose effects can be compared to those of diazepam',* midazolam is expected to compare in the same way with the eligible alternatives as other benzodiazepines.

Could the Committee confirm if midazolam, being a benzodiazepine and due to its specific properties, compares favourably with the eligible alternatives in view of its mode of action at the GABA receptor level and tranquilisation without cardiorespiratory depression which cannot be produced by those alternatives, particularly α-2 agonists?

Scientific rationale for CVMP's answer:

For the Committee to confirm if midazolam compares favourably with the eligible alternatives, the request clearly establishes what the eligible alternatives for comparison are for this exercise, i.e. *substances contained in veterinary medicinal products authorised for food-producing animals of the equine species or in medicinal products referred to in Article 113 of Regulation (EU) 2019/6, i.e. substances listed in Table 1 in the Annex to Commission Regulation (EU) No 37/2010, as amended*.

It follows that, from those mentioned in the Agency's scientific advice, the eligible alternatives are of the α-2 agonist class: detomidine and xylazine. The CVMP had previously indicated that when α-2 agonists are compared to the benzodiazepine pharmacological class, despite α-2 agonists being available in Table 1 with VMPs authorised for food-producing animal of the equine species, *however, its [benzodiazepine] mode of action at the GABA receptor level and unique tranquilisation without cardiorespiratory depression cannot be produced by alternatives, particularly α-2 agonists*. The Committee had also indicated that *midazolam is a benzodiazepine whose effects can be compared to those of diazepam in terms of sedative, hypnotic, anxiolytic, anticonvulsant and muscle relaxant properties. Due to its lipid solubility, its onset is faster with a shorter duration of action due to its fast metabolism*.

CVMP's answer:

The Committee can therefore only confirm that midazolam compares favourably with α-2 agonists in view of its mode of action at the GABA receptor level and tranquilisation without cardiorespiratory depression.

Indication: short-term anticonvulsant for treatment of seizures[4]

2.1.2. Question 2:

The Agency's scientific advice acknowledges that (emphasis added) '[*m*]*idazolam is a benzodiazepine whose effects can be compared to those of diazepam in terms of sedative, hypnotic, anxiolytic, anticonvulsant and muscle relaxant properties (Nordt and Clark, 1997). Due to its lipid solubility, its onset is faster with a shorter duration of action due to its fast metabolism (Mason, 2004; Morant, 2004)*'.

The addendum to the scientific advice reiterates that (emphasis added) "*Midazolam exhibits similar pharmacologic actions as other benzodiazepines; subcortical levels (primarily limbic, thalamic, and hypothalamic) of the CNS are depressed, which produces anxiolytic, sedative, skeletal muscle relaxant, and anticonvulsant effects.*"

Neither the scientific advice nor the addendum to it discuss the indication 'short-term anticonvulsant for treatment of seizures' in respect of midazolam.

However, the additional information recalled those anticonvulsant properties.

As outlined above, benzodiazepines have a common mode of action and similar clinical effects. The scientific advice and the addendum to it recognise that similarities exist between the effects of diazepam and those of midazolam, including in respect of their anticonvulsant properties.

Could the Committee confirm that the conclusions reached for diazepam with respect to the use as short-term anticonvulsant are equally valid for midazolam, that is to say that no satisfactory eligible alternatives to midazolam exist for the indication 'short-term anti-convulsant for treatment of seizures'?

Scientific rationale for CVMP's answer:

In the Agency's scientific advice, diazepam was identified as a *well-recognised sedative/ataractic/neuroleptic substance that has been largely used in equine practice. It is a second generation antiseizure medication with a better clinical profile compared to alternatives such as carbamazepine or primidone*. No alternatives were identified and diazepam was proposed to be qualified as essential for short-term anti-convulsant treatment of seizures.

Midazolam was also discussed for the indication as anti-convulsant for treatment of seizures, particularly in adult horses with tetanus, as mentioned in Regulation (EU) 1950/2006. Diazepam was alluded when the Committee did not propose midazolam to be qualified as essential, nor as bringing added clinical benefit for this indication, since it considered *clinically questionable that a short-acting agent be required as a muscle relaxant for treatment of tetanus or seizures, when a long-acting agent is more clinically useful in these cases*.

With reference to recital (6) and Article 2 of Commission Implementing Regulation (EU) 2025/901, no satisfactory eligible alternatives to midazolam exist for the indication 'short-term anti-convulsant for treatment of seizures'.

CVMP's answer:

The Committee can therefore only confirm that no satisfactory eligible alternatives to midazolam exist for the indication 'short-term anti-convulsant for treatment of seizures'.

[4] In the annex to the European Commission's request, questions 2 and 3 are presented together. Because question 3 is contingent on the answer to question 2, this guidance document separates them for clarity and ease of reference.

2.1.3. Question 3:

In the affirmative, considering the specific pharmacokinetic profile of midazolam and use as CRI, could the Committee confirm if midazolam would also be essential for ensuring more prolonged control of seizure in foals?

Scientific rationale for CVMP's answer:

The CVMP had previously proposed that midazolam should not be qualified as essential, nor as bringing added clinical benefit. However, additional elements were provided to the Commission and presented for assessment. This assessment is based solely on the additional elements appended to the Commission's request, as requested by the Commission.

With reference to the definition of an "essential" substance provided for in Article 2(1) of Commission Implementing Regulation (EU) 2025/901, the additional information provided does not identify any veterinary medicinal product authorised for use in food producing equine animals, nor any medicinal product referred to in Article 113 of Regulation (EU) 2019/6, that could serve as an alternative to midazolam for seizure control in foals. The Committee likewise did not identify any such alternative. A scientific discussion of the elements submitted for 'more prolonged control of seizure in foals' is provided below.

The additional elements contain both published evidence of varying levels of quality and expert statements. Of the relevant publications submitted for this question Eather et al. (2017) is a case series, descriptive in nature and without a control group. Cole et al. (2015) and the expert opinions and consensus expressed in the additional information describe experience based on uncontrolled data.

Some of these additional elements presented are purported to pertain to *the specific pharmacokinetic profile of midazolam and use as CRI for ensuring more prolonged control of seizure in foals*. However, it is worth noting from the outset that the vast majority of these additional elements and statements do not inform about the question posed by the Commission. These statements, which are considered to rely heavily on 'expert consensus' statements, are also noted as being supported by the Member States that submitted additional elements to the Commission. When it comes to ensuring more prolonged control of seizure in foals, the consensus statements are not supported by controlled data from published references.

A book chapter outlines a dose of 0.02-0.1 mg midazolam/kg/h, IV, CRI for 'longer-term seizure control', but it is unclear as to the basis for this recommendation (Cole et al., 2015). A warning to use the lowest possible dose is also indicated, and midazolam is also not to be used if there is increased intracranial pressure. Also, it is recognised that midazolam may not be effective in all cases (Cole et al., 2015). As previously recognised in the Agency's advice, rapid intravenous administration of midazolam can cause hypotension and a period of apnoea. Cole et al. (2015) states that both diazepam (substance not fulfilling the definition for 'eligible alternative' provided in Commission Implementing Regulation (EU) 2025/901, as stipulated in the request) and midazolam can be administered as CRI, and since midazolam bioaccumulates less than diazepam, it is considered to be more conducive to this route of administration at a rate of 0.02–0.1 mg/kg/h. However, it is important to note that the purported prolonged control of seizures will last only until CRI is discontinued.

The additional elements refer to the proceedings of the Bain Fallon Memorial Lectures, where Eather et al. (2017) presented a case series of use of midazolam CRI in foals with neonatal encephalopathy. The document recognises the need for different therapeutic options in such cases, and the use of IV CRI midazolam for rapid titration to obtain the effective dose to provide seizure control. Results indicate

that IV CRI midazolam was of some use for seizure control in foals, but the authors present limited information about clinical benefits for prolonged seizure control.

Other additional elements provided do not inform about the question posed by the Commission: Ambrisko (2011) investigated changes in the distribution of ventilation and regional lung compliance in five anaesthetized warm-blooded horses and reported increasing proportions of distributed tidal volume in left lung regions during alveolar recruitment manoeuvre (ARM); Auer & Moens (2000) evaluated in a prospective clinical study the eyeball position under relaxation with rocuronium during general anesthesia; Huber (2009) investigated in a doctoral thesis the number of attempts to standing after partial intravenous anaesthesia (PIVA) and the quality of recovery phase by using "head and tail ropes" technique; Hopster et al. (2013) and Müller et al. (2017) compared the sedation protocols for standing procedures (tooth extraction), showing that sedation quality was improved and less romifidine was needed when adding ketamine or midazolam to a romifidine-butorphanol based sedation protocol with less chewing during the procedure in the midazolam group, but resulting in increased ataxia; Müller et al. (2017) also reported an improvement of sedation quality and better surgical conditions for tooth extraction when using the combination of romifidine and ketamine; Kushiro et al. (2005) examined the anaesthetic sparring and cardiovascular effects produced by midazolam/ketamine/medetomidine drug infusion during sevoflurane in oxygen (MKM-OS) anaesthesia in six healthy horses; Hubbell et al. (2013) investigated in a randomized crossover study the pharmacokinetics of midazolam after intravenous administration to horses unrelated to seizure control.

A critical requirement for a safe and effective Continuous Rate Infusion (CRI) is that the drug must remain in solution throughout the entire administration process – from the pharmacy preparation to the IV bag for CRI, and ultimately into the patient's bloodstream. As previously stated in the Agency's advice, the often-cited water solubility of midazolam refers to the substance prior to its use when it still has an acidic pH, and not, as argued, under basic neutral pH conditions after intravenous administration. The water solubility properties of midazolam are pH-dependent depending on whether midazolam's imidazobenzodiazepine ring is open or closed (Kanto, 1985). In acidic conditions (pH less than 4), the imidazobenzodiazepine ring is open, resulting in water solubility; however, at physiological pH, the imidazobenzodiazepine ring closes and midazolam becomes lipophilic (not water soluble), which accounts for its rapid onset of action. Upon dilution/mixing into common intravenous fluids for CRI, as well as in the foal's body the pH of the mixture rises and the imidazobenzodiazepine ring closes, causing midazolam to become water insoluble and more lipophilic. This can lead to precipitation of the drug within the IV bag or tubing before it even reaches the patient, as well as in the patient. If the drug precipitates, the patient is not receiving the intended dose, which is counter-intuitive for prolonged seizure control. This also explains why acidic midazolam formulations can be irritating with intramuscular injection, potentially causing pain at the injection site, as the acidic solution will persist longer in muscle tissue before being buffered.

From a scientific perspective, the additional information provided makes the case that the pharmacokinetic profile of midazolam and use as CRI and more prolonged control of seizure in foals supports it being essential. This statement, which relies heavily on 'expert consensus', is also noted as being supported by the Member States that submitted additional elements to the Commission. Moreover, the additional information emphasises the faster onset midazolam and shorter duration of action indicating clinical benefits in terms of better controllable anaesthesia or sedation in general and for foals more specifically, for instance when CRI (constant rate infusion) is used. However, this 'expert consensus' relied upon in the additional information is scarcely supported by the scientific data provided in the accompanying publications.

The only additional published evidence provided in support of the suitability of midazolam for use as CRI for more prolonged seizure control in foals is provided by Cole et al. (2015) and Easter et al. (2017). It is unclear as to what is meant by 'prolonged' seizure control, but to be clear, midazolam is a short-acting substance and thus efficacy is unlikely after cessation of the CRI. The additional evidence provided suggests midazolam could be used in connection with a CRI.

With reference to the definition of an "essential" substance provided for in Article 2(1) of Commission Implementing Regulation (EU) 2025/901, the Committee did not identify any satisfactory alternative fulfilling the definition provided.

CVMP's answer:

Based strictly on the assessment of the specific additional information and publications provided by the Commission, the additional evidence provided suggests midazolam could be used in connection with a CRI. Midazolam is a short-acting substance and thus efficacy is unlikely after cessation of the CRI.

With reference to the definition of an "essential" substance provided for in Article 2(1) of Commission Implementing Regulation (EU) 2025/901, the Committee did not identify any satisfactory alternative fulfilling the definition provided for the suggested use. This conclusion is drawn adhering to the restrictions provided by the European Commission's request and the limited additional elements attached to it.

2.2. Rifampicin

Indication: treatment of [moderate to severe] *Rhodococcus equi* infections[5]

2.2.1. Question 4:

The Agency's advice acknowledges that rifampicin is a '*broad-spectrum, concentration or time-dependent, bactericidal and/or bacteriostatic antibiotic, with activity mainly against mycobacteria, Gram-positive and facultative anaerobic organisms (Wilson et al., 1988). For example, rifampicin demonstrates bacteriocidal, concentration dependent activity against Mycobacterium tuberculosis (Yamori et al., 1992), whereas rifampicin demonstrates bacteriostatic, time-dependent activity against Rhodococcus equi (Giguère et al., 2012).*' The Agency's scientific advice further states that '*Rhodococcus equi infections, may be life-threatening in the severe form of the disease. The severe form of the disease can cause unacceptable suffering of the animal. It does pose a risk for public health since R. equi is considered zoonotic*.'

It is also worth noting that the World Organisation for Animal Health List of Antimicrobial Agents of Veterinary Importance of June 2024 lists rifampicin as a Veterinary Highly Important Antimicrobial Agent as '*Rifampicin is essential in the treatment of Rhodococcus equi infections in foals.*'

Could the Committee confirm that rifampicin, used in combination with a macrolide, remains a necessary component of the treatment of moderate to severe *Rhodococcus equi* infections in foals and provides an added clinical benefit in view of: its properties helping with better treatment outcomes due to mutant prevention thus improving efficacy; its route of administration preserving animal welfare by avoiding the need for repeated injections over the long periods of time required for treatment and associated side effects thus improving safety?

Scientific rational for CVMP's answer:

[5] In the annex to the European Commission's request, questions 4 and 5 are presented together. Because question 5 is contingent on the answer to question 4, this guidance document separates them for clarity and ease of reference.

For the Committee to confirm that rifampicin brings certain added clinical benefits, the Commission identified eligible alternative substances from those captured in the Agency's scientific advice, such as doxycycline, tulathromycin, gamithromycin, erythromycin, since these are listed in Table 1 in the Annex to Commission Regulation (EU) No 37/2010 for *Equidae*. The CVMP had previously proposed that rifampicin should not be qualified as essential, nor as bringing added clinical benefit. However, additional elements were provided to the Commission and presented for assessment. This assessment is based on the additional elements appended to the Commission's request, as requested by the Commission.

These additional elements contain both published evidence of varying levels of quality and expert statements. From the submitted references, Wetzig et al. (2019) presents clinical evidence from Randomised Controlled Trials (RCT), providing an *in vivo* assessment of rifampicin concomitant therapies as well as the azithromycin-doxycycline combination in afflicted foals. Also, historical support for rifampicin concomitant therapies is supplemented by case series (e.g., Hillidge, 1987), which are observational studies more prone to confounding variables. Pharmacokinetic data, such as the peer-reviewed study by Berlin et al. (2017) and the non-peer-reviewed Master's thesis by Leventhal (2021), are case series data useful for understanding drug distribution; in the case of Leventhal, these data is further weakened by the use of an adult horse model for a neonatal disease. Finally, laboratory-based *in vitro* research (e.g., Berghaus et al., 2013; Giguère et al., 2015) and expert consensus summaries (e.g., Bordin et al., 2022; Havemeyer Workshop, 2024) were provided, representing "bench" data or individual clinical experience rather than objective, controlled patient outcomes.

Since the CVMP publication of the scientific advice in 2024 for the essential substances for Equidae, several new publications have provided important clinical and pharmacokinetic observations that support concerns about the continued use of rifampicin in foals. Baptiste et al. (2025) provides evidence through a systematic review and meta-analysis, that critically challenges the long-held belief of rifampicin's necessity by demonstrating that its clinical benefits are not superior to eligible alternatives. This is further supported by case series safety data from Murdock et al. (2025) and Divers (2025), which document serious clinical risks, reporting acute hepatitis and hemolysis in foals treated with rifampicin-doxycycline combinations. Mechanistically, Huguet et al. (2024) and Huguet et al. (2025) provide updated case series laboratory research using advanced microscopy and concentrations achievable in foals. These studies confirm that while macrolide-doxycycline combinations are highly effective, the addition of rifampicin does not significantly increase the bacterial killing rate, suggesting that the traditional reliance on rifampicin may be based on outdated methodologies that do not reflect *in vivo* conditions.

The Committee is requested to confirm that rifampicin, used in combination with a macrolide, remains a necessary component of the treatment of moderate to severe *Rhodococcus equi* infections in foals and provides an added clinical benefit in view of: its properties helping with better treatment outcomes due to mutant prevention thus improving efficacy; its route of administration preserving animal welfare by avoiding the need for repeated injections over the long periods of time required for treatment and associated side effects thus improving safety. Several other points and references are also mentioned in this section.

The Agency's advice already discussed the combination treatment of rifampicin with a macrolide is not necessary for the treatment *Rhodococcus equi* infections in foals. Rifampicin concomitant therapies for *R. equi* in foals have been the subject of several published research studies over the last 15 years. Such published studies include review articles, PK/PD studies, surveys of rifampicin concomitant therapies selecting for global AMR, as well as randomised clinical trials (RCTs), all elucidating new information and raising concerns about rifampicin concomitant therapies for *R. equi* in foals. The

expert opinions stating that rifampicin concomitant therapies "remains a necessary component of the treatment of moderate to severe *Rhodococcus equi* infections in foals" does not reflect the complexity of the wealth of current published evidence.

The World Organisation for Animal Health (WOAH) lists rifampicin as a Veterinary Highly Important Antimicrobial Agent. It is noted that WOAH's methodology for this list is based on a survey among its member states, rather than a science-based assessment, i.e., a different set of criteria is used to define essentiality. The relevance of this information for the response is thus very limited. WOAH does not categorise rifampicin as Veterinary Critically Important Antimicrobial Agents since only one of their non-science criteria are met. The European Medicines Agency's Antimicrobial Expert Group (AMEG) places rifampicin in the 'Avoid' category for veterinary medicine, indicating concerns about its use.

The question focuses on "moderate to severe" *R. equi* infections in foals. However, for *R. equi* pneumonia, the traditional clinical "mild, moderate, severe" triad is blurred, and there are inconsistent field definitions to qualify "severe" *R. equi* infections in foals. The Agency's advice already elaborated on current practices for routine screening and categorisation of clinical cases. As such, there is no recognised category of "moderate" *R. equi* bronchopneumonia, just mild-to-moderate, and no 'moderate' definition was provided by the Member states.

The diagnosis and management of *R. equi* has undergone a paradigm shift over the last two decades with the widespread adoption of thoracic ultrasonography for detection of pulmonary lesions (e.g. abscess), with changing thresholds for initiating rifampicin concomitant therapies that departs from good antimicrobial stewardship. This includes a move away from clinical examinations with transtracheal wash for *R. equi* culture/susceptibility, and radiographs to the routine screening of foals with thoracic ultrasound only, without culture/susceptibility samples. Ultrasonographic screening and RCTs have defined three groups of *R. equi* bronchopneumonia in foals: 'subclinical' as the most common form (> 50% of cases), 'mild-to-moderate' and 'severe' *R. equi* bronchopneumonia. Consequently, the popularisation of thoracic ultrasonographic screening has substantially increased antimicrobial prescriptions of foals with subclinical, or mild-to-moderate *R. equi* pneumonia resulting in overuse of critically important antimicrobials. For example, Wetzig et al. (2020) performed an RCT in foals with mild-to-moderate *R. equi* bronchopneumonia and found 73.1% of the untreated foals from the control group with an abscess score from 10 to 15 cm recovered without antimicrobials.

A recently published meta-analysis by Baptiste et al. (2025) compared RCTs in naturally infected foals with subclinical and mild-to-moderate *R. equi* bronchopneumonia. Results of these RCTs were split into two broad categories, including monotherapy groups (e.g., macrolide or doxycycline) as well as concomitant therapy groups (e.g., rifampin plus a macrolide or doxycycline), and compared to respective placebo groups. Meta-analysis revealed no statistically significant difference in outcomes ($p = 0.28$) between monotherapy versus rifampin concomitant therapy from foals with subclinical or mild-to-moderate pulmonary abscesses, regardless of initial abscess score (no added clinical benefit with rifampicin).

There is still no single, universally standardised clinical definition for the "severe" category. Also, no definition is provided by the Member states. Since ultrasound is the current primary diagnostic tool, then disease severity is usually determined by the total abscess score (the sum of the maximum diameters of all identified lung lesions). Thoracic ultrasound abscess lesion scores have not identified an "ideal cut-off point" for severe *R. equi,* with scores > 15 cm or > 20 cm frequently quoted in the literature. The meta-analysis by Baptiste et al. (2025) included RCTs with thoracic ultrasound abscess lesion scores in foals up to 20 cm. Thoracic ultrasound can only scan the peripherial lung surface and not deeper lung tissues, as well as the fact that individual foal susceptibility varies; as such there is no single lesion score that can differentiate foals that will recover from those requiring antimicrobial

intervention. Unlike subclinical or mild-to-moderate cases, severe cases are characterised by overt respiratory distress, including tachypnea (increased respiratory rate), dyspnea (increased effort), persistent fever, lethargy, failure to thrive or nursing poorly. On that note, the Havemeyer Workshop on *Rhodococcus equi* in foals represents the gathering of global experts in this field to discuss these issues (i.e., expert opinions). The 6th Havemeyer Workshop (2024) concluded to not focus on severity descriptions of *R. equi* bronchopneumonia (subclinical, mild-to-moderate, severe) and proposing characteristics of infected foals that should be treated with antimicrobials – "the current evidence suggests that treatment only be initiated in foals with: (1) an abscess score greater than 20 cm, (2) fever >39.5_C for more than 2 days and pulmonary abscesses, or (3) dyspnoea and pulmonary abscesses.". It must be noted that the foal characteristics laid down by the Workshop has not been tested in RCTs, compared to a monotherapy group. Also, these proposed characteristics are inconsistent with good antimicrobial stewardship as taking samples are not advocated for culture and susceptibility.

Thus, there are inconsistent definitions as to what constitutes 'severe' *R. equi*. Regardless, foals with severe *R. equi* bronchopneumonia represent a gap in the scientific literature where RCTs have not been performed comparing rifampicin concomitant therapies with eligible alternatives. Moreover, rifampicin acts as a "perpetrator drug" that undermines the efficacy of co-administered macrolides due to well known *in vivo* antagonistic drug interactions. As pointed out in the previous CVMP advice on rifampicin there are important PK/PD characteristics of rifampicin concomitant therapies that represent barriers to clinical success, including:

- Strong *in vivo* antagonistic interactions occur between rifampicin and macrolides, preventing therapeutic macrolide target site concentrations. Rifampicin is a nuclear pregnane X receptor activator, resulting in strong negative drug interactions, affecting both its own absorption and that of other drugs via upregulation of presystemic elimination mechanisms (e.g., intestinal and hepatic CYP3A4), functional drug-absorption carriers (e.g., intestinal P-glycoprotein), and/or inhibition of intestinal or hepatic uptake carriers (e.g., OATP1B1, OATP2B1, MRP2). Specifically, rifampicin is a potent inducer of intestinal P-glycoproteins and an inhibitor of intestinal and hepatic OATPs. Macrolides are substrates of intestinal P-glycoproteins; thus, rifampicin induction leads to P-glycoproteins-mediated efflux of macrolides back into the intestinal lumen, reducing oral bioavailability.
- Rifampicin administration over time decreases serum and target site concentrations of many drugs, including itself. Rifampicin induces metabolic enzymes (e.g., CYP450) and transport proteins involved in its own disposition (auto-induction) and that of other antimicrobials (e.g., macrolides). Enzyme induction may occur within 2.5 days of therapy and persist for >2 weeks after discontinuation. Hepatic enzyme auto-induction from multiple rifampicin doses reduces peak serum concentrations and half-life (t½). Systemic exposure of rifampicin decreased by ~60% after chronic oral treatment, due to upregulation of presystemic and systemic elimination. Concomitant treatments (e.g., rifampicin and macrolide) result in elevated concentrations rifampicin and macrolide metabolites (e.g., 25-O-desacetylrifampicin), which are bioactive but generally less potent. MICs of these metabolites against *R. equi* are unknown, so their contribution to clinical efficacy remains unclear.
- Macrolides inhibit uptake transporters (e.g., OATP1A2, OATP1B1, OATP1B3, OAT2), efflux carriers (e.g., P-glycoprotein) and drug-metabolising enzymes (e.g., CYP3A), thereby influencing rifampicin pharmacokinetics. Rifampicin is an OATP1B1 substrate, whereas some macrolides are an inhibitor, leading to reduced rifampicin exposure (AUC) during co-administration.

Gamithromycin significantly lowers rifampicin plasma concentrations likely by inhibiting an unknown intestinal uptake mechanism.

Furthermore, rifampicin has been found to possess potent clinical immunosuppressive effects in humans and animal models. Rifampicin inhibits innate immune function and phagocytic activity through a direct molecular target for binding to myeloid differentiation protein-2 (Baptiste et al., 2025).

The question posed specifically asks if rifampicin concomitant therapies represent an added clinical benefit in view of:

- its properties helping with better treatment outcomes due to mutant prevention thus improving efficacy;
- its route of administration preserving animal welfare by avoiding the need for repeated injections over the long periods of time required for treatment and associated side effects thus improving safety.

With regards *its properties helping with better treatment outcomes due to mutant prevention thus improving efficacy*:

The term "mutant prevention" is not known in clinical studies, where it is assumed this refers to "mutant prevention concentrations" (MPC), defined as an *in vitro* test to determine the lowest antibiotic/s concentration that can prevent all single-step bacterial resistant mutants within a large population size. However, neither mutant prevention nor MPCs have been described as a marker of antimicrobial efficacy, nor used in RCTs to determine treatment outcomes. MPCs along with minimum inhibitory concentrations (MICs) have been previously used as *in vitro* indicators of antimicrobial resistance prevention or synergy between antimicrobial combinations. However, it is noted that antimicrobial resistance considerations are deemed out of scope for this request. Berghaus et al. (2013) found that of 10 antimicrobial agents studied against *R. equi*, rifampicin had the highest MPC, indicating that rifampicin monotherapy is very likely to select for resistance. However, combining rifampicin with erythromycin, clarithromycin, or azithromycin resulted in a significant decrease in MPC for *R. equi* (Berghaus et al., 2013). Lower *R. equi* MICs and MPCs for rifampicin-macrolide combinations are largely the basis for the belief that rifampicin-macrolide combinations are synergistic. However, MICs and MPCs are no longer recommended to explore antimicrobial synergy; modern preferred *in vitro* methods include checkerboard assays (determination of MICs for each antibiotic in combination) as well as bacterial time-kill assays. Checkerboard assays rely on inhibition of visible bacterial growth at a single time point, whereas time-kill assays assess the dynamic activity of the tested drug combination at multiple time points. Using these *in vitro* methods, Huguet et al. (2024) found that checkerboard analysis of *R. equi* against the rifampicin-macrolide combinations were indifferent (i.e., no synergy, antagonism or additive effect). Also, time-kill assays demonstrated only minor differences in the extent of bacterial kill, regardless of whether antimicrobials were tested individually or in combination. These other *in vitro* experiments provide evidence contrary to previously held beliefs that rifampicin-macrolide combinations are synergistic against *R. equi*. Although checkerboard analysis and time-kill assays are preferred *in vitro* methods to explore antimicrobial synergy, there is no true gold standard. While it is interesting that several *in vitro* synergy testing methods incorporate physiological antimicrobial concentrations, these data do not inform as to the clinical outcomes that correlate with these methods (Baptiste et al., 2025). Furthermore, Baptiste et al. (2025) performed a meta-analysis of published RCTs, and found rifampicin concomitant therapies do not demonstrate a significant advantage over monotherapy with macrolides, for subclinical, and mild-to-moderate *R. equi* bronchopneumonia in foals with pulmonary abscesses, regardless of initial

pulmonary abscess score. This demonstrates that whatever antimicrobial synergy between rifampicin and macrolides is suggested by *in vitro* methods (e.g. MPCs) then it does not translate to a clinically relevant *in vivo* added clinical benefit for treatment of *R. equi*. As already indicated, foals with severe *R. equi* bronchopneumonia represent a gap in the scientific literature where RCTs have not been performed comparing rifampicin concomitant therapies with monotherapy or eligible alternatives.

For rifampicin concomitant therapies to reduce the emergence of resistant mutants then it is assumed that both antimicrobials (rifampicin and macrolide) must reach mutant prevention concentrations (MPCs), or above, at the target site of *R. equi* infection (pulmonary abscess, intracellular alveolar macrophages). Both antimicrobials must reach MPCs at the target site because it is believed that the combination only reduces the emergence of resistant mutants and not the individual antimicrobials. Berghaus et al. (2013) compared previously published mean rifampicin-macrolide target site concentrations to their MPCs for *R. equi* and stated concentrations were above the MPCs. However, Berghaus et al. (2013) only considered the results from one short-term administration of clarithromycin-rifampicin, and not other studies, and also did not consider the variability (standard deviations) around the estimate of antimicrobial concentrations. The PK/PD of rifampicin concomitant therapies is dynamic, changing over time with repeated administrations due to auto-induction of drug metabolizing enzymes and p-glycoproteins. Also, these PK/PD studies were performed in healthy foals and thus unable to account for important barriers for antimicrobial penetration (e.g., pyogranulomatous abscess/es); therefore, the true antimicrobial (rifampicin, macrolides) target site concentrations of naturally infected foals are unknown. Considering all PK studies measuring target site antimicrobial concentrations, representing short-to-longer term administrations, reveals high variability as well as decreasing parent antimicrobial target site concentrations, with increasing drug metabolite concentrations for which there is no information about MICs and MPCs (Baptiste et al., 2025).

With regards *its route of administration preserving animal welfare by avoiding the need for repeated injections over the long periods of time required for treatment and associated side effects thus improving safety*:

The main supporting evidence for oral rifampicin concomitant therapies as an added clinical benefit is a study by Hillidge (1987), showing both a high treatment success and low incidence of mild adverse events. Hillidge (1987) is a case series, that lacks a control group and cannot prove that the success was because of rifampicin concomitant therapies. While Hillidge (1987) noted only mild diarrhoea, Giguère (2014) and Divers (2026) highlight that the "list of adverse drug reactions keeps growing". Nearly 40 years later, it is well recognised that rifampicin concomitant therapies are not without serious risk to horses. The majority of foals with *R. equi* pneumonia are still being nursed by their mares. A critical, well-documented risk of oral rifampicin-macrolide therapy in *R. equi* foals is fatal enterocolitis in the nursing mare, both in Europe and globally, associated with infection/proliferation of *Clostridioides difficile* and *Clostridium perfringens* (Giguère, 2014). It is thought that if the mare ingests even trace amounts of macrolides in faeces (coprophagy is common in foals, and mares groom their foals), the drug destroys the mare's normal coecal microflora, leading to overgrowth of *Clostridioides difficile*. Mares could be exposed to low concentrations of macrolides, in foals treated for *R. equi*, possibly due to ingesting small amounts of the active drug during coprophagia or contamination of feeders/water buckets shared by the mare and foal. Since macrolide oral bioavailability is inhibited significantly by rifampicin, then higher macrolide concentrations would be present in the foal's feces (Baptiste et al., 2025). The perceived benefit of oral rifampicin-macrolide administration is offset by the fatal risk of colitis to the mare as well as the high rate of foal diarrhoea. The oral route of administration for rifampicin does not represent a net safety or welfare benefit to the mare-foal unit. Loss of mare is an animal welfare issue, adding further stress to the sick foal and difficult to find a foster-mare for a sick orphan foal. The phenomenon of fatal colitis in mares has only

been described for oral rifampicin concomitant therapies. Currently, the use of injectable macrolides (tulathromycin, gamithromycin) in *R. equi* foals has not been reported as associated with fatal colitis in mares. Furthermore, of the eligible alternatives, both erythromycin and doxycycline are administered orally to foals with *R. equi* bronchopneumonia.

In support of safer oral route of administration, the additional information provided claims that '[t]hree out of thirteen foals treated with doxycycline/azithromycin developed hemolytic anemia' citing Wetzig et al. (2020), which is factually incorrect. This publication included 81 foals treated with this specific combination, but there is no mention of hemolytic anemia or any significant hematological complications. Wetzig et al. specifically monitored for adverse effects and laboratory changes, reporting only mild, self-limiting diarrhoea. On the contrary, the drug combination of doxycycline and rifampicin for *R. equi* pneumonia in foals has been reported to lead to toxic hepatopathy and haemolysis (Murdock et al., 2026; Divers, 2026).

The additional information cites Leventhal (2021) to suggest that tulathromycin is unsuitable due to severe adverse reactions (sweating, recumbency) observed after intramuscular or subcutaneous administration. However, this study exclusively examined six healthy adult horses, a population that is not afflicted by *R. equi* pneumonia (only a foal disease). The fact that adult horses in this study reacted so poorly to IM/SC injections actually leads to the conclusion of tulathromycin being less suitable for use in adults compared to foals, who often tolerate certain macrolides differently than adults. Such severe systemic adverse events have not been confirmed in foals, where administration is typically limited to transient, local injection site reactions. By focusing on the wrong age model, the additional information misrepresents the safety profile of the actual clinical alternatives available for treating foals.

The additional information states that gamithromycin requires diluted intravenous (IV) administration due to local lesions from other routes and asserts it has 'markedly higher plasma exposure' but 'lower distribution into epithelial lining fluid' (ELF) based on Berlin et al. (2017). The issue with this interpretation is that the 'higher plasma exposure' referenced actually pertains to a cross-study comparison between IV administration in foals and IM/SC administration in adult horses, not a comparative failure of the drug in the correct target population. While the study confirmed that gamithromycin penetration into ELF is lower than in bronchoalveolar lavage (BAL) cells (a finding common to most macrolides), this 'mismatch' is of limited clinical relevance for *R. equi*, which is a facultative intracellular pathogen residing in macrophages and abscesses. Furthermore, while the ELF concentrations (1.05 µg/ml) hover at the conservative MIC90 of 1 µg/ml used in the study, they remain significantly above the 0.5 µg/ml MIC90 cited in many other wild-type susceptibility studies. Furthermore, the finding of Huguet et al. (2025) found using advanced in vitro methods that macrolides, alone or in combination with doxycycline, dramatically reduced both intracellular and extracellular *R. equi* populations.

CVMP's answer:

Based on the assessment of the specific additional information and publications provided by the Commission, the Committee cannot confirm that rifampicin, used in combination with a macrolide, remains a necessary component of the treatment of moderate to severe *Rhodococcus equi* infections in foals, nor that it provides a consistent added clinical benefit compared to authorised alternatives. There is no recognised 'moderate' category as well as inconsistent definitions of 'severe' foals with *R. equi* bronchopneumonia. Regardless, foals with severe *R. equi* bronchopneumonia represent a gap in the scientific literature where RCTs have not been performed comparing rifampicin concomitant therapies with eligible alternatives. Regarding the potential added clinical benefits of rifampicin, the Committee concludes the following:

- While historical support for the erythromycin-rifampicin combination exists (Hillidge 1987), constituting evidence lacking a control group, this is directly contradicted by a recent systematic review and meta-analysis (Baptiste et al., 2025) and randomised controlled trials (Wetzig et al., 2020) which show that rifampicin concomitant therapies do not provide an added clinical benefit for certain categories of foals with *R. equi* bronchopneumonia.
- The theoretical benefit of *in vitro* mutant prevention has not been translated into superior clinical outcomes in a meta-analysis (Baptiste et al., 2025) nor in RCTs (Wetzig et al., 2020) *in vivo* evidence. Recent *in vitro* bactericidal studies (Huguet et al., 2024) challenges the belief of synergy between rifampicin and macrolides, finding that rifampicin combinations do not enhance the elimination of *R. equi* compared to macrolide or macrolide-doxycycline alternatives.
- Rifampicin acts as a "perpetrator drug" that undermines the efficacy of co-administered macrolides due to in vivo antagonistic drug interactions. The profound pharmacological antagonism caused by rifampicin induces the rapid elimination of macrolides, undermining the intended benefit of combination therapy.
- While oral administration avoids injection-site stress, cited as an animal welfare benefit, it introduces a well-documented risk of fatal *Clostridioides difficile* enterocolitis in the nursing mare. Since rifampicin suppresses macrolide absorption in the foal, it increases fecal excretion of the drug, heightening the risk to the dam. No such fatal risk to the mare has been documented with injectable alternatives like tulathromycin or gamithromycin. Furthermore, newly documented clinical evidence from case series/reports (Murdock et al., 2026; Divers, 2026) has linked rifampicin-doxycycline combinations to acute toxic hepatopathy and hemolytic anemia in foals. No studies specific for foals were submitted about intolerance issues with injectable alternatives.

In conclusion, because satisfactory clinical results can be achieved using eligible alternatives with established MRLs, rifampicin does not meet the criteria for essentiality or added clinical benefit under Implementing Regulation (EU) 2025/901.

2.2.2. Question 5:

In the affirmative, could the Committee discuss the possible risk for consumers when the substance is used in food-producing animals of the equine species and a six-month withdrawal period is respected?

CVMP's answer:

N/A.

2.3. Griseofulvin

Indication: treatment of ringworm; systemic antifungal use[6]

2.3.1. Question 6:

The Agency's scientific advice acknowledges that '[v]*irtually all dermatophytes of animal origin are inhibited by griseofulvin concentrations of 0.2-0.5 µg/ml.* […] *Actively growing fungi may be killed, but dormant cells are only inhibited, so that cure occurs when infected keratinized cells are shed. For this reason, treatment is prolonged.*'

[6] In the annex to the European Commission's request, questions 6, 7 and 8 are presented together. Because question 8 is contingent on the answer to question 7, and question 7 on the answer to question 6, this guidance document separates them for clarity and ease of reference.

Commission Regulation (EU) 1950/2006, as amended, lists griseofulvin for the following clinical benefits: '*griseofulvin given orally has good activity against trichophyton, microsporum, and epidermophyton*.'

Could the Committee provide information on whether there are any veterinary medicinal products authorised for food-producing animals of the equine species or substances listed in Table 1 in the Annex to Commission Regulation (EU) No 37/2010 which cater to the clinical cases of ringworm in equine animals which require systemic treatment?

Scientific rational for CVMP's answer:

At present, based on the UPD, no veterinary medicinal products authorised for food-producing equine animals have been identified with a specific indication for the systemic treatment of dermatophytosis (ringworm). In addition, no substance listed as an alternative in Table 1 of the Annex to Regulation (EU) No 37/2010 appears to provide an effective oral/systemic treatment option specifically covering ringworm in food-producing equine animals.

Enilconazole is the frequently cited alternative. However, it is explicitly restricted to topical (cutaneous) use in the authorised product information and is not a systemic option. Therefore, while enilconazole is a relevant topical treatment, it does not address the need for an oral/systemic approach in situations where topical therapy is not feasible or does not achieve satisfactory control in practice.

CVMP's answer:

The Committee can inform that there are no veterinary medicinal products authorised for food-producing animals of the equine species or substances listed in Table 1 in the Annex to Commission Regulation (EU) No 37/2010 which cater to the clinical cases of ringworm in equine animals which require systemic treatment.

2.3.2. Question 7:

In the negative, could the Committee confirm whether, due to its oral route of administration, griseofulvin would be considered essential for those cases of ringworm in equines which necessitate such systemic antifungal treatment?

Scientific rational for CVMP's answer:

Equine dermatophytosis is typically a superficial infection of keratinised tissues. While the majority of cases respond to topical therapy, control can be challenging in the field, particularly in group settings or when lesions are extensive or progressive, due to environmental persistence of dermatophytes and practical constraints on repeated topical applications (Chermette et al., 2008; Maurice et al., 2016). Field data show that multiple dermatophyte species may be involved and lesions commonly occur on areas exposed to friction or pressure (e.g saddle, limbs), which further complicates topical only regimens (Chermette et al., 2008; Maurice et al., 2016; Hend et al., 2017). In the field survey by Maurice et al. (2016), identified multiple dermatophyte species in horses, including *Microsporum* and *Trichophyton*, and even a typically human pathogen (*Trichophyton soudanense*) (Maurice et al., 2016). While not a broad public health threat, these findings highlight the importance of rapid control to limit transmission and reduce occupational exposure in collective environments (Maurice et al., 2016). El Damaty et al. note zoonotic potential in equine dermatophytes and link it to close human horse contact, underscoring the need for prompt control in high contact settings (Hend et al., 2017).

In this context, it is appropriate to recognise that while topical management is sufficient in many situations, there is a minority of cases where systemic therapy is clinically justified to achieve reliable

disease resolution when topical therapy does not yield equally satisfactory results (Chermette et al., 2008; Maurice et al., 2016).

Griseofulvin has a specific pharmacological profile that makes it well suited for treating dermatophyte infections. It is a systemic antifungal active against Microsporum, Trichophyton, and Epidermophyton. After oral administration, it preferentially localises in keratinocyte precursor cells, the primary biological niche of dermatophytes. This targeted distribution provides a clear rationale for its use when systemic treatment is required (Mercer, 2022).

For the majority of horses, localised ringworm can be managed effectively with topical therapy, and systemic therapy is not routinely indicated and should be reserved for selected cases, when systemic treatment is required, griseofulvin remains a recognised oral option active against common dermatophytes (Mercer, 2022; Hardefeldt et al., 2025). However, there are no established EU criteria for horses as to when to depart from topical antifungal treatments and include griseofulvin oral therapy. The Agency's advice recognises that systemic treatment may be indicated in rare cases. In these exceptional situations, the decision should be based on a case-by-case clinical assessment, applying the regulatory criterion of alternatives providing "equally satisfactory" results, and considering the documented safety concerns for griseofulvin.

In conclusion, griseofulvin is a well-established systemic antifungal. It is authorised in the European Union, specifically in France. While most equine dermatophytosis cases respond to topical therapy, griseofulvin provides a validated systemic option for exceptional situations such as severe, extensive, or highly contagious infections supporting rapid control, limiting spread, managing zoonotic risk, and safeguarding animal welfare. Its availability ensures veterinarians can respond effectively when topical treatments alone are insufficient. (Chermette et al., 2008; Hardefeldt et al., 2025).

CVMP's answer:

The Committee can agree griseofulvin would be considered essential to treat certain cases of ringworm in equines which necessitate such systemic antifungal treatment. Access to a reference systemic option is needed for situations in which, under real life conditions, a topical only regimen cannot provide satisfactory disease resolution, particularly in extensive, severe, painful, or highly contagious presentations, and in the context of endemic episodes in group settings. Moreover, even if equine dermatophytosis is not necessarily a "public health threat" in the broad sense, the main concern in group settings is local/occupational exposure (e.g. staff, owners, children), which strengthens the rationale for rapid control and limiting spread.

2.3.3. Question 8:

In the affirmative, could the Committee discuss the possible risk for consumers when the substance is used in food-producing animals of the equine species and a six-month withdrawal period is respected?

Scientific rational for CVMP's answer:

Griseofulvin is an orally administered, long-term antifungal against dermatophytoses. Griseofulvin is almost insoluble in water (logP = 2.18). Treatment is carried out over several weeks to prevent recurrence, as it has to be ensured that the skin and appendages such as hair or claws are sufficiently and continuously impregnated with the active ingredient.

The mechanism of the effect is that griseofulvin binds to fungal microtubes, thus, altering the fungal process of mitosis. Griseofulvin has been shown to interact with the spindle apparatus of dividing cells, resulting in a delay to mitosis and cell cycle block (Hebert et al., 1980; Panda et al., 2005; Aris et al., 2022).

The systemically available griseofulvin is mainly deposited in the keratin precursor cells of the epidermis of the skin. The keratinocytes in the epidermis change their characteristics during their lifespan while moving from the bottom of the epidermis to the top of the layers (stratum corneum). Griseofulvin binds firmly to the new keratin, which thus becomes resistant to fungal invasion. The deposition of griseofulvin in keratin of hair, skin, and nails moves gradually to the surface of these appendages. Later, the old fungus-containing keratin is shed and replaced by normal skin and hair.

According to the summary of product characteristics of veterinary medicinal products, the drug is detected after ca. 48–72 hours at the base level of the skin, in 6–12 days in the lower quarter, and in 2–19 days in the middle section of the horny layer. A description of the pharmacokinetics of griseofulvin in the horse is not available but it has been shown that in the horse, the mean epidermal cell renewal time was 17.24 days (range 13 - 25.5 day, determined in eight healthy adult Quarter Horses (Barker et al., 1988). Scott and Miller (2003) reported approximately 17 days. 10%–50% of griseofulvin, dependant on the species, is excreted as metabolites in the urine and the rest in the faeces during ca. 4–5 days after administration. The terminal half-time in animals after oral administration is ca. 24 hours in several species. The metabolic pattern of griseofulvin seems to vary between species. This information is based on the CVMP's 1996 MRL status report following the MRL assessment of griseofulvin. No MRLs could be established, and the application was subsequently withdrawn. The report is not publicly available.

According to summary of product characteristics of some human medicinal products, peak plasma levels depending on the dose are reached in 2-4 hours and are maintained for approximately 10-20 hours after single administration. The volume of distribution is reported as 0.7 L/kg while Rowland et al. (1968) reports a volume of distribution of ca. 100 L, "indicating a marked extravascular concentration", which, however, seems to be a rather theoretical value from prediction modelling, also cited by Coutinho et al. (2024). In the blood, griseofulvin is mainly bound to plasma proteins (80%). It crosses the placenta and reaches breast milk. Griseofulvin undergoes metabolism, mainly hepatic, and the metabolites are inactive. Metabolites are principally 6-desmethylgriseofulvin and its glucuronide conjugate. The majority of the dose is excreted as metabolites in the urine, less than 1% is excreted as unchanged griseofulvin. Excretion takes place also via bile and faeces. The terminal half-time in humans after oral administration is about 9-21 hours. Rowland et al. (1968) reports a bi-exponential plasma-concentration time curve and a half-life for the first exponent of 0.70-1.7 hours and a second of 9.5-21.0 hours.

Griseofulvin absorption from the gastro-intestinal tract is species-dependant, incomplete and highly variable ranging from ca. 25 to 70% of an oral dose in humans and depending on several factors (Lin and Symchowicz, 1975). This variability in oral absorption in humans was previously reported as 27-72.5% by Rowland et al. (1968). Resorption is enhanced with lower particle size and increasing fat content of the food. In the treatment, absorption depends also on the type of formulation, and a repeated administration and high dosages are needed to achieve therapeutic levels (Lin and Symchowicz, 1975). On the contrary, in rats an enterohepatic circulation was demonstrated (CVMP, 1996) enhancing bioavailability. This was not observed in other species (rabbit) (Lin and Symchowicz, 1975).

No publicly available residue depletion data were found for griseofulvin administrated in horses or in closely related species of the *Equidae* family or in other species.

In 1996, an MRL application for griseofulvin was submitted, however, no MRLs could be established. The application was withdrawn. According to the CVMP, the information provided on toxicity of griseofulvin from published data was unsuitable and of insufficient quality for an MRL application. No ADI could be derived. The data for genotoxicity and carcinogenicity was incomplete. Also, the

information on residue depletion was not complete and no data were provided for horses. The original CVMP status report is not publicly available.

According to the industry self-classifications submitted by manufacturers and importers through REACH registrations and CLP notifications to ECHA, griseofulvin has been classified as "Suspected of causing cancer" (Carc. 2, H351), "May damage fertility or the unborn child" (Repr. 1B, H360) and "May cause an allergic skin reaction" (Skin Sens. 1, H317). Moreover, 4.6% of the industry self-classifications are supplemented with the classification "Suspected of causing genetic defects" (Muta 2, H341). (ECHA CHEM, 2025).

Griseofulvin was classified DILI-positive (oral administration route) according to its potential for causing drug-induced liver injury (DILI) by the U.S. Food and Drug Administration (FDA) (FDA, 2026; Thakkar et al., 2020). In a review, De Carli and Larizza (1988) point out that any changes in biochemical pathways associated with griseofulvin administration are related mainly to disturbances to porphyrin metabolism. Griseofulvin causes porphyria in mouse (metabolic disorders in which the body cannot properly convert porphyrins into heme, the red blood pigment; heme synthesis occurs in the liver and in the bone marrow). Also, it was known that griseofulvin can induce Mallory bodies at long treatment (eosinophilic cytoplasmic inclusions in hepatocytes, which serve as histological markers for liver damage).

Oral repeat dose studies with limitations have been performed in mice, rats and cats. Hepatoxicity and renal toxicity were observed in mice at high doses (12500 mg/kg bw/day and 2.5% in the diet; 12- and 22-weeks studies). No adverse effects were observed in rats up to 145 mg/kg bw/day and in cats with 0.1% in the diet. NOAELs could not be derived from these studies. This information is based on the CVMP's 1996 MRL status report on griseofulvin which is not publicly available.

According to summary of product characteristics of some human medical products, griseofulvin was administered to rats and mice during pregnancy and resulted in foetotoxicity and foetal malformations. IARC (2001) reports that griseofulvin was teratogenic in rats and cats. During the MRL evaluation in 1996, CVMP reported that griseofulvin was teratogenic in rats, mice and cats, with only the study in rats conducted according to OECD guidelines. In that study, a NOEL of 750 mg/kg bw/day for teratogenicity and maternal toxicity was derived, with oral administration of 125, 250, 750, 1500 mg/kg bw/day from day 6 to 15 after mating. Fertility was not affected in rats and mice in other studies, although these did not include the two-generation study typically required at this period for testing reproductive toxicity.

IARC last confirmed in 2001, based on their evaluations in 1976 and 1987, that there is sufficient evidence in experimental animals for the carcinogenicity of griseofulvin but there is inadequate evidence in humans. Therefore, griseofulvin was classified to be possibly carcinogenic to humans (Group 2B). Griseofulvin induced aneuploidy in vivo and in vitro (IARC, 2001).

Aneuploidy of griseofulvin as a mitotic inhibitor/spindle poison, thus causing numerical chromosomal aberrations, is confirmed at least in vitro, while griseofulvin is possibly not clastogenic (i.e. causing structural chromosomal aberrations) and does not induce gene mutations for example in the Ames test This information is based on the CVMP's 1996 MRL status report on griseofulvin which is not publicly available. As for aneugens, a threshold may be assumed, and provided the substance is not clastogenic and causes no gene mutation, a quantitative risk characterisation may, in principle, be possible. The EFSA has considered such an approach for aneugens (EFSA, 2021). However, strong evidence is needed to conclude on a threshold mechanism for genotoxicity/carcinogenicity.

IARC reported that griseofulvin induced liver tumours following its oral administration to adult mice or its subcutaneous administration to infant male mice. When given orally to rats and hamsters, it

produced a significant increase in the incidence of thyroid tumours in rats but had no carcinogenic effect in hamsters. (IARC, 1987; IARC, 1976). Rustia and Shubik (1978) found a dose-response relationship for hepatocellular carcinoma induction in mice (NOEL 0.3% in the diet for females, less than 0.1% for males) noting that not much was known about the mechanisms involved.

Regarding the mechanism, De Carli and Larizza (1988) explain that *"it is difficult to define the role of griseofulvin in tumour induction as it can act as a promoting agent, a co-carcinogenic agent, or a carcinogenic agent, depending on the dosage and the circumstances of its administration (Kinsella, 1982). In evaluating the carcinogenic potential of griseofulvin, it is worth considering that most of the tumours induced are hepatomas, which develop as the ultimate event in alterations of the liver metabolism, caused by prolonged treatment with exceedingly high doses of the antibiotic." "However, the carcinogenic risk may also be generated by the capacity of griseofulvin to cause nuclear and chromosomal changes at relatively low concentrations."* Mostly numerical chromosome aberrations are documented but depending on dosage and cell types, also structural chromosomal aberrations may be induced. Griseofulvin may have *"minor clastogenic potential".* However, the data on the probability of neoplastic transformation and induction of tumour development from these aberrations are scarce and inconclusive.

The CVMP (1996) concluded that griseofulvin induced an increase of hepatic tumours in mice and even hepatocarcinomas in young males. In rats of both sexes, griseofulvin induced thyroid carcinomas. In guinea pigs and Syrian hamsters, no tumourigen effects of griseofulvin were reported. However, the studies reported were not of sufficient quality. Regarding a possible mechanism the CVMP reported that *"according to the results of some experiments, griseofulvin could not be considered as an initiator but as a promoter. Therefore, a threshold level dose for the promotion effect reported in rats and mice should be determined. However, other experiments should be conducted to conclude about the possibility of genotoxic mechanism. As the mechanism of tumourigen action of griseofulvin remains unclear, further information is required before forming conclusions."*

Knasmüller et al. (1997), in a review, discussed published carcinogenicity studies from the period 1963-1993 and the possible mechanism of the observed liver tumours. While hepatocellular carcinomas were commonly found in mice, it was not known whether griseofulvin was also a hepatocarcinogen in rats and in other species due to a lack of adequate studies. Thyroid tumours were only observed in rats. It could not be concluded whether griseofulvin could have tumour promoting potential in the liver. After discussing several aspects, they finally assumed that possibly three different phenomena might be causally related to the hepatocarcinogenic properties, the induction of Mallory bodies, aneuploidy or porphyria. But, overall, the knowledge so far was insufficient. Various theories on how aneuploidy is casually related to cancer have been discussed (Knasmüller et al., 1997; De Carli and Larizza, 1988). The possible link between the tumour promoting activity and the aneugenic properties of griseofulvin was investigated experimentally using a medium-term liver carcinogenesis bioassay with rats and the in vivo peripheral blood micronucleus test by Labay et al. (2001). The authors concluded that *"griseofulvin did not initiate the carcinogenic process but rather had a potential in the liver for tumour promoting activity."*

In summary of product characteristics of some human medical products, it is reported that, hepatomas in mice and thyroid tumours in rats but not hamsters were induced through long-term administration of high doses of griseofulvin with food. It was assumed that the effects in mice may be due to a species-specific effect on porphyrin metabolism. The relevance of carcinogenicity of griseofulvin for humans has not yet been conclusively clarified but is often listed as a potential risk in the warnings.

Some recent supportive evidence for carcinogenicity of griseofulvin in humans was found for patients receiving griseofulvin in a cohort study. Increased risk for breast cancer was confirmed; increased risk

for liver cancer and thyroid cancer could not be excluded (Friedmann, 2009a and 2009b). Breast cancer was previously also observed in mice (El-Mofti et al., 1994).

In the CVMP status report following the MRL assessment of griseofulvin in 1996, the available information on genotoxicity was summarised as follows: "*Griseofulvin gave negative results in two in vitro tests (Ames test and the sister chromatid exchange test on V79 Chinese hamster lung fibroblasts) and in three in vivo tests (translocation test in mal mice spermatocytes, the bone marrow micronucleus test in mice and the dominant lethal test in male mice). However, griseofulvin induced aneuploidy in two in vitro tests (CREST test and the in situ hybridisation with chromosome-selective DNA probes assay) and in one in vivo test (cytogenetic analysis in zygote cells). A threshold concentration (0.5 µg/ml) for which no aneuploidy can be induced could be retained in the micronucleus assay on human lymphocytes (CREST test). However, as all information on the mutagenicity of griseofulvin has not been provided, no conclusion on the genotoxicity potential of griseofulvin can be drawn.*" The CVMP did not agree that griseofulvin is a purely non-genotoxic substance due to its strong aneugenic effect and that a threshold value can be established. Overall, the issue on the clastogenic potential of griseofulvin had not been resolved (Leonard et al., 1979; Kinsella, 1982; Raimondi et al., 1989; Kolachana and Smith, 1994; Mori et al., 1984; Marchetti and Mailhes, 1994).

At the time, the available literature indicated that griseofulvin acts predominantly as a strong inducer of aneuploidy rather than as a clastogen in peripheral human lymphocytes (Kolachana and Smith, 1994). However, when lymphocytes were treated with griseofulvin in whole blood, the portion of kinetochore negative micronuclei was increased, indicating structural chromosomal aberrations. Kolachana and Smith (1994) also reported conflicting findings regarding the induction of structural chromosomal aberrations (e.g. De Carli and Larizza, 1978; Leonard et al., 1979). DeCarli and Larizza (1988) concluded that the primary effect of griseofulvin is on chromosome number; however, depending on dose and cell type, the induction of structural chromosomal aberrations could not be excluded. Griseofulvin was not effective in inducing gene mutations or direct DNA damage, as it gave negative results in the Salmonella mutagenicity assay and in DNA repair assays. On this basis, the authors concluded that griseofulvin exhibits only minor clastogenic potential.

In contrast, Migliore et al. (1996) evaluated griseofulvin in the in vitro human lymphocyte micronucleus (MN) assay combined with fluorescence in situ hybridisation (FISH) using a centromeric probe. Griseofulvin induced micronuclei that were predominantly centromere-positive (94%), partly in agreement with the observations of Kolachana and Smith (1994), supporting a specific aneugenic activity with limited potential to induce structural chromosomal damage. The authors noted that even following exposure to a purely aneugenic compound, 100% centromere-positive micronuclei are not achievable. In contrast, griseofulvin was clearly positive in a mouse lymphoma assay (Sofuni et al., 1996). The publication states that a classification of large and small colonies was performed, indicative for gene mutation and chromosomal aberrations, respectively. However, this is not reflected in the results and no definitive conclusion on the mechanism of genotoxicity can be drawn from this study.

IARC (2001) reported that no data were available on the genetic and related effects of griseofulvin in humans. In experimental animals, griseofulvin induced sister chromatid exchange in bone-marrow cells and chromosomal aberration in spermatocytes, but it did not cause micronucleus formation or chromosomal aberrations in bone-marrow cells of mice. It induced aneuploidy in vivo and in vitro and micronucleus formation in cells in vitro. Griseofulvin did not induce recombination or mutation in fungi, but it induced DNA damage and somatic mutation or mitotic recombination in insects. Griseofulvin was not mutagenic and did not induce DNA damage in bacteria.

In vivo, griseofulvin caused aneuploidy without indication of structural chromosomal aberrations in mouse sperm cells (Shi et al., 1999). An increase of hyperploid cells was seen in the bone marrow of female mice (Pacchierotti et al., 2002), whereas in another study no micronuclei were detected in the bone marrow but were observed in gut epithelial cells (Vanhauwaert et al., 2001). Griseofulvin was used as an aneugen in this study and no distinction between aneuploidy and structural chromosomal aberrations was made. Guideline and GLP compliant in vivo studies investigating structural and numerical chromosomal aberrations are not available.

More recently, griseofulvin was used as a training substance for the mechanism of aneuploidy in studies with the intention to improve discrimination between aneugenicity and clastogenicity. From selected literature 2010 – 2019, it can be seen that the predominant genotoxic mode of action reported for griseofulvin was aneuploidy, and a threshold was partially examined (Wilde et al., 2019; Takeiri et al., 2019; Bryce et al., 2014, 2016, 2017; Hashimoto et al., 2010). However, griseofulvin also induced structural chromosomal aberrations (e.g. at one of 20 concentration levels; Bryce et al., 2017) or the tests could not completely distinguish between clastogens and aneugens, i.e. a certain degree of uncertainty remains depending on the test system and/or database (Wilde et al., 2019). Overall, the available data indicate that griseofulvin may also induce structural chromosomal aberrations.

CVMP's answer:

Griseoulvin accumulates in the keratinocytes of the skin but due to shedding of the outer skin layers of the horse about every 2-3 weeks no more residues are to be expected after six months. However, griseofulvin also takes a few weeks to migrate into the keratinocytes and no data is available to demonstrate skin elimination in horses. The minor portion that is metabolized or directly excreted is quickly eliminated. The metabolites did not appear to be active.

However, griseofulvin is known to be very toxic to the liver as well as being genotoxic, carcinogenic and teratogenic. As no direct DNA damage was seen, a threshold mechanism of action concerning the carcinogenicity was discussed in the past, so that previous and current literature on this subject was reviewed here. In the case of a threshold mechanism, a toxicological risk assessment would be permissible but as long as evidence is insufficient, it must be assumed that even a single molecule can lead to carcinogenicity.

Mechanistically, griseofulvin has been shown to be a mitotic inhibitor. Therefore, the main genotoxic mechanism exerted by griseofulvin is considered to be the induction of aneuploidy. However, based on the available toxicity data, the lack of guideline compliant genotoxicity studies and the more recent mechanistic data, structural chromosomal aberrations caused by griseofulvin cannot be entirely ruled out.

Overall, it can be summarised that tumour development with griseofulvin may be a multifactorial process involving various mechanisms that are still not fully understood, making it difficult to conclude on a threshold effect. Thus, as, in particular, it cannot be ruled out that chromosomes/DNA are also structurally damaged to a small extent, it must therefore be emphasised that without sufficient and clear mechanistic studies, no threshold argument can be accepted.

Therefore, considering that griseofulvin is a possible carcinogen to humans based on sufficient evidence in experimental animals (group 2B IARC), the mechanism is still not fully understood, that structural chromosomal aberrations may play a role, albeit to a minor extent, so that a non-threshold mode of action cannot be ruled out, i.e. a threshold approach cannot be accepted, it is concluded that griseofulvin will pose an unacceptable risk for the consumers when used in food producing animals of the equine species and a six-month withdrawal period is respected.

2.4. Ketoconazole

Indication: adjunctive therapy in the treatment of guttural pouch mycosis; topical use[7]

2.4.1. Question 9:

Could the Committee confirm if ketoconazole, when used topically as an adjunctive therapy in the treatment of guttural pouch mycosis, brings added clinical benefits over the eligible alternatives?

Scientific rationale for CVMP's answer:

There is no approved veterinary medicinal product for the treatment of guttural pouch mycosis. Treatment approaches described in case reports and reviews often combine surgical and medical treatment. The role of topical antifungals for guttural pouch mycosis is not fully understood, and protocols are available to resolve guttural pouch mycosis without topical antifungal treatments (Baptiste, 2004). Nevertheless, topical antifungals can prevent the fungal mass from spreading as well as killing the visible fungal infection. They are used after surgery to kill and avoid the spread of *Aspergillus fumigatus* which is the most frequently associated pathogen found in mycotic lesions (plaques) in the pouch. Topical medication with antifungals were reported in some cases to help hastening the regression of the mycotic lesions after ligation of the ipsilateral common carotid artery.

Enilconazole can be considered as an 'eligible alternative' since the substance is listed in Table 1 in the Annex to Commission Regulation (EU) No 37/2010. Veterinary medicinal products containing enilconazole are approved in the EU for another indication i.e., the treatment of local dermatomycoses. Good results have been reported with the enilconazole veterinary product used as adjunctive therapy for guttural pouch mycosis. For some equine practitioners, the use of enilconazole is an effective option while others prefer ketoconazole mainly because of its safety and its ease of administration. Some other equine practitioners report issues with the use of enilconazole veterinary medicinal products. Firstly, it must be diluted in order to administer the product via the catheter used during endoscopy, but then it is so liquid that the product runs out of the guttural pouches. Secondly, the product can induce inflammation of the mucous membrane in the guttural pouch, if administered daily. Treatments are therefore given every other day, and endoscopic examinations are needed to assess the degree of inflammation before instillation of enilconazole. Consequently, some equine practitioners prefer the use of a ketoconazole gel formulation authorised in human medicine. They consider that the gel is a more practical pharmaceutical form and raises less issues in terms of tolerance. It is used as an adjunctive topical treatment after ligation of the ipsilateral common carotid artery to reduce the time before regression of the fungal plaques.

It should be noted that the level of evidence provided for the assessment of this question is low as only expert opinions form the basis for discussing the role of different topical antifungal options especially as this answer could solely be based on the additional elements appended to the Commission's request, as requested by the Commission.

In conclusion, according to the clinical experience of some equine experts, the added benefit of the ketoconazole over enilconazole is mainly related to its safety and its ease of administration when used topically as an adjunctive therapy in the treatment of guttural pouch mycosis.

CVMP's answer:

[7] In the annex to the European Commission's request, questions 9 and 10 are presented together. Because question 10 is contingent on the answer to question 9, this guidance document separates them for clarity and ease of reference.

Topical administration of an antifungal on mycotic plaques may be needed as an adjunctive therapy after surgery to kill and avoid the spread of fungi and reduce the time before regression of the fungal plaques. For some equine practitioners, the use of enilconazole is an effective option while others prefer ketoconazole mainly because of its safety and its ease of administration.

It should be noted that the level of evidence provided for the assessment of this question is low as only expert opinions form the basis for discussing the role of different topical antifungal options.

Considering the overall objective of this exercise, which contributes to the VMP Regulation's policy aim of ensuring the availability of VMPs for food-producing equine species, and in line with the legal framework provided, ketoconazole is considered to bring added clinical benefits over the eligible alternatives when used topically as an adjunctive therapy in the treatment of guttural pouch mycosis.

2.4.2. Question 10:

If in the affirmative, could the Committee discuss the possible risk for consumers when the substance is used in food-producing animals of the equine species and a six-month withdrawal period is respected?

Scientific rational for CVMP's answer:

Please refer to question 13.

CVMP's answer:

Please refer to question 13.

Indication: treatment of fungal pneumonia; systemic use[8]

2.4.3. Question 11:

The Agency's scientific advice acknowledges that the clinical significance of fungal pneumonia in equids in the Union is unclear but indicates that various fungal pathogens have been associated with pneumonia.

Could the Committee confirm if and what eligible alternatives exist to the systemic use of ketoconazole for treatment of fungal pneumonia in equids?

Scientific rationale for CVMP's answer:

The Committee confirms that there is no eligible alternative to the systemic use of ketoconazole for the treatment of fungal pneumonia in equids. The Committee is aware of the exceptional case of pneumocystosis, for which the combination trimethoprim-sulfonamide is used. That substance combination is considered an eligible alternative for that specific case, with MRLs established for trimethoprim in *Equidae*, and all sulphonamides in all food-producing species. It would, however, not be appropriate to be considered as an alternative for the wider treatment of fungal pneumonia.

The additional information provided does refer to the oral administration via nasogastric tube of ketoconazole as recommended for the treatment of Scopulariopsis pneumonia (Stewart et al., 2008; Stewart and Cuming, 2015). Ketoconazole is also mentioned for the treatment of cryptococcosis (Cafarchia et al., 2013; Figueredo et al. 2013).

[8] In the annex to the European Commission's request, questions 11, 12 and 13 are presented together. Because question 13 is contingent on the answer to question 12, and question 12 on the answer to question 11, this guidance document separates them for clarity and ease of reference.

CVMP's answer:

The Committee confirms that there is no eligible alternative to the systemic use of ketoconazole for the treatment of fungal pneumonia in equids.

2.4.4. Question 12:

Could the Committee confirm if ketoconazole, when used systemically in the treatment of fungal pneumonia, brings added clinical benefits over the eligible alternatives, if any?

Scientific rationale for CVMP's answer:

In absence of other eligible alternatives in food-producing horses (with the exception of Trimethoprim-sulfonamide for the exceptional case of pneumocystosis) the discussion related to the "added clinical benefit" as defined in the legal framework is not relevant. Therefore, in absence of alternatives, ketoconazole would qualify as an essential substance for the treatment of fungal pneumonia.

CVMP's answer:

In absence of an eligible alternative (except Trimethoprim-sulfonamide for pneumocystosis) to the systemic use of ketoconazole for the treatment of fungal pneumonia, the substance would qualify as an essential substance in accordance with the definition provided in Article 2(1) of Implementing Regulation (EU) 2025/901.

2.4.5. Question 13:

In the affirmative, could the Committee discuss the possible risk for consumers when the substance is used in food-producing animals of the equine species and a six-month withdrawal period is respected?

Scientific rationale for CVMP's answer:

Ketoconazole is an antifungal imidazole derivative that can be administered either orally or topically. Intravenous application is only performed for study purposes.

The substance exerts its pharmacological effects by suppression of ergosterol biosynthesis (through inhibition of lanosterol 14α-demethylase, a cytochrome (CYP) P-450 enzyme). Ergosterol is an essential component of the cytoplasmic membranes of fungi (Ghannoum and Rice, 1999; Lewis, 2011).

Ketoconazole is a strong inhibitor of CYP3A4/5 and P-glycoprotein (P-gp, a transmembrane efflux pump; Cornwell, 1991; Juranka et al., 1989). Interaction has been shown for a large number of CYP- and other enzymes (also for its metabolite N-deacetyl ketoconazole, DAK), e.g. ketoconazole and DAK inhibit CYP3A4, CYP2D6, CYP2C19, P-gp, and organic anion transporting polypeptides (OATP)1B1 and 1B3 and breast cancer protein (Weiss et al., 2022; Deng et al., 2023; Vermeer et al., 2016). As both ketoconazole and DAK inhibit CYP3A4, the same enzyme primarily responsible for ketoconazole metabolism, a process of auto-inhibition takes place, where the drug slows its own clearance upon repeated administration. For example, in dogs, multiple high doses have been documented to double the terminal half-life of the parent compound from 3.6 to 6.8 hours within just five days of treatment (Daneshmend et al., 1983). In humans, a similar increase from 1.25 hours to 2.6 hours has been observed. Thus, over the long-term treatment course in horses, the elimination of the parent substance becomes non-linear and significantly more persistent than single-dose studies suggest.

In the EU, as a result of a referral under Article 31 of Directive 2001/83/EC as amended, the EMA recommended the suspension of the marketing authorisations of oral ketoconazole-containing human

medicinal products (e.g. Nizoral, Fungoral) due to the seriousness of occurred liver injury (EMA/458028/2013). In human medicine ketoconazole continues to be used in topical formulations.

Ketoconazole is lipophilic (log P = 3.73) in an octanol-water system and a weak dibasic compound (pKa1 = 6.51; pKa2 = 2.94; Van Tyle, 1984). Acidity is therefore required for dissolution and absorption (solubility 0.006 mg/ml at a pH of 7.5), which greatly limits the substance's oral bioavailability. Antacids consequently reduce gastric absorption (Piscitelli et al., 1991).

Topical administration of ketoconazole for the treatment of guttural pouch mycosis in horses will most likely be restricted to one, or very few, applications per individual. Only one publication by Cousty et al. (2016) was found which describes the use of ketoconazole for this indication. Multiple topical medications of a ketoconazole formulation (20 g in 20 ml of saline) were administrated, however, combined with a second topically applied antifungal (amphotericin B, nystatin).

Regarding oral administration, long-term treatment for fungal pneumonia in horses has been reported at doses of 3 – 10 mg ketoconazole/kg bw once a day (Higgins and Pusterla, 2006; Ziemer et al., 1992). Ziemer et al. (1992) reported treatment periods with ketoconazole from 20 days up to seven weeks. Nappert et al. (1996) utilised acidification in the ketoconazole treatment of a fever associated with consistent pulmonary isolation of Scopulariopsis sp. in a mare and applied a higher dose (30 mg/kg bw, mixed in 0.2 N HCI, administered twice a day intragastrically for 9 days). Also, Prades et al. (1989) in an experiment have administered this dose five times twice a day. General recommendations for duration of treatment of fungal pneumonia in horses are a minimum of usually six to 12 weeks and in severe cases over six months to over a year (Stewart et al., 2015).

Pharmacokinetics (other species than the horse)

According to Heykants et al. (1980, cited from Van Tyle, 1984), oral bioavailability in humans is 76%. However, original peer-reviewed literature referencing absolute oral bioavailability of ketoconazole in humans is not publicly available.

Absolute bioavailability was investigated in a random cross-over study in dogs and determined as 50 and 57% (400 mg oral as tablets versus tablets diluted in acidified solution), which confirms the overall limited oral bioavailability of ketoconazole (Baxter et al., 1986).

Relative bioavailability has been shown to vary between individuals and is, among other factors, influenced by the presence of food (Ghazal et al., 2015; Daneshmend and Warnock, 1988). Overall, food appears to increase the extent of absorption (noted up to doses of 600 mg, not for higher doses in humans, also notable delay of T_{max}; Daneshmend et al., 1984; contradictory study: Männistö et al., 1982). The AUC was documented to increase disproportionately with high oral doses (≥ 400 mg), which has been interpreted to suggest more complete absorption, nonlinear elimination, saturation of presystemic elimination/hepatic metabolism or a decreased volume of distribution (Huang et al., 1986; Brass et al., 1982).

Ketoconazole is highly bound to plasma proteins following absorption (99% bound at a drug concentration of 1 µg/ml in vitro in human plasma; Heel et al., 1982), and the volume of distribution is moderate (given as 25.41 l in humans, apparent Vd 0.36 l/kg; Daneshmend et al., 1981). Huang et al. (1986) calculated a higher mean apparent Vd of 88.31 l (± 68.72). In the body, the substance is distributed widely, and has been documented, among other tissues/fluids, in human saliva (Brass et al., 1982) and milk (Moretti et al., 1995), and is able to cross the placental barrier (Furukawa et al., 2008; inferred from studies on foetal/placental effects; for references regarding embryotoxicity or teratogenicity see below). Penetration into the CNS (the substance crosses the blood-brain barrier in cases of meningitis; Brass et al., 1982; Craven et al., 1983) and bones (Horsburgh et al., 1983)

appears limited. The substance was also found in the fur of both rats and guinea pigs (Van Cutsem et al., 1980).

Metabolism mainly takes place in the liver. Numerous metabolites have been described in vitro using human, mouse and rat liver microsomes (Fitch et al., 2009; Kim et al., 2017). In vivo experiments in mice and rats also documented several metabolites, of which DAK (also named M1) represented the major metabolite formed in the deacetylation pathway mediated by arylacetamide deacetylase (AADAC) (Whitehouse et al., 1990, 1994; Rodriguez and Acosta, 1997a, 1997b). DAK was also present in rabbits (Rodriguez and Miranda, 2000). DAK was demonstrated to be more metabolically cytotoxic than the parent substance in rat hepatocytes (Rodriguez and Acosta, 1997b). It is also formed in vitro in human liver microsomes and hepatocytes and is further metabolised by flavin-containing monooxygenases (FMOs) to highly reactive dialdehyde intermediates, which accumulate in liver tissue by forming covalent adducts with cellular proteins and macromolecules. Unlike the parent drug, these adducts are permanently bound to the tissue and are only cleared as the modified proteins are naturally turned over by the body. These adducts are thought to be responsible for the hepatotoxicity of ketoconazole (Rodriguez et al., 1999; Rodriguez and Miranda, 2000; Rodriguez and Buckholz, 2003; Fukami et al., 2016; Foti and Dalvie, 2016). Different O-dealkylated metabolites were detected in the cornea, retina, vitreous humour and plasma of rats subsequent to topical ocular administration, with the parent substance (cornea 98.3%, retina 98.8%, vitreous humour 99.4%, plasma 85.4%) as the dominant component (Pu et al., 2024).

Because DAK can cause phospholipidosis (the accumulation of phospholipids in cells), it can become sequestered within lysosomal structures in the liver (Whitehouse et al., 1994). DAK and ketoconazole are cationic amphiphilic drugs (CADs), a class of molecules known to induce and be sequestered by phospholipidosis. Mechanistically, CADs like DAK diffuse into the acidic environment of the lysosomes, where they become protonated (the "ion trapping" effect). Once protonated, they are unable to diffuse back across the lysosomal membrane (Saito et al., 2014). This sequestration physically shields the metabolite from rapid elimination, potentially leading to a residency time of weeks rather than hours. In mammalian models, while plasma clears quickly, the liver tissue can show signs of DAK-induced stress (like vacuolisation and ballooning) long after the drug is no longer detectable in the blood (Whitehouse et al., 1994).

Studies in rodents have demonstrated that DAK concentrations in the liver can be many times higher than those found in the blood, so that the liver acts as a reservoir (Weiss et al. 2022).

In male wild-type mice, Nagaoka et al. (2022) demonstrated the time-dependent concentration curve of ketoconazole and DAK in plasma following administration of a single high dose of 150 mg ketoconazole/kg bw, with DAK predictably lagging behind the parent substance (AUC_{48h} 412.4 ± 164.9 µg x h/ml, T_{max} 2.0 ± 1.4 h, versus AUC_{48h} 38.8 ± 13.8 µg x h/ml, T_{max} 10.0 ± 2.0 h; DAK representing 9.4% of the plasmatic parent substance concentrations when calculated from the mean AUC_{48h}). Tissue samples were also taken in this study 48 h post application, proving that DAK is mainly found in the liver, and that the extent of hepatic DAK-formation seen here in wild-type mice does become apparent in the plasma, especially when compared to plasma concentration curves and liver tissue contents of ketoconazole and DAK in simultaneously treated arylacetamide deacetylase (AADAC) knockout mice (responsible for deacetylation of ketoconazole to form DAK).

Still little is known on the in vivo metabolism and the significance of metabolites in humans, where the formation of DAK has only recently been proven (at concentrations in plasma of max. 3.1% of the parent compound following oral application). Despite the low plasmatic levels, it was hypothesised that, in agreement with data in rodents, DAK might be the main hepatic metabolite in humans (possibly accumulating in the liver and hardly reaching systemic circulation). Of 12 metabolites

studied, ketoconazole M23 represented the only other metabolite (aside from DAK) detectable in human plasma (estimated concentration of about 1% of the parent substance; Weiss et al., 2022).

Therefore, toxicologically relevant concentrations of DAK may persist in liver tissue in all species although not always detectable in plasma.

In contrast, regarding the parent compound, pharmacokinetic studies overall demonstrate a rapid systemic elimination of ketoconazole following oral treatment in animals and humans.

In dogs, oral administration of a high dose of 400 mg of ketoconazole in tablet form (2 x 200 mg) or as an acidified solution (random cross-over study design) led to a C_{max} of 17.4 ± 16.7 µg/ml, range 1.10 – 45.6 µg/ml (tablet) or 15.4 ± 9.4 µg/ml, range: 8.8 – 34.0 µg/ml (solution), showing considerable interindividual variation especially for the tablet form. Maximum mean plasma concentrations were reached at 2.7 or 2.4 hours, and the absolute bioavailability (also cited above) was determined at 0.5 and 0.57 (the same dogs had received 400 mg of ketoconazole hydrochloride intravenously, equivalent to 376 mg of ketoconazole; Baxter et al., 1986).

Cited mean half-lives in humans range from 2.0 to 7.9 hours after oral intake of 200 mg (in different formulations), with peak serum concentrations between 2.8 and 6.2 µg/ml (Huang et al., 1986; Brass et al., 1982). Following administration of a higher single dose of 400 mg of ketoconazole as tablets (2 x 200 mg tablets with 240 ml of orange juice) or in the form of an acidified solution (2 x 200 mg tablets dissolved in 10 ml of 0.1 M HCl, subsequently mixed with 240 ml of orange juice), the half-life was rapid at 2.38 or 2.57 hours, respectively (calculated from study data using K_{el}; Baxter et al. 1986).

Administration of multiple high doses of ketoconazole to dogs (400 mg/d per os for 5 days, given with two tablespoons of peanut butter to six greyhounds, 28.1 – 41.9 kg bw (≙ 9.5 – 14.2 mg/kg)) led to a significantly reduced elimination of ketoconazole (ca. doubling of mean terminal half-life from 3.6 to 6.8 h) with a decrease of the mean apparent volume of distribution from 0.97 to 0.67 l/kg after the first versus after the fifth dose (Kukanich and Hubin, 2010). A similar plasma profile of ketoconazole on the fifth dose was reported earlier in greyhound dogs (Kukanich and Borum, 2008). The substance is known to inhibit its own metabolism in humans (increase of terminal half-life from 1.25 h on day 1 to 2.6 h on day 5; Daneshmend et al., 1983).

Literature cites a major part of the applied dose in humans to be eliminated unchanged via faeces (≈ 10 – 37%), only 2 – 4% are found in the urine (Daneshmend and Warnock, 1988; Heel et al., 1982). In rats, > 80% of radioactivity was excreted via faeces after an intravenous dose of 5 mg/kg of 3H-ketoconazole (with biliary excretion amounting to 54.3 ± 18.0 % of the dose over 7.5 – 8 h). Faecal excretion took place over a 7-day period, while urinary excretion was essentially complete after 48 hours (Remmel et al., 1987).

The substance is retained in the skin for longer periods of time following topical or oral application (concentration decreased from median 3.6 µg/g at 2 hours to 1.2 µg/g measured 10 days following completion of a 10-day treatment with 200 mg ketoconazole/day to 17 human volunteers; Haneke, 1987). Studies indicate that mainly eccrine sweat delivers ketoconazole across the blood-skin barrier to the skin surface, where it is concentrated in the formed elements of skin and sebum. Rapid passive diffusion may play an additional role, as well as delivery via sebum in longer treatments (Rheney and Saddler, 1998; Harris et al., 1983).

Systemic absorption of ketoconazole after topical application to the skin is minimal to negligible (2%). In most cases, the active ingredient is not measurable in plasma after dermal application

(Daneshmend and Warnock, 1988). The absorption and distribution of ketoconazole after topical application to fungal lesions in cases of equine guttural pouch mycosis has not been studied.

Toxicology

According to the harmonised classification and labelling (CLH) under the CLP Regulation[9], ketoconazole is classified as "toxic if swallowed" (Acute Tox. 3, H301), "may damage fertility" (Repr. 1B, H360F) and "may cause damage to organs through prolonged or repeated exposure" (STOT RE 2, H373; no details are available regarding organ specification; ECHA CHEM, 2026).

Regarding organ toxicity, it is known that azole antifungals bear a common potential for hepatotoxicity, but both incidence and seriousness of hepatotoxicity is higher with ketoconazole than with other antifungal agents (reflected in the EU referral procedure (EMA/458028/2013); oral ketoconazole HMPs have also been withdrawn in other countries). According to the DILI rank 2.0 dataset, the U. S. Food and Drug Administration (FDA) classify ketoconazole among the 217 drugs (out of 1,336 currently FDA-approved) with the highest potential for developing Drug Induced Liver Injury. The classification in this system is derived from analysing the hepatotoxic descriptions presented in FDA-approved drug labelling documents and assessing causality evidence in literature. The substance was assigned the "Most-DILI-concern" regarding causal relationship, and a "severity class 8" (of classes 0 to 8; FDA, 2026; Olubamiwa et al., 2025; Chen et al., 2016).

In the 2013 referral of oral ketoconazole HMPs (EMA/458028/2013), the following non-clinical toxicity studies were referenced, however these are not publicly available: single dose toxicity after oral (mouse, rat, Guinea-pig, dog), and i.v. (mouse, rat, Guinea-pig, dog) administration; repeat dose oral toxicity up to 12 months in the dog and 18 months in the rat (the derivation of a NOAEL is not reported), and carcinogenicity evaluations after a life-time of exposure in the mouse (18 months dosing) and rat (24 months dosing); oral reproduction studies testing fertility and general reproduction performance in the rat, teratogenicity and embryotoxicity in mice, rats and rabbits, and perinatal/postnatal reproduction in rats. Mutagenicity was also evaluated in an extensive battery of studies. From the results it was concluded that the liver and endocrine system represented primary target organs (EMA/CHMP/580489/2013).

Preclinical safety data are reported in the summary of product characteristics of human medicinal product authorised for oral treatment of Cushing's disease. Toxicity to reproduction is noted, with doses of 25 mg/kg and higher in male rats and dogs producing sperm abnormalities and decreased fertility in rats. While doses up to 40 mg/kg showed no effects on female fertility in the rat, doses of 75 mg/kg and higher decreased the pregnancy rate and the number of implantation sites. Higher doses (80 and 160 mg/kg) inhibited ovulation in immature rats. Evidence of embryotoxicity and teratogenicity is cited at doses of 40 mg/kg/day and higher in rats and rabbits (named teratogenic effects: mainly skeletal anomalies, including cleft palate, brachydactylia, ectrodactylia and syndactylia). In some rodent studies, implantation loss was observed at doses as low as 10 mg/kg. In juvenile rats, puberty onset was delayed by treatment for 30 days, beginning at 21 days of age. Studies in pregnant rats and in guinea pigs with 3H-ketoconazole indicate that ketoconazole crosses the placenta. It was inferred that effects on human reproduction cannot be excluded.

In a teratogenicity study, daily high doses of 80 mg ketoconazole/kg to female rats on days 6 – 15 of gestation induced signs of maternal toxicity (reduction of body weight gain and food consumption, the latter significantly decreased on treatment days), caused reproductive toxicity (significant increase: mean percentages of post-implantation loss, number of both early and late post-implantation

[9] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006

resorptions, mean number of implantation sites; significant decrease: mean foetal body weights per litter), and resulted in 61% of cleft palate in foetuses, high numbers of reduced ossification of skull bones, and axial skeletal defects (de Santana Amaral and Nunes, 2008).

In another study, after daily administration of 50 mg ketoconazole to rats in 2 ml of corn oil on gestation days 7 to 21, Taxvig et al. (2008) saw a significant reduction in body weight and plasma oestradiol of dams, a decrease in the number of live foetuses and a high frequency of post-implantation losses, significantly decreased anogenital distances in both male and female foetuses, a decrease of testicular testosterone levels in male foetuses, and a tendency towards a decrease in ex vivo testosterone production.

In vivo and in vitro teratogenicity or embryotoxicity in rodents has also been described by other authors (Dimopoulou et al., 2017; Mineshima et al., 2012; Bechter und Schmid, 1987).

From a study assessing the in vivo predictability of in vitro tests, Dreisig et al. (2013) report from data on in vivo embryotoxicity that the lowest LOAEL referenced for ketoconazole was 10 mg/kg bw (implantation loss, increase).

In a population-based case-control study, an increased risk for congenital malformations in infants from mothers who received oral ketoconazole treatment (200 mg tablets, 1 or 2 per day) during the second and third month of gestation was not demonstrated (Kazy et al., 2005). Nevertheless, ketoconazole is contraindicated during pregnancy, and its use is advised against in women with childbearing potential not using an effective method of contraception, and when breastfeeding. The same contraindications are referenced for ketoconazole veterinary medicinal products for oral application.

Ketoconazole has not been examined or classified for carcinogenic hazards by the International Agency for Research on Cancer (IARC), and a literature review did not suggest that the substance is carcinogenic. According to the Hazardous Substances Data Bank (HSDB, 2026; within PubChem), the substance revealed no mutagenic potential when evaluated using the dominant lethal mutation test or the Ames Salmonella microsomal activator assay. While ketoconazole is generally considered non-mutagenic at therapeutic exposures in humans, DNA effects were shown in vitro at high, cytotoxic concentrations (rather indicating toxicity than direct mutagenicity; Casley et al., 2007).

Human cases of liver damage

The pharmacologically effective dose in humans is 200 mg/day over a period of e.g. 10 days. The severity of liver damage caused by ketoconazole ranges from mild and temporary enzyme elevations to symptomatic acute liver damage with jaundice to severe hepatitis, liver cirrhosis and acute liver failure and death. The damage may still appear months after treatment. Recovery is very slow (LiverTox, 2017). The FDA stated that e.g. for Nizoral tablets, the serious hepatic injury was noted to be unrelated to dose, duration, or indication for treatment. It was assumed that possibly some combination of dose, concentration, and duration of exposure is more severe than others (Greenblatt and Greenblatt, 2014).

Pharmacokinetics in horses

There is very limited information available regarding the pharmacokinetics of ketoconazole in equines, and no other studies on the pharmacokinetics of ketoconazole in animal species intended for human consumption appear publicly available (some studies exist, relating to co-administration of ketoconazole with other drugs).

Gastrointestinal absorption of the substance in horses is slow and also benefits from acidification (equines show a variable intragastric pH dependant on a number of animal- and feed-related factors (Damkel et al., 2015; Merritt, 1999; Murray and Grodinsky, 1992; Coenen, 1990; Sangiah et al., 1989; Campbell and Merritt, 1987). This was shown by Prades et al. (1989), who gave a single intragastric dose of 30 mg ketoconazole/kg bw in 50 ml of corn syrup to a healthy mare, and could not detect ketoconazole serum levels at any time point (11 samplings from 0.25 – 12 h post application).

In contrast, a second experiment with repeated administration by Prades et al. (1989), in which five consecutive intragastric doses of 30 mg ketoconazole/kg bw (q12h) in 0.2 N HCl were administered to six healthy, non-fasted adult horses, resulted in detectable concentrations in serum, synovial fluid, peritoneal fluid, urine and endometrium a few hours after each administration.

Furthermore, the authors gave a single i.v. injection of ketoconazole (10 mg/kg bw) to one of the six mares used in the multiple application oral study, which resulted in an estimated overall elimination rate constant of 0.22 h^{-1} (from serial serum samples obtained at 0, 3, 6, 10, 15, 20, 30, 45 minutes and 1, 1.5, 2, 3, 4, 5, 6 and 8 h), and an AUC of 161.4 µg/min/ml. The authors concluded that, although the bioavailability was calculated for only one individual (= 23% after intragastric administration), the low serum ketoconazole concentrations seen (even after acidification) would support the theory of poor gastrointestinal absorption of the drug in horses (Prades et al., 1989). The half-life was not given, but can be calculated as 3.15 h (ln(2)/kel).

The overall plasma concentration profile does not suggest accumulation of ketoconazole with repeated oral administration even of high doses in horses. However, urinary excretion increased to its peak at 50 hours, and peritoneal fluid as well as synovial concentrations also slightly, but steadily increased between each 12-hour time point (right before application). Upon termination of treatment, a rapid decrease of peritoneal and synovial fluid concentrations was seen (to 0.17 ± 0.116 µg/ml and 0.03 ± 0.021 µg/ml, respectively, at 60 hours), and urinary excretion also dropped. It can therefore be expected that, with a disproportionately (to the dose) rising AUC over the course of treatment when administering multiple high oral doses to horses (increasing concentrations over time also seen in repeated dosing of humans and dogs), the overall elimination will be steady once treatment is terminated, assuming a fast rate, however, peritoneal and synovial fluid concentrations would probably be delayed after a long treatment and thus possibly also elimination from tissues.

Further, horses would probably metabolise ketoconazole to the main and toxic metabolite DAK, similar to rodents and humans. Information on its distribution and elimination in horses is not available. According to studies in the mouse, DAK accumulates in the liver, and concentrations increase with higher doses (21 days of administration (Whitehouse et al. 1994). Therefore, high DAK concentrations can theoretically rise with long-term administration in any species and persist in liver tissue, probably also in the horse.

There is also uncertainty in understanding equine-specific data for this arylacetamide deacetylase (AADAC) enzyme responsible for forming DAK. While the human AADAC gene is highly expressed in the liver and gastrointestinal tract, rat and mouse AADAC exhibit high expression in the kidney as well, highlighting a fundamental difference in tissue distribution (Diaz-Vidal T et al., 2022). Furthermore, the catalytic efficiency of AADAC orthologs varies significantly; for instance, human AADAC can hydrolyze rifampicin, but mouse and rat AADAC cannot (Diaz-Vidal T et al., 2022). Without experimental verification using horse liver microsomes, it is impossible to predict whether the horse is a "high producer" of the toxic DAK metabolite.

Ketoconazole remains in the skin and hair long after systemic clearance (Haneke, 1987). The diffusion of potentially long-lasting ketoconazole residues from the horse's skin into underlying structures

(edible tissue), which would pose a risk to consumer safety, is unlikely. In addition, skin is also shed to the outside. It has been shown that the average renewal time of epidermal cells in horses is 17.24 days (range 13–25.5 days, determined in eight healthy adult Quarter Horses; Baker et al., 1988). However, sequestration in deep tissue cannot be completely ruled out.

No studies on the metabolism of ketoconazole in equine species were found.

Tissue distribution and residue depletion

There are no residue studies for ketoconazole openly accessible for any animal species.

Tissue concentrations have been only described in a study in male rats, where the highest mean ketoconazole concentration (n = 3/sampling point) was found in the adrenal, followed by liver, plasma and lungs (195.9 / 90.3 / 44.9 / 24.7 µg/g following oral application of a single dose of 150 mg/kg bw in corn oil (75 mg/ml)). Peak concentrations occurred after 2 h in the liver and adrenal and after 4 h in the plasma and lungs (Riley and James, 1986).

CVMP's answer:

Topical applications of ketoconazole result in very low systemic concentrations, and therefore no residues are to be expected after 6 months. It can be accepted that topically administered ketoconazole used as an adjunctive therapy in the treatment of guttural pouch mycosis will not pose an unacceptable risk for consumers when used in food-producing animals of the equine species and a six-month withdrawal period is respected.

For oral administration of ketoconazole, it is assumed that the severe hepatotoxicity is caused not only by the substance itself, but also significantly by its metabolic conversion in the liver. Official reports that led to the suspension of oral ketoconazole tablets in human medicine highlighted the role of liver metabolites in the development of hepatoxicity.

N-deacetylketoconazole (DAK) has been identified as a major metabolite in rodents, rabbits, and humans. DAK is biologically active and inhibits similar cytochrome P450 enzymes (CYP3A4) and transporters such as ketoconazole, in some cases even more strongly (e.g. CYP2D6). DAK can also be further metabolised by flavin monooxygenases (FMO) and these reactive metabolites have a high tendency to form covalent bonds with proteins in hepatocytes (liver cells), altering their function, which can lead to cell stress, mitochondrial damage, and finally to hepatocyte death. Covalent binding is a hallmark of the severe drug-induced liver injury (DILI) associated with ketoconazole.

There have been reports of patients who were treated with the usual dose of at least 200 mg/day over a period of e.g. 10 days and experienced severe liver damage, such as cirrhosis, months after finishing the treatment. Other severe liver injury have been reported leading to deaths. A correlation with the dose is not precisely known.

Although no data are available for horses, there is no indication that DAK is not also the main metabolite in horses, as it is in several other mammalian species and in humans. Given the low bioavailability of ketaconazole in horses (23%), high doses (30 mg ketoconazole/kg body weight) and a long-term administration (possibly over months and up to one year) are often required for a successful treatment. These higher doses increase the potential for non-linear elimination and saturation of hepatic metabolic pathways. Ketoconazole inhibits its own metabolism, leading to a doubling of the half-life after multiple doses in humans and dogs. Therefore, high DAK concentrations can theoretically arise with long-term administration for any species and persist in liver tissue, probably also in horses.

Since pre-clinical studies are not publicly available and from the human (therapeutic) dose no threshold or toxicological reference value can be derived, a quantitative risk characterization is not possible. Also, the exposure of DAK to humans cannot be estimated. However, the hazard is severe and occurs long-term.

Therefore, assuming that the main metabolite DAK is also systemically available in consumers after consumption of horse liver, the high uncertainty in this regard, combined with the potentially occurrence of a very serious effect in liver, is so pronounced that a serious risk cannot be ruled out in a worst-case and plausible scenario. It must therefore be assumed that there is a potential risk to consumers regarding liver toxicity if ketoconazole is administered orally to horses used for food production. In addition, ketoconazole may damage fertility and is teratogenic and embryotoxic.

Considering the above it can be concluded that orally administered ketoconazole in horses, due to its very toxic main metabolite, will pose an unacceptable risk for the consumers when used in food producing animals of the equine species and a six-month withdrawal period is respected.

2.5. Sevoflurane

Indication: inhalation anaesthesia for horses with limb fractures and other orthopaedical injuries and mask induction of anaesthesia in foals[10]

2.5.1. Question 14:

Commission Regulation (EC) No 1950/2006 lists sevoflurane due the following added clinical benefits: 'sevoflurane is a *volatile anaesthetic with minor metabolism and fast excretion; while there is an MRL for isoflurane in the EU, isoflurane is not suitable for all equine anaesthetic cases due to its recovery characteristics where excitement may lead to the horse breaking a leg; sevoflurane is essential in certain equine surgeries where a smooth recovery is vital, as it has been shown to produce a smoother, more controlled recovery in horses; it is therefore selected in preference to isoflurane for horses with limb fractures and other orthopaedical injuries; sevoflurane is essential for mask induction of anaesthesia in foals as it is completely non-irritant as opposed to isoflurane, which is irritant and therefore causes coughing and breath holding*.'

Given that sevoflurane and isoflurane are comparable agents, could the Committee confirm that sevoflurane brings certain added clinical benefits in terms of control of anaesthesia and duration and quality of recovery in some clinical settings requiring rapid induction and recovery, or better controllability, and for certain populations, such as foals?

Scientific rational for CVMP's answer:

For the Committee to confirm that sevoflurane brings certain added clinical benefits, the substance isoflurane is identified as an eligible alternative for comparison, as it is listed in Table 1 in the Annex to Commission Regulation (EU) No 37/2010 for *Equidae* and there are veterinary medicinal products authorised for food-producing animals of the equine species. The CVMP had previously proposed that sevoflurane should not be qualified as essential, nor as bringing added clinical benefit. However, additional elements were provided to the Commission and presented for assessment. This assessment is based solely on the additional elements appended to the Commission's request, as requested by the Commission.

[10] In the annex to the European Commission's request, questions 14, 15 are presented together. Because question 15 is contingent on the answer to question 14, this guidance document separates them for clarity and ease of reference.

The additional elements are presented in two sub-groups, pertaining to *efficacy and safety* and *suitability for mask induction*. This assessment follows the same structure.

These additional elements contain both published evidence of varying levels of quality and expert statements. Of the publications submitted for this question, Read et al. (2002), Driessen et al. (2006) and Matthews et al. (1998) present evidence from RCTs: all three are prospective, randomised studies, and use direct comparisons. Aida et al. (2000) is a case study describing 18 horses undergoing thoracotomy with sevoflurane but provides no control group receiving isoflurane for comparison. Matthews (2001) represents an expert opinion, being a 'How-to' clinical impression summarizing experience without controlled data. Similarly, the expert opinions expressed in the additional information describe experience based on uncontrolled data.

1.Efficacy and safety: recovery time, recovery quality and anaesthesia control.

The Commission indicates that the additional information highlighted several advantages of sevoflurane over isoflurane. The first quotation indicates that '*Sevoflurane has a lower blood-gas partition coefficient than isoflurane, leading to faster onset and recovery times, allowing for more precise anaesthetic depth adjustments, particularly crucial for foals and critical care patients.*' The second quotation indicates that '*Sevoflurane is particularly a safer choice for young and compromised animals due to better maintaining cerebral perfusion and better cardiovascular stability.*' These statements, which are considered to rely heavily on "expert consensus", are also noted as being supported by the Member States that submitted additional elements to the Commission. However, this consensus is not corroborated by the objective, controlled clinical trials provided for this assessment (see below).

Further peer-reviewed information is provided. Matthews et al. (1998) compared recovery from sevoflurane and isoflurane anaesthesia in horses in a prospective, randomized cross-over study involving nine Arabian horses (3 mares, 3 geldings, and 3 stallions) weighing 318-409 kg, with ages of 4-20 years. The study did not involve surgery. Under the conditions of this study, the authors found that recovery times varied widely between individual horses, but were significantly shorter with sevoflurane than with isoflurane, while sevoflurane followed by xylazine did not differ from isoflurane. Recoveries from sevoflurane, with or without xylazine, were of better quality than those from isoflurane. While the study found a statistically significant reduction in "time to standing" for sevoflurane compared to isoflurane (13.9 vs 17.4 minutes), the clinical relevance of a 3.5-minute difference in healthy, non-surgical horses is debatable. The authors concluded that sevoflurane anaesthesia in horses may contribute to a shorter and safer recovery from anaesthesia. In a clinical setting, a 3.5-minute faster recovery does not necessarily translate to a "safer" recovery or a reduced risk of injury (like a broken leg), which is the primary safety claim made. However, practitioner observations regarding anaesthetic recovery quality must be viewed with caution due to high individual variability. As noted by the authors, 'recovery times varied widely between individual horses' and some horses 'recovered badly with all treatments'. This suggests that perceived differences in practice may be attributed to individual horse temperament or sedative protocols rather than the intrinsic properties of the volatile agent used, further complicating the reliance on expert consensus.

Driessen et al. (2006) investigated whether haemodynamic function in horses, particularly mean arterial blood pressure (MAP), is better maintained with sevoflurane than with isoflurane, thereby requiring less pharmacological support. This was assessed in a prospective, randomised clinical study involving 39 racehorses undergoing arthroscopy in lateral recumbency. The results showed that average inhalant anaesthetic time and dose, volume of crystalloid solution infused, and cardiopulmonary parameters including cardiac output were similar between groups, with the exception that heart rate was 8% higher in horses receiving isoflurane compared with those receiving

sevoflurane. To maintain MAP > 70 mmHg, horses in the isoflurane group required dobutamine for a significantly longer duration and at a 51% higher total dose than those in the sevoflurane group. Dobutamine infusion rates were consistently lower in the sevoflurane group, with differences reaching statistical significance during the 0–30 minute period ($p < 0.01$) and the 61–90 minute period ($p < 0.05$). The authors concluded that horses anaesthetised with sevoflurane may require less pharmacological support in the form of dobutamine than those anaesthetised with isoflurane, possibly due to reduced suppression of vasomotor tone. These findings originate from a study limited to a homogenous population of racehorses. In contrast, other objective comparative research provided in the additional information contradicts this. Matthews et al. (1998) concluded that the cardiopulmonary effects of sevoflurane 'did not appear to be different from those produced by isoflurane' in adult horses, with similar requirements for dobutamine support (4/9 horses for isoflurane vs. 5/9 for sevoflurane). Similarly, in the only controlled study in foals provided, Read et al. (2002) found that 'arterial blood pressures were not significantly different between drugs at any time point'. Given these inconsistent results across the limited provided literature, there is insufficient evidence to demonstrate a robust 'added clinical benefit' regarding cardiopulmonary safety in a diverse clinical setting.

Aida et al. (2000) evaluated sevoflurane as an inhalation anaesthetic for thoracotomy in eighteen horses aged 2 to 15 years. This study was descriptive and did not include a control group receiving isoflurane for comparison. Anaesthesia was induced with xylazine followed by ketamine and maintained with sevoflurane, with ventilation controlled to maintain $PaCO_2$ at approximately 45 mmHg. Neuromuscular blocking agents (succinylcholine or atracurium) were administered to eliminate spontaneous breathing and facilitate the surgical procedures, while cardiovascular function was monitored and supported as required. Among the 14 horses not euthanised, two died due to ventricular fibrillation. The authors subjectively reported that sevoflurane offered good control of anaesthetic depth during induction, maintenance and recovery, and concluded that sevoflurane, in combination with neuromuscular blocking drugs, provides stable and readily controllable anaesthetic management for horses undergoing elective thoracotomy and cardiac manipulation. The authors' conclusion that sevoflurane provided "good control" is subjective and cannot be used to demonstrate superiority over isoflurane in the absence of a direct comparison within the study.

Evidence provided in the additional information confirms that anaesthetic recovery quality is a multifactorial process. Matthews et al. (1998) explicitly state that recovery is influenced by 'many factors including individual variability and the use of sedatives'. Crucially, this study noted that 'individual variation between horses appeared to be large' and that 'some individual horses recovered well regardless of the treatment, whereas other horses recovered badly with all treatments'. This supports the conclusion that factors such as pre-anaesthetic temperament and sedative protocols are likely more influential on the smoothness of recovery than the choice of volatile agent itself.

The Committee notes that the peer-reviewed publications submitted date from 1998 to 2008, and is aware of additional, more recent peer-reviewed studies that contribute to the broader scientific evidence concerning similarities and differences between isoflurane and sevoflurane in equine anaesthetic procedures; however, as these were not submitted to the Commission, they are not considered in this assessment, in line with terms of the request.

The Committee distinguishes between evidence arising from prospective, randomised, controlled clinical trials, and expert opinions and anecdotal reports based on uncontrolled data. The objective data provided for this assessment regarding foals (Read et al., 2002) explicitly demonstrate that sevoflurane and isoflurane provide 'comparable anaesthetic quality' with no significant differences. Furthermore, while Matthews et al. (1998) found statistical differences in recovery times, they confirmed that cardiopulmonary effects were not different from isoflurane, and the clinical relevance of

the recovery difference is debatable given the high individual variability observed. Driessen et al. (2006) reported that sevoflurane maintained mean arterial pressure with less dobutamine support than isoflurane in a specific population of racehorses. However, this hemodynamic advantage reported in Driessen et al. (2006) was not replicated in the other RCT studies (Matthews et al., 1998; Read et al., 2002), which found comparable dobutamine requirements and blood pressures.

Conversely, the claims of 'added clinical benefit' are almost exclusively supported by subjective summaries (Matthews, 2001) or uncontrolled descriptive reports (Aida et al., 2000). In the view of the Committee, high-level controlled data showing comparable safety and quality takes precedence over expert consensus that lack a comparative scientific control.

The Committee's previous advice on sevoflurane for *Equidae* relied on a range of scientific literature. The systematic review by Robinson et al. (2023) appraised multiple trials and found no clinically significant improvement in recovery quality. This was bolstered by evidence from White et al. (2021) and Grosenbaugh & Muir (1998), which used randomised, controlled, and blinded designs to demonstrate that both agents provide comparable hemodynamic stability and recovery outcomes. While narrative reviews and pharmacokinetic summaries by Steffey (2002) and Behne et al. (1999) discussed the theoretical benefits of sevoflurane's low solubility, these expert opinions were ultimately outweighed by the clinical data that failed to translate those theoretical properties into superior surgical results.

The Agency's scientific advice had already indicated that, *even though sevoflurane is cited as being superior to isoflurane, there is evidence that their differences may not be significant*, and more recent published evidence was provided in support of this. The advice continues stating that *total intravenous anaesthesia (TIVA) nowadays is the best option with the lowest risk for anaesthetic complications as compared to volatile agents, which do cause dose dependent cardiopulmonary depression and hypoventilation*.

2.Suitability for mask induction in foals and for use in compromised patients

The Agency's scientific advice does not address the relevance of using sevoflurane in foals for mask induction, as alluded to in Commission Regulation (EC) No 1950/2006.

Mask induction with sevoflurane comes from observations in human medicine. Isoflurane has a pungent, "ether-like" odour that triggers airway irritation, coughing, and breath-holding. Sevoflurane is significantly less pungent and used for mask induction in human pediatrics. However, it is unclear if these differences in pungent odour are recognised by foals using mask induction.

The additional information provided makes the case that sevoflurane is a safer choice for foals and for compromised animals. This statement, which again relies heavily on "expert consensus", is also noted as being supported by the Member States that submitted additional elements to the Commission. This "expert consensus" relied upon in the additional information is not consistently supported by the scientific data provided in the accompanying publications.

Further peer-reviewed information is provided. Matthews (2001) describes the use of sevoflurane for maintenance of anaesthesia in 571 equine clinical patients over a three-year period. The author reports that sevoflurane provides cardiovascular stability comparable to isoflurane, but with shorter and smoother recoveries, suggesting particular utility in outpatient procedures, geriatric and high-risk patients, prolonged surgeries, and foals. Matthews further states that sevoflurane is better accepted than isoflurane for mask induction, making it suitable for rapid inductions in young foals. It must be highlighted that Matthews (2001) is a clinical review article based on subjective practitioner experience. No controlled data comparing mask induction with sevoflurane versus isoflurane were

provided. Therefore, the authors' statement regarding the better acceptability of sevoflurane, is an anecdotal observation. Although the number of foals or specific cases is not indicated, it appears that anaesthesia was induced using an inhalant agent alone, administered via a face mask or nasotracheal intubation. Reported complications were similar to those associated with other inhalant anaesthetics and were managed appropriately. The publication also notes that, due to its lower solubility relative to other inhalants, the depth of anaesthesia with sevoflurane can be adjusted more rapidly, necessitating close monitoring.

Read et al. (2002) conducted a prospective crossover study involving six healthy foals. Induction and recovery characteristics and cardiopulmonary effects of isoflurane and sevoflurane were evaluated. Each foal was anaesthetised twice, at one month and again at three months of age, with anaesthesia induced via nasotracheal administration of the agent in oxygen and maintained under intermittent positive-pressure ventilation in dorsal recumbency. Induction and recovery were smooth and unremarkable with both agents, and no significant differences were observed between them. Cardiopulmonary variables did not differ significantly between treatments, although both agents initially caused hypotension that resolved over time. The study findings from Read et al. (2002), however, explicitly states that both isoflurane and sevoflurane provide "comparable anaesthetic quality" for general anaesthesia in 1- to 3-month-old foals. This is aligned with the original advice from the Agency. This study does not contribute to the analysis of the relevance of using sevoflurane in foals for mask induction.

The only evidence provided in support of the suitability of sevoflurane for mask induction in foals belongs to experience based on uncontrolled data. No additional evidence was made available to the Committee with this request. Additionally, it is noted that the findings from the peer-reviewed publications provided under this heading show contradictory findings regarding overall anaesthetic quality.

The additional information provided for this question asserts that sevoflurane provides an added clinical benefit for mask induction in foals because isoflurane is an 'irritant'. The claim regarding mask induction in foals relies entirely on expert observations and Matthews (2001), a "How-To" clinical review. This publication offers anecdotal observations but provides no controlled data comparing coughing, breath-holding, or anaesthetic induction times between the two agents. A key part of the additional information argument is that isoflurane is irritating, leading to coughing and breath holding. However, the provided RTC study in foals (Read et al., 2002) describes induction with both agents as "smooth and unremarkable". While Read et al. (2002) used only nasotracheal intubation, the absence of respiratory adverse events, before and after anaesthesia, in this controlled trial directly contradicts the "irritant" profile described in the anecdotal reports for isoflurane. The Committee notes that the same expert consensus (e.g., Matthews, 2001) claiming sevoflurane advantage for mask induction also claimed sevoflurane superiority in general anaesthetic maintenance and recovery—claims that were explicitly disproven by the objective RCT data in Read et al. (2002) and Matthews et al. (1998), which found "comparable anaesthetic quality" or insufficient evidence of clinical superiority. Since the expert opinion is demonstrated to be inaccurate regarding general anaesthetic maintenance and recovery when compared to controlled data, it constitutes a fragile and insufficient basis to justify "added clinical benefit" status for mask induction. Consequently, the Committee concludes that the evidence provided is very weak for an added clinical benefit for sevoflurane mask induction in foals.

CVMP's answer:

Based strictly on the assessment of the specific additional information and publications provided by the Commission, the Committee cannot confirm that sevoflurane brings a consistent added clinical benefit in terms of control of anaesthesia, duration and quality of recovery, or better controllability for the

populations specified. This is also consistent with the previous Committee conclusions on sevoflurane. The Committee concludes the following:

- Although expert consensus suggests a benefit for foals, the provided study data by Read et al. (2002) explicitly demonstrates that both sevoflurane and isoflurane provide "comparable anaesthetic quality" for general anaesthesia in foals with no significant differences observed between them.
- The hemodynamic advantage (reduced dobutamine requirement) reported by Driessen et al. (2006) was limited to a specific, homogenous population of racehorses. This advantage was not replicated in other RCT studies—Matthews et al. (1998) and Read et al. (2002)—which found comparable dobutamine requirements and blood pressures between agents.
- Although Matthews et al. (1998) found a statistically significant reduction in "time to standing" for sevoflurane (3.5 minutes), the clinical relevance of this in healthy non-surgical horses is debatable and does not necessarily translate to a "safer" recovery or reduced risk of injury.
- Evidence indicates that recovery quality is a multifactorial process; Matthews et al. (1998) noted large individual variations, where some horses recovered badly with any treatments, suggesting temperament and sedative protocols are more influential than the volatile agent itself.
- Claims of "good control" or "better controllability" (e.g., Aida et al., 2000) are based on uncontrolled, descriptive reports and lack a direct comparison with isoflurane, rendering them insufficient to demonstrate superiority.
- Evidence supporting the suitability of sevoflurane for mask induction in foals remains limited to anecdotal observations and clinical reviews with uncontrolled data. This suggests a fragile scientific basis to conclude on sevoflurane for mask induction in foals. For example, when this evidence is compared to RCT evidence on control of anaesthesia, duration and quality of recovery, or better controllability then objective evidence could not corroborate anecdotal observations.

The objective high-level data provided for this assessment fails to translate theoretical pharmacokinetic benefits—such as a lower blood-gas partition coefficient—into superior clinical results. Even when confined to the additional elements provided, the evidence remains inconsistent and contradictory. Consequently, the Committee finds that the data does not support a definitive "added clinical benefit" over isoflurane.

For the indication “mask induction of anaesthesia in foals” only, and with reference to the relevant legal framework and recital (6) of Commission Implementing Regulation (EU) 2025/901, the following is noted. As already indicated, the scientific evidence reviewed by the Committee, including prospective, randomised, controlled clinical trials, indicates that sevoflurane and the existing alternative, isoflurane, provide comparable anaesthetic quality and safety in horses and foals. The assertion of an added clinical benefit for sevoflurane for mask induction for foals relies primarily on anecdotal observations and expert opinion, which is not corroborated by the available controlled data. When controlled data is available then sevoflurane and isoflurane are comparable in anaesthetic quality and safety for *Equidae*.

However, the Committee also acknowledges the regulatory perspective that interprets "added benefit" under recital (6) of CIR (EU) 2025/901 not as a requirement for proven clinical superiority, but as an increase in the availability of authorised substances based on the provision of an additional possible use or route of administration.

Consequently, sevoflurane for this specific indication meets that definition based on this particular regulatory application of the legal framework. The Committee wishes to emphasize that a scientific demonstration of clinical superiority over isoflurane has not been provided. This decision does not alter the Committee's fundamental commitment to its objective structured appraisal framework, where robust scientific data demonstrating a clear clinical advantage over authorised alternatives remains the primary basis for its recommendations, which are grounded in proven clinical benefits and a high-level of consumer safety.

2.5.2. Question 15:

In the affirmative, could the Committee discuss the possible risk for consumers when the substance is used in food-producing animals of the equine species and a six-month withdrawal period is respected?

Scientific rational for CVMP's answer:

Sevoflurane is an ether inhalation anaesthetic agent used to induce and maintain general anaesthesia in humans. It is also commonly used in veterinary practice, in non-food producing species.

Sevoflurane is absorbed on inhalation, and its most important property is its low solubility in the blood, which results in a rapid uptake and induction of anaesthesia, and faster elimination and recovery. Similar to uptake, the elimination of a given volatile anaesthetic is related to its solubility in blood and tissues. In humans, the blood/gas partition coefficient is low. Between 95 and 98% of the amount of sevoflurane taken up is eliminated through the lungs (Behne et al., 1999). Up to 5% of the absorbed dose of sevoflurane is metabolised in the liver by the cytochrome P450 isoenzyme CYP2E1 and defluorinated to its major metabolites hexafluoroisopropanol (HFIP), inorganic fluoride, and carbon dioxide. HFIP is rapidly conjugated with glucuronic acid and excreted rapidly via the kidneys (Sweetman, 2009; Riviere and Papich, 2018). Using noncompartmental analysis, the half-life for the early elimination phase has been calculated as 9.47 ± 4.47 mins (Behne et al., 1999). In another study, the terminal elimination half-life of sevoflurane from the peripheral fat compartment was about 20 hours, using a 3-compartment model (Holaday and Smith, 1981).

Some pharmacokinetic parameters have been studied in the horse. The blood/gas partition coefficient is also low in horses, approximately 0.47 (Bergadano et al., 2003), so a rapid induction and recovery is expected also in this species. The elimination half-life and the mean residence time were calculated in 6 healthy adult horses using a 2-compartment model (14.11 ± 18.65 mins and 9.33 ± 3.91 mins, respectively) (Valente et al., 2014). Regarding degradation products of sevoflurane, inorganic fluoride was measured in horses during and after a 5.2-hour anaesthesia; serum inorganic fluoride concentration reached 50.8 ± 7.1 µmol/L by the end of anaesthesia and then rapidly decreased to near baseline two days after anaesthesia (Steffey et al., 2005).

In dogs administered with sevoflurane 4% in oxygen, blood concentration of sevoflurane was found to decrease by 50% over an average period of 64 minutes, during the postexposure period. No anaesthetic was found in blood 24 hours after anaesthesia. In dogs administered with 3 and 4% in oxygen, sevoflurane was metabolised to inorganic fluoride and hexafluoroisopropanol. Serum fluoride concentrations reached peak values after 2 to 3 hours and returned to normal values within 24 hours after anaesthesia. HFIP was excreted in the urine as glucuronide conjugate, with its elimination essentially complete within 48 hours after the end of exposure to sevoflurane (Martis et al., 1981).

The summary of product characteristics of some human medicinal products report that preclinical data on single and repeated dose toxicity of sevoflurane showed no specific organ toxicity. In a reproductive toxicity study, the pregnancy rate, the number of foetuses (foetal mice) and general body weight were substantially lower in treated female rats when compared with control; the investigators concluded

that long-term inhalation of trace amounts of sevoflurane is toxic to female reproductive system (Wang et al., 2024).

Regarding the genotoxic potential, there are conflicting results between the studies. First of all, the substance is not expected to be mutagenic in the Ames test (ECHA; Baden et al., 1982). A DNA damage capacity of sevoflurane using comet assay in whole blood cells of Wistar rats treated with 4% sevoflurane for 120 mins has been described; however, in this study no significant differences regarding DNA damage were found between isoflurane and sevoflurane groups or between isoflurane and control group (Rocha et al., 2015). A dose-dependent genotoxic effect has also been described using the micronucleus test in cytokinesis-blocked human lymphocytes irradiated with X-rays (Alcaraz et al, 2014). Brozovic et al. (2017) observed that DNA damage in kidney cells of mice exposed to sevoflurane increased continuously before it reached its peak 24 hours after exposure; the alkaline comet assay was used. On the other hand, the summaries of product characteristics of authorised human medicinal products report that, extensive in vitro and in vivo mutagenicity studies with sevoflurane yielded negative results, and there are studies that support these conclusions (Krause et al., 2003; Szyfter et al., 2004; Braz et al., 2011).

No carcinogenicity studies have been found. The International Agency for Research on Cancer (IARC), has classified volatile anaesthetics as Group 3, i.e. non-classifiable as to their carcinogenicity to humans (IARC, 1987).

No information on residues depletion from tissues has been found. However, considering the route of administration and the low blood/gas partition coefficient together with the available pharmacokinetic information, no tissue accumulation and a rapid elimination are expected. Therefore, it can be assumed that sevoflurane will not pose an unacceptable risk for consumers when used in food-producing animals of the equine species and a six-month withdrawal period is respected.

CVMP's answer:

Sevoflurane is an ether inhalation anaesthetic agent used to induce and maintain general anaesthesia. The summaries of product characteristics of authorised human medicinal products indicate a low toxicity profile, which is also supported by literature references. However, there are some contradictory results between the different literature sources regarding its genotoxic potential. According to the IARC it is classified as Group 3, i.e. 'not classifiable as to its carcinogenicity to humans'.

No residues depletion data are available, but, considering the route of administration and the low blood/gas partition coefficient together with the available pharmacokinetic information that reflects a low metabolism of the substance in the liver, and a short half-life, no tissue accumulation and a rapid elimination are expected. Therefore, it can be assumed that sevoflurane will not pose an unacceptable risk for consumers when used in food-producing animals of the equine species and a six-month withdrawal period is respected.

3. Conclusion

Please refer to section 2 for the responses to each individual question.

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Note that for some questions, the response did not required reviewing scientific literature.

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