18 July 2024
EMA/CVMP/159047/2023-Corr.[2]
Committee for Veterinary Medicinal Products

Scientific advice under Article 115(5) of Regulation (EU) 2019/6 on veterinary medicinal products, regarding the list of substances which are essential for the treatment of equine species and for which the withdrawal period for equine species shall be six months

[2] This version includes a correction in the conclusion section (Section 6): for quinapril under cardiology, phenylephrine under gastrointestinal disorders, and diazepam under nervous system disorders, the superscript is changed from 'b' to 'a' to correctly reflect the reason for the proposed recommendation; for phenylephrine, atropine is removed from the identified alternatives. Parallel changes are made on p.130 (Section 4.5.3), p.151 (Section 4.7.2), p.159 (Section 4.7.4), and p.186 (Section 4.10.2). Additionally, the standard sentences used to conclude the essentiality assessment of the substances have been revised to better distinguish when a substance is deemed 'essential' or when it 'brings added clinical benefit'. Other minor changes of editorial nature are implemented.

A previous version corrected the table on page 260 (Section 4.15.1).

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Introduction

On 9 February 2023, the European Medicines Agency (EMA, the Agency) received from the European Commission a request[1] to provide scientific advice for the establishment, under Article 115(5) of Regulation (EU) 2019/6, of 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.

According to the request from the Commission, the objective was to develop of a proposed list of substances along with the aimed indication, explanation of use, identification of alternatives and a justification for the inclusion of each of the substances in the list.

The Committee for Veterinary Medicinal Products (CVMP) of the Agency formed an expert group to prepare the scientific advice. The experts that joined the group had either expertise on the clinical setting with animals of the equine species, expertise on antimicrobial resistance (AMR) and with knowledge of the ongoing work of EMA on its scientific advice to the Commission with regards to Article 107(6) of Regulation (EU) 2019/6, or expertise on consumer safety.

The expert group submitted their report to the CVMP on 11 June 2024.

The CVMP adopted the scientific advice on 18 July 2024.

Considerations and rationale for the recommendations

Increasing availability of veterinary medicinal products and maintaining a high level of consumer protection are two overarching objectives of Regulation (EU) 2019/6[2] on veterinary medicinal products.

Article 115 paragraph 5 of the Regulation foresees that "*by way of derogation from Article 113(1) and (4), the Commission shall, by means of implementing acts, establish 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*".

In summary, the European Commission indicated that points to consider when preparing the scientific advice were:

- the overall objective of Regulation (EU) 2019/6 to increase availability of veterinary medicinal products and of maintaining a high level of consumer protection,
- experience gained with the application of the current list of substances essential for the treatment of *Equidae* as listed in Regulation (EU) 1950/2006 and amended by Regulation (EU) 122/2013[3],
- indications provided in the mandate as to when to consider a substance as essential or bringing added clinical benefit, and as regards eligibility for inclusion or non-inclusion of substances listed in

[1] The request for scientific advice from the European Commission related to the adoption of implementing measures under Article 115(5) of Regulation (EU) 2019/6 is accessible via this link.
[2] Regulation (EU) 2019/6 of the European Parliament and the Council of 11 December 2018 on veterinary medicinal products and repealing Directive 2001/82/EC.
[3] Commission Regulation (EC) No 1950/2006 of 13 December 2006 establishing, in accordance with Directive 2001/82/EC of the European Parliament and of the Council on the Community code relating to veterinary medicinal products, a list of substances essential for the treatment of *equidae* and of substances bringing added clinical benefit, amended by Commission Regulation (EU) No 122/2013 of 12 February 2013.

tables 1 and 2 of the Annex to Commission Regulation (EU) No 37/2010[4], or in the Annex to Commission Implementing Regulation (EU) 2022/1255[5] (see footnote 1 for further details),

- and ensuring coherence with the ongoing work of EMA on its scientific advice to the European Commission, and the resulting implementing act, setting a list of antimicrobials which shall not be used in accordance with Articles 112, 113 and 114 or which may be used in accordance with these articles subject to certain conditions.

In addition, the request from the Commission included the recommendation to carry out a survey among national competent authorities and relevant stakeholders on the possible need to add other substances as a result of newly available evidence and the need for updating the information on use, advantages and alternatives of the entries in the current list.

For the preparation of the advice, EMA took into account all the points mentioned by Commission in their request and agreed, as explained in the following sections, on a working methodology to produce a (revised) 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.

Overview of recommendations

The list of substances which are essential for the treatment of equine species was prepared in fulfilment of the mandate of the European Commission under Article 115(5) of Regulation (EU) 2019/6.

Based on the evaluations presented in sections 4 and 5 below, the scientific advice from the CVMP is made relating to the 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, according to Article 115(5) of Regulation (EU) 2019/6, as follows:

1. The scientific advice following the review of the entries in Regulation (EU) 1950/2006, as amended by Regulation (EU) 122/2013, with due consideration of the stakeholders survey results, and considering also the assessment of new proposed substances, is[6]:

- Anaesthetics:

	Active substance (ATCvet code)
Substances in Regulation (EU) 1950/2006 **proposed to be retained**, with or without amendment of the current entry	Oxybuprocaine (QS01HA02); Prilocaine (QN01BB04)
Substances in Regulation (EU) 1950/2006 **proposed to be removed**	Bupivacaine (QN01BB01); Sevoflurane (QN01AB08)
Substances from stakeholders' survey **proposed for inclusion**	None

[4] Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin.
[5] Commission Implementing Regulation (EU) 2022/1255 of 19 July 2022 designating antimicrobials or groups of antimicrobials reserved for treatment of certain infections in humans, in accordance with Regulation (EU) 2019/6 of the European Parliament and of the Council.
[6] A revised categorisation is proposed to account for the results of the survey of stakeholders. It does not coincide entirely with that in Regulation (EU) 1950/2006, as amended by Regulation (EU) 122/2013, which explains why 'none' is used in some cases.

	Active substance (ATCvet code)
Substances from stakeholders' survey **not proposed for inclusion**	Ropivacaine (QN01BB09)

- Analgesics:

	Active substance (ATCvet code)
Substances in Regulation (EU) 1950/2006 **proposed to be retained**, with or without amendment of the current entry	Fentanyl (QN02AB03; QN02AB03); Ketorolac (QS01BC05); Morphine (QN02AA01); Triamcinolone acetonide (QS01BA05)
Substances in Regulation (EU) 1950/2006 **proposed to be removed**	Buprenorphine (QN02AE01); Flumethasone (QH02AB90); Gabapentin (QN02BF01); Pethidine (QN02AB02)
Substances from stakeholders' survey **proposed for inclusion**	Bromfenac (S01BC11); Methocarbamol (M03BA53)
Substances from stakeholders' survey **not proposed for inclusion**	Methadone (QN02AC90); Phenylbutazone (QM02AA01); Pregabalin (QN02BF02);

- Antimicrobials:

	Active substance (ATCvet code)
Substances in Regulation (EU) 1950/2006 **proposed to be retained**, with or without amendment of the current entry	Acyclovir (QD06BB03); Amikacin (QJ01GB06); Azithromycin (QJ01FA10); Miconazole (QD01AC02); Nystatin (QD01AA01); Ofloxacin (QS01AE01); Polymyxin B (QS01AA18)
Substances in Regulation (EU) 1950/2006 **proposed to be removed**	Griseofulvin (QD01BA01); Idoxuridine (QS01AD01); Isometamidium (QP51DX04); Ketoconazole (QJ02AB02); Ponazuril (QP51BC04); Pyrimethamine (QP51BX56); Rifampicin (QJ04AB02); Ticarcillin(QJ01CA13)
Substances from stakeholders' survey **proposed for inclusion**	Amphotericin B (QJ02AA01); Clarithromycin (QJ01FA09); Fusidic acid (QS01AA13); Ganciclovir (QJ05AB06; QS01AD09); Moxifloxacin (QS01AE07); Valacyclovir (QJ05AB11); Voriconazole (QJ02AC03)
Substances from stakeholders' survey **not proposed for inclusion**	Buparvaquone (QP51EX03); Ciprofloxacin (QS03AA07); Fluconazole (QJ02AC01); Rifamycin (QS01AA16); Tenoic acid (no ATCvet code identified); Tobramycin (QS01AA12)

- Substances for respiratory disorders:

	Active substance (ATCvet code)
Substances in Regulation (EU) 1950/2006 **proposed to be retained**, with or without amendment of the current entry	Ambroxol (QR05CB06); Fluticasone (QR03BA05); Ipratropium bromide (QR03BB01); Oxymetazoline (QR01AA05); Phenylephrine[7] (QS01FB01)
Substances in Regulation (EU) 1950/2006 **proposed to be removed**	Budesonide (QR03BA02)
Substances from stakeholders' survey **proposed for inclusion**	None
Substances from stakeholders' survey **not proposed for inclusion**	None

- Substances for cardiology:

	Active substance (ATCvet code)
Substances in Regulation (EU) 1950/2006 **proposed to be retained**, with or without amendment of the current entry	Amiodarone (C01BD01); Quinidine sulfate/gluconate (QC01BA01)
Substances in Regulation (EU) 1950/2006 **proposed to be removed**	Digoxin (QC01AA05); Procainamide (QC01BA02); Propranolol (QC07AA05)
Substances from stakeholders' survey **proposed for inclusion**	Propafenone (QC01BC03); Quinapril (QC09AA06); Sotalol (QC07AA07); Verapamil (QC08DA01)
Substances from stakeholders' survey **not proposed for inclusion**	Flecainide (QC01BC04)

- Substances for diagnostic procedures:

	Active substance (ATCvet code)
Substances in Regulation (EU) 1950/2006 **proposed to be retained**, with or without amendment of the current entry	Barium sulfate (QV08BA01, QV08BA02); Fluorescein (QS01JA01); Iohexol (QV08AB02); Phenylephrine[8] (QS01FB01); Rose bengal (no ATCvet code identified); Thyrotropin releasing hormone (QH01A)
Substances in Regulation (EU) 1950/2006 **proposed to be removed**	Iopamidol (QV08AB04); Radiopharmaceutical Tc99m (QV09BA)
Substances from stakeholders' survey **proposed for inclusion**	None
Substances from stakeholders' survey **not proposed for inclusion**	Disodium oxidronate (no ATCvet code identified); Methylene diphosphonate (no ATCvet code identified);

[7] This substance is discussed in detail in section 4.11 (substances for ophthalmology).
[8] This substance is discussed in detail in section 4.11 (substances for ophthalmology).

	Active substance (ATCvet code)
	Tetrasodium dihydrogenbutedronate, diphosphono-1,2-propanedicarboxylic acid (no ATCvet code identified)

- Substances for gastrointestinal disorders:

	Active substance (ATCvet code)
Substances in Regulation (EU) 1950/2006 **proposed to be retained**, with or without amendment of the current entry	Metoclopramide (QA03FA01); Phenylephrine[9] (QS01FB01); Ranitidine (QA02BA02); Sucralfate (QA02BX02)
Substances in Regulation (EU) 1950/2006 **proposed to be removed**	Bethanechol (QN07AB02); Codeine (QR05DA04); Loperamide (QA07DA03); Phenoxybenzamine (QC04AX02); Propantheline bromide (QA03AB05)
Substances from stakeholders' survey **proposed for inclusion**	Misoprostol (QA02BB01)
Substances from stakeholders' survey **not proposed for inclusion**	None

- Substances for metabolic disorders:

	Active substance (ATCvet code)
Substances in Regulation (EU) 1950/2006 **proposed to be retained**, with or without amendment of the current entry	Insulin (QA10AC03; QA10AC01)
Substances in Regulation (EU) 1950/2006 **proposed to be removed**	None
Substances from stakeholders' survey **proposed for inclusion**	Pergolide[10] (QN04BC02)
Substances from stakeholders' survey **not proposed for inclusion**	Canagliflozin (QA10BK02); Ertugliflozin (QA10BK04); Flutamide (QN01BB09); Goserelin (QN01BB09); Metformin (QN01BB09); Velagliflozin (QA10BK90)

- Substances for musculoskeletal disorders:

	Active substance (ATCvet code)
Substances in Regulation (EU) 1950/2006 **proposed to be retained**, with or without amendment of the current entry	Atracurium (QM03AC04); Dantrolene sodium (QM03CA01); Edrophonium (QV04CX07); Guaifenesin (QM03BX90)

[9] This substance is discussed in detail in section 4.11 (substances for ophthalmology).
[10] Please refer to the section 'Points for further consideration' within this scientific advice for additional scientific considerations regarding this substance. It is proposed that despite the substance fulfilling the criteria established in the European Commission's request, it is not listed.

	Active substance (ATCvet code)
Substances in Regulation (EU) 1950/2006 **proposed to be removed**	Phenytoin[11] (QN03AB02)
Substances from stakeholders' survey **proposed for inclusion**	Cisatracurium (QM03AC11)
Substances from stakeholders' survey **not proposed for inclusion**	Chondroitin sulfate (QM01AX25); Glucosamine (M01AX05); Pentosan polysulphate (QM01AX90); Rocuronium (QM03AC09); Thiocolchicoside (QM03BX05)

- Substances for nervous system disorders:

	Active substance (ATCvet code)
Substances in Regulation (EU) 1950/2006 **proposed to be retained**, with or without amendment of the current entry	Diazepam[12] (QN05BA01)
Substances in Regulation (EU) 1950/2006 **proposed to be removed**	Carbamazepine (QN03AF01); Phenytoin (QN03AB02); Primidone (QN03AA03)
Substances from stakeholders' survey **proposed for inclusion**	None
Substances from stakeholders' survey **not proposed for inclusion**	Alprazolam (QN05BA12); Fluoxetine (QN06AB03); Phenobarbital (QN03AA02); Trazodone (QN06AX05)

- Substances for ophthalmology:

	Active substance (ATCvet code)
Substances in Regulation (EU) 1950/2006 **proposed to be retained**, with or without amendment of the current entry	Cyclosporine A (QL04AD01); Phenylephrine (QS01FB01); Timolol maleate (QS01ED01); Triamcinolone acetonide[13] (QS01BA05); Tropicamide (QS01FA06)
Substances in Regulation (EU) 1950/2006 **proposed to be removed**	Dorzolamide (QS01EC03); Latanoprost (QS01EE01)
Substances from stakeholders' survey **proposed for inclusion**	Acetazolamide (QS01EC01); Cyclopentolate (QS01FA04); Synephrine (QS01GA06); Tetryzoline (QS01GA02)
Substances from stakeholders' survey **not proposed for inclusion**	Brinzolamide (QS01EC04); Pilocarpine (QS01EB01); Tacrolimus (QL04AD02)

[11] This substance is discussed in detail in section 4.10 (substances for nervous system disorders).
[12] This substance is discussed in detail in section 4.12 (substances for sedation and premedication (and antagonism)).
[13] This substance is discussed in detail in section 4.2 (analgesics).

- Substances for sedation and premedication (and antagonism):

	Active substance (ATCvet code)
Substances in Regulation (EU) 1950/2006 **proposed to be retained**, with or without amendment of the current entry	Acepromazine (QN05AA04); Atipamezole (QV03AB90); Diazepam (QN05BA01); Flumazenil (QV03AB25); Naloxone (QV03AB15); Propofol (QN01AX10)
Substances in Regulation (EU) 1950/2006 **proposed to be removed**	Midazolam (QN05CD08); Sarmazenil (QV03AB91); Tiletamine (QN01AX99); Zolazepam (QN01AX99)
Substances from stakeholders' survey **proposed for inclusion**	Dexmedetomidine (QN05CM18)
Substances from stakeholders' survey **not proposed for inclusion**	Medetomidine (QN05CM91); Vatinoxan (no ATCvet code identified)

- Substances for systemic disorders:

	Active substance (ATCvet code)
Substances in Regulation (EU) 1950/2006 **proposed to be retained**, with or without amendment of the current entry	Allopurinol (M04AA01); Dobutamine (QC01CA07); Dopamine (QC01CA04); Ephedrine (QC01CA26); Glycopyrrolate (QA03AB02); Noradrenaline/norepinephrine (QC01CA03) ; Vasopressin (QH01BA01)
Substances in Regulation (EU) 1950/2006 **proposed to be removed**	Hydroxyethyl-starch (QB05AA07); Pentoxifylline (C04AD03)
Substances from stakeholders' survey **proposed for inclusion**	Dalteparin (QB01AB04); Gelatinpolysuccinate (B05AA06)
Substances from stakeholders' survey **not proposed for inclusion**	Aminocaproic acid (QB02AA01); Clopidogrel (QB01AC04); Enoxaparin (QB01AB05); Nadroparin (QB01AB06); Tranexamic acid (QB02AA02)

- Substances for tumours:

	Active substance (ATCvet code)
Substances in Regulation (EU) 1950/2006 **proposed to be retained**, with or without amendment of the current entry	None
Substances in Regulation (EU) 1950/2006 **proposed to be removed**	None
Substances from stakeholders' survey **proposed for inclusion**	Imiquimod (D06BB10)
Substances from stakeholders' survey **not proposed for inclusion**	5-Fluorouracil (L01BC02); Cisplatin (L01XA01); Mitomycin (QL01DC03); Sarcoid Cream (no ATCvet code identified);

- Miscellaneous:

	Active substance (ATCvet code)
Substances in Regulation (EU) 1950/2006 **proposed to be retained**, with or without amendment of the current entry	Domperidone (QA03FA03, noting the proposed use is not adequately covered by any ATCvet code for this substance)
Substances in Regulation (EU) 1950/2006 **proposed to be removed**	Cyproheptadine (QR06AX02); Imipramine (QN06AA02, noting the proposed use is not adequately covered by any ATCvet code for this substance)
Substances from stakeholders' survey **proposed for inclusion**	Cetirizine (QR06AE07, QS01GX12); Sulpiride (QN05AL01)
Substances from stakeholders' survey **not proposed for inclusion**	CBD (no ATCvet code identified); Fipronil (QP53AX15); Hydroxyzine (QN05BB01)

2. The scientific advice following the review of the entries in Table 2 of the Annex to Commission Regulation (EU) No 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal, is:

- that none of these substances, currently not included in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), should be included in the list.

Points for further consideration

Additional scientific considerations are proposed for the substance 'pergolide', the assessment of which is presented in section 4.8.3 of this scientific advice.

Considering the European Commission's request and the criteria given for assessment, it is concluded that pergolide is qualified for inclusion in the list. However, it is advised that pergolide should be excluded from the list of essential substances for *Equidae* for the reasons detailed below. Pergolide is not listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013).

Pergolide is used for the symptomatic treatment of pituitary pars intermedia dysfunction in horses (PPID, also known as Equine Cushing's disease). PPID is the most common endocrine disorder of geriatric horses, affecting 20-25% of horses over 15 years of age (Kirkwood et al., 2022[14]). Pergolide is the treatment of choice for PPID and is marketed in the EU for the management of clinical signs associated with PPID; pergolide-containing veterinary medicinal products are authorised for use in non-food-producing equine species.

Since PPID was first described in 1932 considerable research has been conducted investigating PPIDs pathophysiology, prevalence of clinical signs, appropriate diagnostic techniques, and treatment options (Kirkwood et al., 2022). It is now well-known that the pathogenesis of this disease leads to chronic elevations in adrenocorticotropic hormone (ACTH) and blood cortisol levels. This further leads to serious clinical abnormalities including lethargy, muscle atrophy (due to atrophy of types 2A and 2B muscle fibres), polydipsia, polyuria, recurrent infections, insulin dysregulation, or laminitis. Other clinical abnormalities associated with PPID include hypertrichosis, impaired shedding, pendulous

[14] Kirkwood, N.-C., Hughes, K.-J., Stewart, A.-J., 'Pituitary Pars Intermedia Dysfunction (PPID) in Horses', Vet Sci, 2022, Vol. 9, p. 556.

abdomen, abnormal fat deposition, hyperhidrosis, infertility, or behavioural changes (Aleman et al., 2006[15]; Kirkwood et al., 2022).

PPID is a chronic metabolic disorder of *Equidae* and incurable, but manageable; appropriate life-time treatment is necessary for quality of life of the horse, which includes use of pergolide mesylate and husbandry practices (Tatum et al., 2020[16]).

In fulfilling the conditions laid out in Article 115(5), a 6-months withdrawal period must be respected for any substance included in the list. During the time without treatment, the available evidence on the disease pathogenesis indicates that all clinical manifestations of PPID, including the most serious ones, will re-appear, which poses an unacceptable scenario from an animal welfare point of view. Pergolide-treated animals suffering from PPID will never be in a position to adhere to the 6-months withdrawal period and enter the food-chain, unless the animal is to experience the full clinical manifestations. This has additional implications towards the suitability of such an animal for human consumption.

As previously indicated, elevated blood cortisol is both a manifestation of untreated PPID as well as related to painful clinical manifestations (recurrent infections, laminitis). It is well established that high cortisol levels prior to slaughter have a major negative impact on meat quality leading to depletion of glycogen reserves in muscles and adversely impacting on water-holding capacity of meat (Colditz et al., 2006[17]; Ekiz et al., 2012[18]; Maghfiroh et al., 2014[19]). Modern slaughterhouse management aims at minimizing stress, cortisol levels, and its adverse effects on meat quality. In addition, research studies have demonstrated that there is an effect of the age of the horse on the slaughter value and quality of horsemeat (Stanisławczyk et al., 2023[20]). PPID is a disease of old horses. As horses age, their meat contains decreasing amounts of water, with increasing amounts of fat and minerals; this change is largely related to the properties of muscle fibers. Stanisławczyk et al., (2023) revealed that young horses had the most desired meat quality characteristics as compared to older horses from 10 to 20 years of age.

Overall, considering that

- PPID is incurable and requires life-long management,
- as per the disease pathogenesis, PPID horses/ponies will never be in a position to adhere to the 6-months withdrawal period and enter the food-chain, unless the animal is to experience the full clinical manifestations,
- clinical manifestations include very serious painful conditions including recurrent infections, laminitis, lethargy and muscle atrophy,
- the pathogenesis of untreated PPID include chronic elevations in blood cortisol, which further leads to generalised muscle wastage and poor meat quality,

[15] Aleman, M., Watson, J.-L., Williams, D.-C., LeCouteur, R.-A., Nieto, J.-E., Shelton, G.-D., 'Myopathy in horses with pituitary pars intermedia dysfunction (Cushing's disease)', Neuromuscul Disord, 2006, Vol. 16, pp. 737-744.
[16] Tatum, R.-C., McGowan, C.-M., Ireland, J.-L., 'Efficacy of pergolide for the management of equine pituitary pars intermedia dysfunction: A systematic review', Vet J, 2020, Vol. 266, p. 105562.
[17] Colditz, I.-G., Watson, D.-L., Kilgour, R., Ferguson, D.-M., Prideaux, C., Ruby, J., Kirkland, P.-D., Sullivan, K., 'Impact of animal health and welfare research within the CRC for Cattle and Beef Quality on Australian beef production', Aust J Exp Agr, 2006, Vol. 46, pp. 233-244.
[18] Ekiz, B., Ekiz, E.-E., Kocak, O., Yalcintan, H., Yilmaz, A., 'Effect of pre-slaughter management regarding transportation and time in lairage on certain stress parameters, carcass and meat quality characteristics in Kivircik', Meat Sci, 2012, Vol. 90, pp. 967-976.
[19] Maghfiroh, K., Latif, H., Santoso, K., 'Cortisol Hormone Concentration and Meat Quality of Beef Cattle Stunned by Captive Bolt Stun Gun before Slaughtering', Media Peternakan, 2014, Vol. 37(3), pp. 155-160.
[20] Stanisławczyk, R., Zurek, J., Rudy, M., Gil, M., 'Influence of Horse Age on Carcass Tissue Composition and Horsemeat Quality: Exploring nutritional and Health Benefits for Gourmets', Appl Sci, 2023, Vol. 13, p. 11293.

- in accordance with Commission Implementing Regulation (EU) 2019/627[21] laying down uniform practical arrangements for the performance of official controls on products of animal origin intended for human consumption, a horse suffering from a generalised disease such as PPID would likely not fulfil the requirements as regards ante-mortem inspection at the slaughterhouse in accordance with Article 11, and be declared as not healthy for slaughter at the end of the six-month withdrawal period,

it is advised that pergolide be excluded from the list of essential substances for *Equidae*.

Acknowledging the European Commission's request could not anticipate all proposed clinical scenarios for the substances to be considered for inclusion, the proposal to exclude pergolide is consistent with the spirit of the European Commission's request and protects animal welfare. Animals suffering from PPID horses should be excluded from the food-chain and treated appropriately to avoid unnecessary suffering of the animals.

[21] Commission Implementing Regulation (EU) 2019/627 of 15 March 2019 laying down uniform practical arrangements for the performance of official controls on products of animal origin intended for human consumption in accordance with Regulation (EU) 2017/625 of the European Parliament and of the Council and amending Commission Regulation (EC) No 2074/2005 as regards official controls.

Scientific advice under Article 115(5) of Regulation (EU) 2019/6 on veterinary medicinal products, regarding the list of substances which are essential for the treatment of equine species and for which the withdrawal period for equine species shall be six months

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1. Terms of reference and scope

1.1. Request from the European Commission for scientific advice regarding implementing measures under Article 115(5) of Regulation (EU) 2019/6

On the 9 February 2023, the European Medicines Agency (EMA, the Agency) received from the European Commission a request to provide scientific advice for the establishment of 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.

This request is in accordance with the provision in Article 115(5) of Regulation (EU) 2019/6, which indicates that *"by way of derogation from Article 113(1) and (4), the Commission shall, by means of implementing acts, establish 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. Those implementing acts shall be adopted in accordance with the examination procedure referred to in Article 145(2)*".

The European Commission indicated that the Agency was to consider the following when preparing the scientific advice:

- *The overall objective of the VMP Regulation to increase the availability of VMPs.*
- *Experience gained with the application of the current list of substances essential for the treatment of Equidae as listed in Regulation (EU) 1950/2006 as amended by Regulation (EU) 122/2013. For this purpose, the Agency should review the existing entries in the current list and carry out a survey among national competent authorities and relevant stakeholders, on the possible need to add other substances as a result of newly available evidence and the need for updating the information on use, advantages and alternatives of the entries in the current list.*
- *A substance should only be considered as essential if i) no satisfactory alternative treatment for an indication is authorised for food-producing animals of the equine species or ii) it brings added clinical benefit compared to other treatment options and where the condition would, if untreated, put at risk animal or public health, or cause unacceptable suffering for the animal.*
- *A substance should only be considered as bringing added clinical benefit where no medicinal product authorised for food producing animals of the equine species would yield equally satisfactory results based on robust evidence in terms of successfully treating the animal, avoiding unnecessary suffering for the animal, or ensuring the safety of those treating the animal.*
- *Substances listed in Table 1 of the Annex to Commission Regulation (EU) 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin, including those not having an MRL status in equine species, should not be considered for inclusion in the list. Substances included in Table 2 of that Annex should be considered unless there are concerns related to consumer protection.*
- *Substances listed in the Annex to Commission Implementing Regulation (EU) 2022/1255 designating antimicrobials or groups of antimicrobials reserved for human treatment of certain infections in humans, should not be considered.*
- *Coherence should be ensured with the ongoing work of the EMA on its scientific advice to the Commission, and the resulting implementing act, setting a list of antimicrobials, which shall not be*

used in accordance with Articles 112, 113 and 114 or which may be used in accordance with these articles subject to certain conditions.

- *The overall objective of maintaining a high level of consumer protection should be kept in mind. Therefore, substances can only be included in the list of essential substances when they do not compromise consumer safety.* As clarified by the Commission, this reference to compromising consumer safety is to be considered in the context of the possible presence of residues of the substance at levels which may be of concern beyond the six-month withdrawal period set by Article 115(5).
- *The advice should consist of a proposed list of substances along with the aimed indication, explanation of use, identification of alternatives and a justification for the inclusion of each of the substances.*

The deadline for providing the advice was set by the European Commission by 31 March 2024. In February 2024 the Commission agreed to a four-month extension of the deadline until 31 July 2024, with due consideration of the additional workload emanating from the survey results.

1.2. Legislative background

Regulation (EU) 2019/6 lays down rules for the placing on the market, manufacturing, import, export, supply, distribution, pharmacovigilance, control and use of veterinary medicinal products in the EU.

Article 113 lays down the conditions for the use of medicinal products outside the terms of the marketing authorisation in food-producing terrestrial animal species. Paragraph 1 provides the rules to exceptionally treat food-producing terrestrial animal species under the direct personal responsibility of the veterinarian responsible, and in particular to avoid causing unacceptable suffering. Paragraph 4 establishes that the pharmacologically active substances included in the medicinal product used in accordance with paragraphs 1 (and 2) shall be allowed in accordance with Regulation (EC) No 470/2009 and any acts adopted on the basis thereof.

Article 115 establishes criteria to set a withdrawal period for medicinal products used outside the terms of the marketing authorisation in food-producing animal species. Paragraph 5 of the same provision foresees, as indicated above, a derogation from Article 113(1) and (4), and establishes that the withdrawal period for equine species shall be six months for the active substances which are on the list to be established.

The current list of substances essential for the treatment of *Equidae* and of substances bringing added clinical benefit established by Commission Regulation (EU) 1950/2006, as amended by Commission Regulation (EU) 122/2013, was considered. The main principles from this Commission Regulation remain.

Commission Regulation (EU) 37/2010[22] provides a classification of pharmacologically active substances regarding their maximum residue limits in foodstuffs of animal origin in accordance with Regulation (EC) No 470/2009[23] and any acts adopted on the basis thereof. Substances are classified in two categories, allowed and prohibited substances, and presented in tables 1 and 2 of the Annex,

[22] Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin.
[23] Regulation (EC) No 470/2009 of the European Parliament and of the Council of 6 May 2009 laying down Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin, repealing Council Regulation (EEC) No 2377/90 and amending Directive 2001/82/EC of the European Parliament and of the Council and Regulation (EC) No 726/2004 of the European Parliament and of the Council.

respectively. The published consolidated version of this legal act was considered when addressing the request from the European Commission at the time of submission of the present scientific advice.

Commission Implementing Regulation (EU) 2022/1255[24] designates antimicrobials or groups of antimicrobials reserved for treatment of certain infections in humans, in accordance with Regulation (EU) 2019/6. This Regulation follows the scientific advice[25] provided by the Agency on 25 May 2022, in relation to implementing measures under Article 37(5) of Regulation (EU) 2019/6 on veterinary medicinal products. It was considered when preparing the present scientific advice.

1.3. Other documents referenced in the request for scientific advice

In its request, the European Commission indicated coherence should be ensured with the ongoing work of EMA on its scientific advice to the Commission, and the resulting implementing act, setting a list of antimicrobials, which shall not be used in accordance with Articles 112, 113 and 114 of Regulation (EU) 2019/6 or which may be used in accordance with these articles subject to certain conditions. The European Commission clarified that coherence between both lists will ultimately be ensured when drafting the associated implementing acts.

The Agency's recommendation[26] (scientific advice) was sent to the European Commission on 16 June 2023. The recommendation was considered, as appropriate, for the preparation of this advice.

[24] Commission Implementing Regulation (EU) 2022/1255 of 19 July 2022 designating antimicrobials or groups of antimicrobials reserved for treatment of certain infections in humans, in accordance with Regulation (EU) 2019/6 of the European Parliament and of the Council.
[25] Advice on the designation of antimicrobials or groups of antimicrobials reserved for treatment of certain infections in humans - in relation to implementing measures under Article 37(5) of Regulation (EU) 2019/6 on veterinary medicinal products (accessible via this link).
[26] Advice under Article 107(6) of Regulation (EU) 2019/6 for the establishment of a list of antimicrobials which shall not be used in accordance with Articles 112, 113 and 114 of the same Regulation or which shall only be used in accordance with these articles subject to certain conditions(accessible via this link).

2. Methodology

2.1. Expert group composition

Upon receipt of the request from the European Commission, the Committee for Veterinary Medicinal Products (CVMP) of the Agency formed an expert group to prepare the scientific advice. The experts that joined the group had either expertise on the clinical setting with animals of the equine species, expertise on antimicrobial resistance (AMR) and with knowledge of the ongoing work of EMA on its scientific advice to the European Commission with regards to Article 107(6), or expertise on consumer safety. The group was composed of 8 experts selected from the European network of experts on the basis of recommendations from the national competent authorities, supported by the Agency.

2.2. General considerations and two-tiered assessment approach

In the European Commission's request, it was stated that *the advice should consist of a proposed list of substances along with the aimed indication, explanation of use, identification of alternatives and a justification for the inclusion of each of the substances*. To fulfil this request, the expert group agreed the approach to:

- revise the existing entries in the list of substances essential for the treatment of *Equidae* and of substances bringing added clinical benefit, as established in Commission Regulation (EU) 1950/2006, as amended by Commission Regulation (EU) 122/2013, and
- carry out the survey proposed by the European Commission and assess the substances proposed therein.

For the assessment of all substances the expert group applied a standardised approach. This consisted of the development of a monograph that followed the same assessment steps for all entries. To that effect, all the considerations as presented in the request from the European Commission were taken into account in a two-tiered assessment.

First, an assessment was carried out to determine if the substance was considered essential for the treatment of animals of the equine species. As provided for in the European Commission's request, a substance should only be considered as essential if:

- no satisfactory alternative treatment for an indication is authorised for food-producing animals of the equine species, or
- it brings added clinical benefit compared to other treatment options and where the condition would, if untreated, put at risk animal or public health, or cause unacceptable suffering of the animal.

The text of the European Commission's request specified that a substance should only be considered as bringing added clinical benefit where no medicinal product authorised for food producing animals of the equine species would yield equally satisfactory results based on robust evidence in terms of successfully treating the animal, avoiding unnecessary suffering of the animal, or ensuring the safety of those treating the animal.

In order to determine whether a substance fulfilled the above criteria, the following aspects of the substance were considered as part of this first assessment:

- The clinical characteristics of the condition (or disease) to be treated or diagnosed, as clarified by the European Commission, and how the substance is used in the disease process. This included a

consideration of whether the condition (or disease) would, if untreated, put at risk animal or public health, or cause unacceptable suffering of the animal.

- The pharmacodynamic properties of the substance(s), including the (main) mechanism of action.
- When the substance is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), the specific indication(s) for use of the substance as detailed in the Regulation, as well as other known clinical use(s) in animals of the equine species.
- The availability of satisfactory alternative treatment(s) (for the indication or procedure) authorised for food-producing animals of the equine species. The Union Product Database[27] was consulted. It was scientifically discussed whether any available treatment could constitute a satisfactory alternative treatment as detailed in the request; in the context of such considerations, substances with an equivalent clinical profile and an entry in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 were considered as satisfactory alternatives, even if an authorised treatment for the proposed indication could not be found specifically for food-producing animals of the equine species. Discussion also entailed, when appropriate, elaboration of the added clinical benefit of the substance compared to the existing alternative treatment options, including those already listed or proposed for addition to the list.
- The overall objective to increase availability was also considered as part of these considerations.
- Any information arising from the survey to stakeholders (see below and section 3 for further details).

Considering all these, a conclusion for the substance regarding its 'essentiality' was reached. The conclusion included the indication that the expert group was ready to accept and that could, in justified cases, be different from that currently captured in Commission Regulation (EU) 1950/2006, as amended by Commission Regulation (EU) 122/2013. In all cases, appropriate justification supporting the proposal was provided.

When the substance was concluded by the expert group as essential, then a further assessment was required, focusing on the consumer safety aspects of the proposed substance (see below). On the contrary, when the substance was concluded by the expert group as not qualifying as essential, no further assessment was carried out.

The request from the European Commission indicated that substances can only be included in the list when they do not compromise consumer safety. For that purpose, the provision in Article 115(5) of Regulation (EU) 2019/6 indicates that the withdrawal period for equine species shall be six months.

As provided for in the European Commission's request, *substances listed in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin, including those not having an MRL status in equine species, should not be considered for inclusion in the list. Substances included in Table 2 of that Annex should be considered unless there are concerns related to consumer protection*.

For the consumer safety assessment, the following aspects relevant to the substance were considered:

- If there are similar molecules or substances belonging to the same class of molecules for which a MRL entry is available in Table 1 of the Annex to Commission Regulation (EU) No 37/2010.

[27] Union Product Database (https://medicines.health.europa.eu/veterinary)

- If there are published pharmacokinetic/pharmacodynamic studies in *Equidae* or other animal species, or other key data (e.g. half-life) allowing for an understanding of persistence of the substance within the animal's body.
- If there are published studies in laboratory animals or in vitro tests investigating toxicity of the substance (e.g. AMES test).
- If the SPC of human or veterinary medicinal products containing the proposed substance contains any relevant information related to its toxicity properties (e.g. teratogenicity or carcinogenicity).

Considering all available evidence, a conclusion regarding the acceptability of a six-months withdrawal period for the substance to not compromise consumer safety was reached. Evidence of genotoxicity or carcinogenicity led to a conclusion that the substance is not appropriate for use in food producing *Equidae*; other pharmacological and toxicological properties (and related effects in humans, as appropriate) were considered in the context of the possible presence of residues of the substance at levels which may be of concern beyond the six-month withdrawal period set by Article 115(5) (see section 2.5 for further details).

Only substances that were considered essential and for which a six-months withdrawal period was deemed sufficient to protect consumer safety were proposed to be included in the list.

The individual assessments conducted in accordance with the two-tiered approach are presented in sections 4 and 5. Each individual assessment contains a justification, as requested by the European Commission, for the proposed outcome and, when the recommendation is to include the substance in the list, the conclusion states the intended indication, the explanation of use, and the identification of alternatives for each substance. With regards to the identification of alternatives, it is noteworthy that only substances listed in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 or deemed essential following the assessment presented in sections 4 and 5 of this report, i.e. proposed for inclusion in the list, were considered as alternatives.

The proposed list of substances is presented in tabular format as an annex to this scientific advice report (see section 6).

2.3. Other considerations arising from the European Commission's request

The European Commission's request made explicit that when providing the scientific advice, the Agency should take into account the overall objective of Regulation (EU) 2019/6 to increase the availability of VMPs and the overall objective of maintaining a high level of consumer protection. Both objectives have been considered by the expert group when preparing the scientific advice.

The request included the suggestion to carry out a survey among national competent authorities and relevant stakeholders, on the possible need to add other substances as a result of newly available evidence and the need for updating the information on use, advantages and alternatives of the entries in the current list. The survey is discussed in detail in section 3 (see below).

It was also stated in the European Commission's request that substances listed in the Annex to Commission Implementing Regulation (EU) 2022/1255 designating antimicrobials or groups of antimicrobials reserved for human treatment of certain infections in humans, and substances listed in Table 1 of the Annex to Commission Regulation (EU) No 37/2010, were not to be considered. No assessment was thus warranted in either case.

Consideration was given to substances listed in Table 2 of the Annex to Commission Regulation (EU) No 37/2010, as indicated in the European Commission's request. These had been previously assessed

by the Committee for Veterinary Medicinal Products (CVMP), in accordance with Regulation (EC) No 470/2009 (or its predecessor, Regulation (EC) No 2377/90), and it was concluded that an "MRL cannot be established" (a high-level assessment for each substance is publicly available in their respective European Public MRL Assessment Reports, EPMARs, accessible via the Agency's corporate website[28]). For one substance in this group for which a well-documented use in equine practice was recognised, a complementary safety assessment was undertaken and is presented in section 7.4 (see annex).

2.4. Revised classification of substances

The list of substances laid down in Regulation (EU) 1950/2006, as amended by Regulation (EU) 122/2013, contains eighty-eight substances that are classified, primarily, according to its intended therapeutic use. When considering the results of the survey to stakeholders it was considered that a revision of the former classification of substances would be warranted.

The new proposed classification considers both the intended therapeutic use of the substance and the body system in which its therapeutic effect is foreseen. This allows for a classification that reduces the 'miscellaneous' group to a minimum. The assessment presented in section 4 follows this proposed reclassification.

2.5. Data sources, data gaps and uncertainties

To conduct the review of the existing entries in the current list, and to consider the possible need to add other substances or to update the information on use, advantages and alternatives to the entries in the current list, it was considered necessary to backup any proposal with reference to scientific evidence. However, as indicated in the European Commission's request, any other robust evidence like e.g. expert judgment has also been considered, as appropriate, and thoroughly discussed.

Published scientific knowledge has been derived, primarily, from textbooks, review articles, case studies, and retrospective studies. When available, clinical trials were also referenced. Peer-reviewed scientific literature was preferred. While data from *Equidae* species was prioritised, data from other species was also considered, when relevant. Furthermore, international equine guidelines, consensus statements, monographs from international organisations (e.g. OIE, WHO) were also considered as reliable sources of scientific reference for the assessments.

When any member of the expert group preparing this scientific advice had clinical experience and expertise with any of the assessed substances, such knowledge was considered; similarly, statements made by responders to the stakeholder survey were taken into account for the discussions. In either case, such expert judgement was discussed along with any available published scientific evidence.

The quality of the evidence used in the assessment is discussed at the end of each assessment section (i.e. it is stated how much of the assessment relies on published evidence and on expert judgement).

For the consumer safety assessments, the expert group noted that there are generally no residue depletion studies available (nor other dedicated scientific assessments with the substances) on which to base a conclusion regarding consumer safety following a 6-month withdrawal period. Thus, it was accepted that the expert opinion would be largely based on expert judgement considering all the available evidence regarding the intrinsic characteristics of the substance that could contribute to a consumer safety risk. Such judgement was made on a per-substance basis, and the proposed conclusions based on the available evidence at the time of assessment. The experts aimed to always

[28] European Medicines Agency, Maximum residue limit assessment reports: https://www.ema.europa.eu/en/find-medicine/maximum-residue-limit-assessment-reports

ensure the overall objective to maintain a high level of consumer protection. However, it is acknowledged that all proposed conclusions entail a degree of uncertainty, mainly due to the absence of dedicated residue depletion studies for each relevant tissue. Therefore, these conclusions could be overturned if new evidence arises.

3. Survey conducted among national competent authorities and stakeholders, on the possible need to add other substances as a result of newly available evidence and the need for updating the information on use, advantages and alternatives to the entries in the current list.

3.1. Background

As indicated above, the request from the European Commission included the recommendation *to carry out a survey among national competent authorities and relevant stakeholders on the possible need to add other substances as a result of newly available evidence and the need for updating the information on use, advantages and alternatives to the entries in the current list*.

The expert group appointed by the CVMP prepare the scientific advice discussed and adopted a survey during its second meeting held on 3 April 2023. The CVMP discussed and adopted the survey at its meeting held 18-20 April 2023.

The survey, which was published shortly after its adoption by CVMP, was available until 30 June 2023. Its design and the results obtained are presented hereinafter.

3.2. Survey design

The survey was designed and released using EU Survey[29].

It contained an introduction with general information about the rationale for conducting this survey and the objectives; the legal framework and an excerpt of the general terms established by the European Commission in their request were also stated, and hyperlinks to relevant documents or legislative text provided for ease of reference. It was clearly indicated that the information collected would be used to formulate scientific advice.

The survey itself contained five questions: two general questions and three specific. Before the specific questions, a note was added indicating the terms of the European Commission's request for inclusion of any substance in the list together with hyperlinks to the relevant documents for ease of reference.

Questions were as follows:

- The first question aimed to identify the responder's stakeholder group. Options given were equine animal healthcare practitioner, non-equine animal healthcare practitioner, academia, regulator (e.g. from NCA) and other (e.g. from pharmaceutical industry).
- The second question offered a dropdown menu to select the responder's country among the EU-27 countries, 'EU/EEA country' or 'non-EU country'.
- The third question was specific, designed to get the opinion of the responder as to whether a substance should be 'added', 'removed', 'updated' or 'none of the above' (more than one option could be selected). A tabulated response model was offered, customized depending on the selected option. Responders were encouraged to provide scientific or other duly justified evidence (e.g. no alternatives available); it was stated that published peer-reviewed scientific articles were preferred.

[29] https://ec.europa.eu/eusurvey/home/welcome

- The fourth question focused on identifying alternatives to substances included in the current list or proposed to be added.
- The fifth and final question was a blank box to provide any additional information relevant to this exercise.

3.3. Survey results

A total of 152 responses were recorded in the EU survey tool. However, a deeper look at these allowed identifying consecutive responses added by the same responder; it was determined that the correct number of responses is 136.

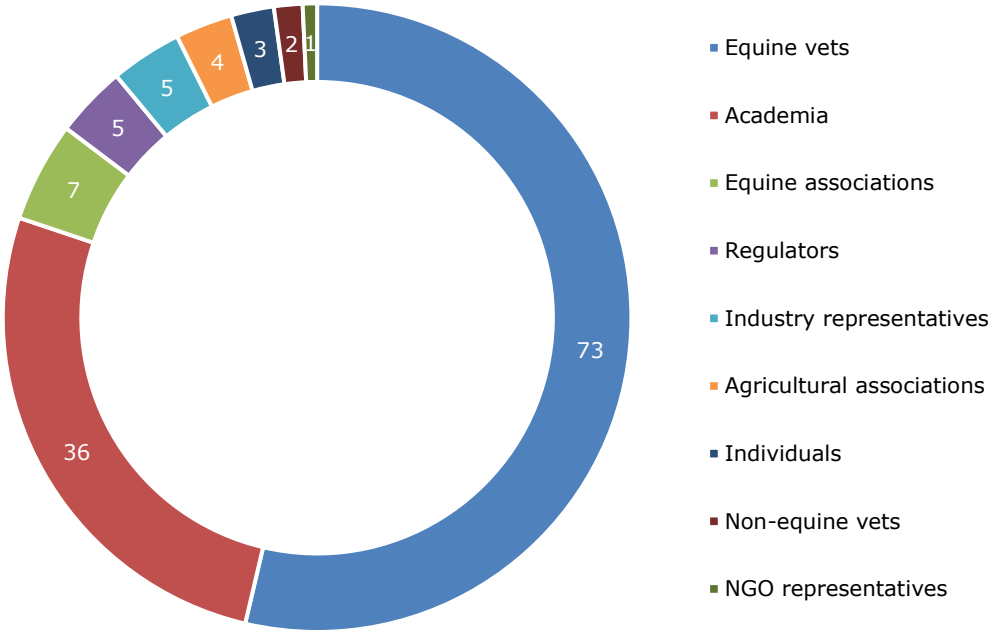

Figure 1. Number of responders to the survey per stakeholder group, with equine animal healthcare practitioners and representatives from academia accounting for approximately 80% of them.

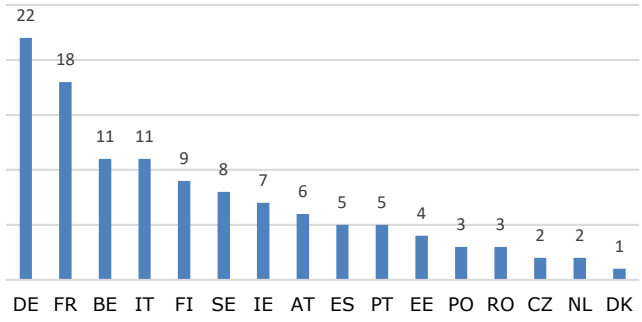

Figure 2. Distribution of responders per EU-27 country. In addition, 2 responders were from EEA countries and 17 from non-EU/EEA countries.

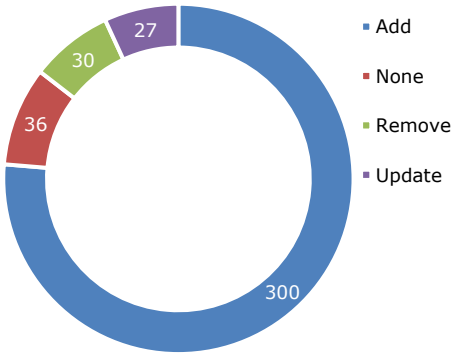

Figure 3. Distribution of proposals from responders.

All targeted stakeholders were represented (see Figure 1). Most responders (i.e. 117) were from countries within the EU-27; only two were from EEA countries and seventeen from non-EU/EEA countries (see Figure 2). The responders made a total of 393 proposals relating to the list (see Figure 3), to either 'add', 'remove' or 'update' an entry for a substance in the list; or to indicate that 'none' of the previous categories was deemed appropriate, meaning that in the responders' view the current list in Regulation (EU) 1950/2006, as amended by Regulation (EU) 122/2013, could remain unchanged.

It was noted that these 393 proposals do not translate directly into 'substances' proposed, since several of these constitute repetitions for a given substance. Therefore, it was considered warranted to review the survey results to identify repetitions, as well as proposals not in line with the request from the European Commission. In reviewing the results, the following was noted:

- Proposals relating to 24 substances currently in Regulation (EU) 1950/2006, as amended by Regulation (EU) 122/2013, were received. Some of these were inadequately characterised (i.e. the responder proposed to add a substance that is already included in the current list). Overall, updates and/or removals were proposed for 19 substances (see table below for further details).
- Several proposals corresponded to substances included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010. As indicated in the European Commission's request, these are not eligible for inclusion in the list and no assessment was warranted. The following were proposed: acetylsalicylic acid, amoxicillin, atropine, benzylpenicillin, bupivacaine, butylscopolaminium bromide, detomidine, dimethyl sulphoxide, doxycycline, enilconazole, epinephrine, flunixin, furosemide, gentamicin, imidocarb, isoflurane, ivermectin, ketamine, levothyroxine, lidocaine, mepivacaine, moxidectin, neogstigmine, omeprazole, paracetamol, sodium 2-methyl-2-phenoxypropanoate, tetracaine, thiopental, trimethoprim and tulathromycin.
- A substance, polyacrylamide hydrogel (CAS No: 9003-05-8), was proposed. However, the CVMP had previously concluded it does not exert pharmacological effect, thus an entry was added to the list of substances not falling within the scope of Regulation (EC) No 470/2009 with regards to residues of veterinary medicinal products in foodstuffs of animal origin[30]; therefore, it was considered that no further assessment was warranted. Similarly, an immunological substance (BCG vaccine) was proposed. However, immunological substances are not within the scope of Regulation (EC) No 470/2009; therefore, no further assessment was warranted.
- Salbutamol is included on the List B (prohibited substances with derogations) of Annex II of the Council Directive 96/22/EC concerning the prohibition on the use in stockfarming of certain substances having a hormonal or thyrostatic action and of beta-agonists, and repealing Directives 81/602/EEC, 88/146/EEC and 88/299/EEC. Its administration to *Equidae* for therapeutic purposes is only possible if used within the terms of the marketing authorisation of an authorised veterinary medicinal product. Hence, this substance being a beta-agonist cannot be considered for inclusion in the list.
- Four proposals were inadequately characterised (e.g. "anthelmintic"), and it was not possible to relate any of these to a given substance.
- Finally, proposals were identified for two substances included in Table 2 of the Annex to Commission Regulation (EU) No 37/2010. According to the European Commission's request, consideration is given to all substances in Table 2 in a separate assessment (see section 5 for further details).

[30] Substances considered not to exert pharmacological effects: https://www.ema.europa.eu/en/veterinary-regulatory/research-development/maximum-residue-limits-mrl#substances-considered-not-to-exert-pharmacological-effects-section

A hundred and thirty-one (131) substances were mentioned in the survey (see tables 1 and 2 below):

- twenty-four (24) correspond to substances that are currently in Regulation (EU) 1950/2006, as amended by Regulation (EU) 122/2013; however, only 19 of these were adequately characterised as revisions to the current entries;
- seventy-five (75) correspond to new substances proposed to be added to the list; and
- thirty-two (32) to substances that do not fulfil the criteria for inclusion in the list as per the European Commission's request, or that cannot be considered for inclusion within the current legal framework.

It is worth noting that the number of individual proposals per substance ranged from 1 to 27.

With their proposals, responders mainly referred to 'other duly justified evidence' (i.e. clinical experience or expertise of responders, or absence of alternatives) as a justification; only in 25% of the cases (i.e. in 98 out of the 393 individual proposals) was scientific evidence, mainly peer-reviewed scientific articles, referenced.

Only 8 alternatives to substances included in the current list or proposed to be added were identified.

Overall, the expert group concluded that the survey had served to its purpose; the number of responses received is significant and all targeted stakeholders were represented in the responses received. The results are thus considered relevant.

Table 1. Substances currently in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) mentioned in the survey; A = add, U = update, R = remove; numbers indicate times proposed.

Active substance	A	U	R	Active substance	A	U	R	Active substance	A	U	R
Ambroxol			5	Latanoprost			5	Sarmazenil			1
Amikacin		5		Midazolam		1		Sevoflurane		1	1
Budesonide	1		1	Pethidine			1	Sucralfate		1	
Bupivacaine	1			Phenylephrine	3			Ticarcillin	1		5
Domperidone	1			Polymyxin B	9	5		Tiletamine		1	
Fluorescein	1			Propofol		1		Timolol maleate	1		
Fluticasone	1		1	Ranitidine			6	Tropicamide	1	1	
Griseofulvin			1	Rifampicin		5	1	Zolazepam		1	

Table 2. New substances proposed to be added to the list, i.e. not in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013); n = number of times proposed.

Active substance	n	Active substance	n	Active substance	n	Active substance	n
5-fluorouracil	1	Dalteparin	2	Methadone	9	Sarcoid cream 5Fu	1
Acetazolamide	1	Dexmedetomidine	5	Methocarbamol	2	Sotalol	1
Alprazolam	1	Disodium oxydronate	1	Methylene diphosphonate	1	Sulpiride	5
Aminocaproic acid	1	Enoxaparin	2	Metronidazole	3	Synephrine	12
Amphotericin B	1	Ertugliflozin	2	Misoprostol	9	Tacrolimus	1
Brinzolamide	1	Fipronil	1	Mitomycin	3	Tenoic acid	16
Bromfenac	1	Flecainide	1	Moxifloxacine	1	(Acid) Tetrasodium dihydrogenbutedronate diphosphono-1,2-propanedicarboxylic	1
Buparvaquone	2	Fluconazole	1	Nadroparin	1	Tetryzoline	13
Canagliflozin	1	Fluoxetine	2	Pentosan Polysulphate	1	Thiocolchicoside	1
CBD	2	Flutamide	1	Pergolid	6	Tobramycin	2
Cetirizine	4	Fusidic acid	10	Phenobarbital	1	Tranexamid acid	1
Chloramphenicol	3	Gancyclovir	2	Phenylbutazone	27	Trazodone	1
Chondroitin sulphate	1	Gelatinpolysuccinat	1	Pilocarpine	1	Valacyclovir	5
Ciprofloxacin	8	Glucosamine	1	Pregabalin	2	Vatinoxan	1
Cisatracurium	1	Goserelin	1	Propafenone	1	Velagliflozin	1
Cisplatin	1	Hydroxyzine	1	Quinapril	1	Verapamil	1
Clarithromycin	8	Imiquimod	1	Rifamycine	14	Voriconazole	3
Clopidogrel	1	Medetomidine	3	Rocuronium	1		
Cyclopentolate	1	Metformin	2	Ropivacaine	2		

4. Review of the entries in Regulation (EU) 1950/2006, as amended by Regulation (EU) 122/2013 – considering the survey results – and assessment of new proposed entries as per the stakeholders survey

4.1. Anaesthetics

4.1.1. Overview

	Active substance (ATCvet code)
Substances in Regulation (EU) 1950/2006 **proposed to be retained**, with or without amendment of the current entry	Oxybuprocaine (QS01HA02); Prilocaine (QN01BB04)
Substances in Regulation (EU) 1950/2006 **proposed to be removed**	Bupivacaine (QN01BB01); Sevoflurane (QN01AB08)
Substances from stakeholders' survey **proposed for inclusion**	None
Substances from stakeholders' survey **not proposed for inclusion**	Ropivacaine (QN01BB09)

4.1.2. Review of the existing entries in Regulation (EU) 1950/2006, as amended by Regulation (EU) 122/2013, considering the survey results

A. Considerations on the essentiality of the substance(s)

Bupivacaine is an amide-type local anaesthetic. It is a derivative of mepivacaine but has longer side chains than mepivacaine, is more lipid soluble, and displays greater protein binding; this translates as slower onset, higher potency and longer duration of action (Dugdale, 2020). It has approximately four times the potency of lidocaine for myocardial depression and is 16 times more potent as arrhythmogenic. Levobupivacaine, which is less cardiotoxic, is available in human medicine (Dugdale, 2020).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for local anaesthesia. Lidocaine is identified as an alternative.

Bupivacaine was mentioned (once) in the survey to stakeholders and proposed for addition to the list.

Bupivacaine is listed in Table 1 of the Annex to Commission Regulation (EU) 37/2010 pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin with a 'no MRL required' status for porcine and bovine species. As stated in the mandate received from the European Commission on the 9 February 2023, *substances listed in Table 1 […], including those not having an MRL status in equine species, should not be considered for inclusion in the list*.

Thus, the substance bupivacaine cannot be considered for inclusion in the list.

Oxybuprocaine is an amide-type local anaesthetic. It binds to sodium channels and reversibly stabilizes the neuronal membrane, which decreases its permeability to sodium ions. Depolarization of

the neuronal membrane is thus inhibited, blocking the initiation and propagation of action potentials along the nerve fiber. Suppression of neurotransmission of nerve fibers mediates the primarily desired analgesic effects. It is used primarily in ophthalmology and otolaryngology.

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for local anaesthesia for use in eyes. No alternatives are identified, and the specific advantage captured in the current list reflects the wide clinical experience with the substance.

Oxybuprocaine has been shown to induce significant corneal anaesthesia (Lelescu et al., 2020). It has also been described to provide a greater and longer anaesthetic effect as compared to other ophthalmic anaesthetics making it more suitable for potentially painful ophthalmologic procedures (Lelescu et al., 2020).

The substance is not intended for the treatment of a specific condition. However, failure to adequately anesthetise the eye could cause unacceptable suffering of the animal.

Oxybuprocaine was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any oxybuprocaine-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance oxybuprocaine is proposed to be qualified as essential because no satisfactory alternative treatments are authorised for food-producing animals of the equine species for the following indication: for local topical anaesthesia for use in eyes.

Prilocaine is an amide-type local anaesthetic generally used (both in human and veterinary medicine) for topical anaesthesia of e.g. skin, genital mucosa and in dental procedures.

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for local anaesthesia prior to intravenous catheterisation. No alternatives are identified, and the specific advantages captured in the current list are its use in specific preparations (i.e. eutectic mixture of local anaesthetics) for topical application to skin where it is absorbed intradermally in 40 min, and that it can be used to facilitate intravenous catheterisation, especially in foals.

Prilocaine produces local nerve blocks with very little tissue reaction (i.e. little swelling is observed due to minimal effects on vasomotor tone) (Dugdale et al., 2020) and it's commonly used in a eutectic mixture with lidocaine (Aronson, 2014), so that it can be used to facilitate intradermal puncturing. Lidocaine could constitute an alternative treatment for local anaesthesia prior to intravenous catheterisation for food-producing animals of the equine species; it has an MRL entry in Commission Regulation (EU) No 37/2010 for *Equidae* and there are veterinary medicinal products authorised for food-producing animals of the equine species. However, in randomised trials, combinations of local anaesthetics in eutectic mixtures rendered slightly better results in reducing thermal nociception and mechanical sensation in horses (Söbbeler and Kästner, 2018). Interventional studies with prilocaine also showed slightly prolonged duration of the effects when compared to other local anaesthetics (Harcourt et al., 2021). Additionally, combination of lidocaine/prilocaine (as topical anaesthetic cream) was as effective as lidocaine infiltration and its use decreased the adverse events associated with monotherapy (Erkert et al., 2005).

The substance is not intended for the treatment of a specific condition. However, failure to adequately anesthetise the local area could cause unacceptable suffering of the animal.

Prilocaine was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any prilocaine-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance prilocaine is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: for local topical anaesthesia prior to intravenous injection or catheterisation. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Sevoflurane is an inhalation anaesthetic agent used for induction and maintenance of general anaesthesia during surgical procedures (Behne et al., 1999). It has a lower blood/gas partition coefficient, indicating lower solubility of the agent in blood, resulting in a good control of anaesthesia depth during induction, maintenance, and recovery (Grosenbaugh and Muir, 1998). Sevoflurane accumulates in the adipose tissue during long periods of general anaesthesia which may be an important factor during the recovery phase, where horses can experience emergence delirium and dysphoria due to the continued presence of a volatile agent in their system (Robinson et al., 2023).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), for inhalation anaesthesia for horses with limb fractures and other orthopaedical injuries and mask induction of anaesthesia in foals. Isoflurane is identified as its alternative and the specific advantages captured are as follows: 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.

Even though sevoflurane is cited as being superior to isoflurane, there is evidence that their differences may not be significant (White et al., 2021). Moreover, 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 (Steffey, 2002).

The substance is not intended for the treatment of a specific condition. However, failure to adequately anesthetise the animal could cause unacceptable suffering.

Sevoflurane was mentioned (two times) in the survey to stakeholders. Contradictory proposals are noted: one responder suggested to 'modify' the entry expanding its use to the maintenance of all adult horses and foals under anaesthesia while a second responder proposed that the substance be removed from the list. Arguments were provided in support of the latter, quoting a single centre, prospective, randomised, blinded clinical investigation that recruited 101 healthy client owned horses undergoing elective surgery where there was no significant difference between groups administered either sevoflurane or isoflurane in terms of haemodynamic support required during anaesthesia nor in quality or duration of recovery (White et al., 2021). Isoflurane has an MRL entry in Commission Regulation (EU) No 37/2010 for *Equidae* and there are veterinary medicinal products authorised for food-producing animals of the equine species.

A search in the veterinary medicines database does not retrieve any sevoflurane-containing veterinary medicinal product authorised for use in equine species. There are veterinary medicinal products authorised for use in species other than the equine (i.e. cat, dog).

The substance sevoflurane is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully anesthetising the animal, avoiding unnecessary suffering of the animal.

Knowledge regarding inhalation and local anaesthetics in horses for this assessment was derived from textbooks, review articles, retrospective studies, and clinical trials in horses.

B. Considerations regarding consumer safety

Oxybuprocaine is mainly used as a local anaesthetic in ophthalmology and otolaryngology cases in humans. It is also used for topical anaesthesia of the cornea in both large and small animals (Lelescu et al., 2020).

Anaesthetics of the ester type are hydrolysed by esterases in the plasma and, to a lesser extent, in the liver; there is little protein binding with this type of anaesthetics (Scriba, 2009). Its metabolism in humans is extensive. The drug is almost completely absorbed and is rapidly excreted in the urine. Nine metabolites and the parent drug were isolated from the urine (Kasuya et al., 1987a). After a 100 mg single oral dose, mean urinary excretion of oxybuprocaine and (five) metabolites after 9 hours was 89.2%. Only about 6% of the dose was excreted as unconjugated metabolites in urine (Kasuya, et al., 1987b).

The most common toxicities of topical ocular anaesthetics are to the ocular surface itself and systemic side effects are considered rare (McGee and Fraunfelder, 2007). The acute toxicity by oral administration is low when compared to the doses applied topically to the eye; no repeated-dose toxicity studies are available in the literature.

Considering the topical use of the substance, the rapid excretion reported and the apparent low systemic toxicity, it can be concluded that oxybuprocaine 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.

Prilocaine, is considered a local anaesthetic with low toxicity; its absorption is relatively slow, and its metabolism is very rapid. These features should reduce any potential for systemic toxicity. However, its metabolism, especially for the R-enantiomer, produces ortho-toluidine, which can cause methaemoglobinaemia.

Data in humans, from human medicinal products information, suggests that following intravenous administration the steady state volume of distribution is approximately 0.7-4.4 L/Kg; 55% of prilocaine is bound to plasma proteins, with the parent drug rapidly metabolized (mainly in liver) and excreted via urine. At doses of 8 mg/kg or above, the metabolite ortho-toluidine (or o-toludine) is believed to be responsible for adverse events such as methaemoglobinaemia and cyanosis. The metabolism of prilocaine is primarily due to the hydrolysis of the amide bond forming o-toluidine; the primary urinary metabolite is p-hydroxytoluidine (34% of dose), followed by o-hydroxytoluidine (2.7%) and o-toluidine (Scriba, 2009).

Pharmacokinetics from R(-)- and S(+)-prilocaine molecules after intravenous administration in humans have been evaluated. Differences in plasma clearance and terminal half-life were observed, indicating a faster elimination of the R(-) enantiomer. The longest half-life was observed for the S(+) enantiomer,

at 293 minutes only, and can be explained by different enzyme affinity (Van der Meer et al., 1999). In healthy Chinese volunteers, the pharmacokinetics of topical prilocaine were assessed after application of 15 g of 2.5%lidocaine / 2.5% prilocaine cream for 4 hours (test and positive control groups were included). The active components absorbed into skin layers were relatively low. For prilocaine, values for test and control groups were as follows, respectively: AUC_{0-t} was 97.29 and 95.27 ng.h/ml; C_{max} was 12.3 and 12.2 ng/ml; T_{max} was 7.5 and 8 h; and $t_{1/2}$ was 6.5 and 6.3 h (Lingjun et al., 2023).

In piglets, the systemic bioavailability of prilocaine following topical penile exposure to 1 g of lidocaine/prilocaine cream was low (7.2 ± 5.7%) and the ratio between exposure to o-toluidine with cream versus intravenous administration was also low (4.2 ± 9.3%) (Gazarian et al., 1995).

From a pharmacokinetic study in mice that compared a prilocaine and lidocaine-containing products when applied in lacerated and non-lacerated tissue, prilocaine absorption was very rapid, reaching peak plasma concentrations 20 minutes after application of 0.45 mg prilocaine in lacerated tissue and 60 minutes after application in non-lacerated tissue; C_{max} values also increased from 193.3 ng/ml in intact tissue to 658.1 ng/ml in lacerated tissue. The volume of distribution was high when applied to non-lacerated tissue (50.77 L vs 17.53 L). The elimination half-life was also rapid, being 15.25 minutes in intact skin and 26.12 minutes in lacerated tissue; clearance was 0.91 and 0.3 L/min in intact and lacerated tissue, respectively (Al-Musawi et al., 2016).

Regarding the o-toluidine metabolite in humans, this has only been investigated after subcutaneous administration of prilocaine or after administration of eutectic mixtures of prilocaine and lidocaine. After administration of an anaesthetic periodontal gel, Richter (2015) noted an average half-life elimination from plasma of 4 hours for the o-toluidine metabolite. It is noted that in rats, the biological half-life of o-toluidine bound to albumin or haemoglobin was observed to be 2.6 and 12.3 days, respectively, following an intraperitoneal single dose of [14C]-labelled o-toluidine (IARC, 2010).

The toxicology of prilocaine, and its metabolites, is well documented in the scientific literature. Data in human medicinal products information shows that prilocaine has low systemic toxicity, with its major disadvantage being the formation of methaemoglobin by its metabolites; an overdose increases the risk for methemoglobinemia and local anaesthetic systemic toxicity. No major findings in reproductive organs have been observed from repeat-dose toxicity studies. The toxicity of o-toluidine was addressed in mice fed with diets containing o-toluidine for a period of up to 14 days. The doses tested were 56.9, 239.6 and 668.6 mg/kg. No adverse clinical observations were reported. Prilocaine was not genotoxic either in vitro or in vivo genotoxicity tests. The o-toluidine metabolite has genotoxic potential in vitro, and from carcinogenicity studies in rats, mice and hamsters; tumours were observed in several organs. However, the clinical relevance of tumour findings with respect to short-term/intermittent use of prilocaine in humans is unknown. Human exposure is 1000-fold less than the minimum dose used in these studies. Ortho-toluidine (a constituent of tobacco smoke) was considered by IARC and classified as carcinogenic to humans (group 1).

Using the data available to estimate a worst-case scenario for consumers exposure, the expert group concluded that 99.9% of any o-toluidine residue would be eliminated from the treated animal after 123 days (please refer to the annex for further details on this estimate).

Considering the topical use of the prilocaine, the very limited exposure anticipated, the pharmacokinetic data reported, the margin of safety noted in humans for the most toxic metabolites and the worst-case estimate performed, it can be concluded that prilocaine 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.

4.1.3. Assessment of new substances proposed to be added to the list in the stakeholders survey

A. Considerations on the essentiality of the substance(s)

Ropivacaine was mentioned (two times) in the survey to stakeholders for addition to the list for long-acting local anaesthesia. The responders stated it is required for perioperative analgesia and treatment of chronic severe pain (e.g. laminitis). Its longer effect was compared to that of lidocaine (four to five hours vs one hour); however, no scientific references were provided.

It is a long-acting amide local anaesthetic agent; a pure S-enantiomer, with a high pKa and relatively low-lipid solubility (Hansen, 2014). It is known to have comparable effects to bupivacaine in terms of onset, quality and duration of sensory block and is of increasing interest in human medicine due to its increased safety profile particularly in terms of central nervous system and cardiovascular toxicity, when compared to bupivacaine (Hansen, 2014). In equine medicine, it is becoming more widely used particularly for diagnostic anaesthesia (Schumacher and Boone, 2021). In vitro studies have suggested that ropivacaine may be less toxic to chondrocytes (Breu et al., 2013; Jayaram et al., 2019) than bupivacaine, however there is yet no clear evidence as to the detrimental effect of a single intra-articular dose of any local anaesthetic solution in vitro (Schumacher and Boone, 2021). Bupivacaine could thus constitute an alternative treatment for long-acting local anaesthesia; is listed in Table 1 of the Annex to Commission Regulation (EU) 37/2010 pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin with a 'no MRL required' status for porcine and bovine species. There are veterinary medicinal products authorised for non-food-producing animals of the equine species.

The substance is not intended for the treatment of a specific condition. However, failure to adequately anesthetise the local area could cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any ropivacaine-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance ropivacaine is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Knowledge regarding inhalation and local anaesthetics in horses for this assessment was derived from textbooks, review articles and retrospective studies.

B. Considerations regarding consumer safety

Not warranted since the above-mentioned substance is not considered essential.

4.1.4. Conclusion

Based on the above assessment and justifications, the following recommendations are proposed:

1. The following active substances, currently in Regulation (EU) 1950/2006 as amended by Regulation (EU) 122/2013, are proposed to be retained in the list, either without modification or with an amendment of the current entry as shown below:

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Oxybuprocaine	local topical anaesthesia for use in eyes	none identified	wide clinical experience
Prilocaine	local topical anaesthesia prior to intravenous injection or catheterisation	lidocaine	in specific preparations (eutectic mixture of local anaesthetics), for topical application to skin; can be used to facilitate intravenous injection or catheterisation

2. The following active substances, currently in Regulation (EU) 1950/2006 as amended by Regulation (EU) 122/2013, are proposed to be removed from the list: sevoflurane, bupivacaine.

3. The following active substance, suggested for addition to the list in the survey to stakeholders, is not proposed for inclusion: ropivacaine.

4.2. Analgesics

4.2.1. Overview

	Active substance (ATCvet code)
Substances in Regulation (EU) 1950/2006 **proposed to be retained**, with or without amendment of the current entry	Fentanyl (QN02AB03; QN02AB03); Ketorolac (QS01BC05); Morphine (QN02AA01); Triamcinolone acetonide (QS01BA05)
Substances in Regulation (EU) 1950/2006 **proposed to be removed**	Buprenorphine (QN02AE01); Flumethasone (QH02AB90); Gabapentin (QN02BF01); Pethidine (QN02AB02)
Substances from stakeholders' survey **proposed for inclusion**	Bromfenac (S01BC11); Methocarbamol (M03BA53)
Substances from stakeholders' survey **not proposed for inclusion**	Methadone (QN02AC90); Phenylbutazone (QM02AA01); Pregabalin (QN02BF02);

4.2.2. Review of the existing entries in Regulation (EU) 1950/2006, as amended by Regulation (EU) 122/2013, considering the survey results

A. Considerations on the essentiality of the substance(s)

Buprenorphine is a weak partial μ-opioid receptor agonist and a weak κ-opioid receptor antagonist used for the treatment of severe pain. It dissociates from opioid receptors very slowly, resulting in a long duration of action and relief from pain upwards of 24-36 hours (Johnson et al., 2003). Buprenorphine interacts predominately with the opioid μ-receptor. These μ-binding sites are discretely distributed in the brain, spinal cord, and other tissues. In clinical settings, buprenorphine exerts its principal pharmacologic effects on the CNS. Its primary actions of therapeutic value are analgesia and sedation (Lutfy et al., 2003; Lufty and Cowan, 2004).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for analgesia, used for sedatives for restraint. Alternatives that are identified in the current list include butorphanol, fentanyl, morphine, and pethidine and the specific advantages captured are as follows: it is a partial μ-agonist opioid analgesic; μ-receptor activity producing better analgesia than κ- agonist opioids such as butorphanol; it is a long-acting analgesic; due to partial agonist characteristic, it has limited addictive

and respiratory depressant properties; long and short-acting opioids have different indications, hence the need for more than one alternative substances as choice.

It is noted that the substance butorphanol, which is listed as current alternative, is included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with a 'No MRL required' entry for *Equidae* for which there are several veterinary medicinal products authorised for food-producing animals of the equine species. Butorphanol is indicated for relief of moderate to severe abdominal pain (e.g. associated with colic of gastrointestinal origin), for sedation after administration of certain alpha2-adrenoceptor agonists (e.g. detomidine or romifidine), and for therapeutic and diagnostic procedures such as minor standing surgery. Butorphanol thus qualifies as satisfactory alternative treatment. Moreover, morphine and fentanyl are retained in the list of essential substances which are considered more potent clinical alternatives to buprenorphine, which is not considered to bring added clinical benefit compared to the options previously listed.

The substance is not intended for the treatment of a specific condition. However, failure to adequately sedate the animal could cause unacceptable suffering for the animal.

Buprenorphine was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database retrieves at least twenty-eight buprenorphine-containing veterinary medicinal products authorised for use in equine species (non-food-producing horses) as sedative. There are veterinary medicinal products authorised for use in species other than the equine (i.e. cat, dog).

The substance buprenorphine is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering for the animal.

Fentanyl is a potent opioid agonist causing strong analgesia through its activation of opioid receptors, especially the μ-opioid receptor (Al-Hasani and Bruchas, 2011). It is characterized by its fast and short duration of action. Its analgesic properties are 50-100 as potent as morphine, but with a shorter duration of action (Clotz and Nahata, 1991; Löscher, 2010).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for analgesia. Alternatives that are identified in the current list include butorphanol, buprenorphine, morphine, and pethidine and the specific advantages captured include its μ-agonist opioid, μ- receptor activity, which produces better analgesia than κ-agonist opioids such as butorphanol; it is very short acting due to rapid metabolism and excretion; fentanyl is the only opioid used in horses that is suitable for infusion and skin patch administration and highly effective for pain management.

As stated in the current list, fentanyl produces better analgesia than certain other opioids and can be used for very painful conditions. There is a recognized value for the use of the substance in multi-modal approaches. Addition of fentanyl transdermal therapeutic systems (TTS) in horses with inadequate analgesic response to NSAIDs alone demonstrated enhanced analgesic effect (Thomasy et al., 2004). Thus, a change to the indication is proposed (see below).

The substance is not intended for the treatment of a specific condition. However, failure to achieve adequate levels of analgesia could cause unacceptable suffering to the animal.

Fentanyl was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any fentanyl-containing veterinary medicinal product authorised for use in equine species. There are veterinary medicinal products authorised for use in species other than the equine (i.e. dog).

The substance fentanyl is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: multimodal approach for moderate to severe acute painful conditions. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating certain painful conditions in the animal or avoiding unnecessary suffering for the animal.

Flumethasone is a synthetic corticosteroid and is also considered a potent anti-inflammatory. When administered intravenously to exercised horses, it has been shown to significantly suppress endogenous hydrocortisone for at least 72 hours (Knych et al., 2019). Flumethasone is used in animals of the equine species as a systemic anti-inflammatory and anti-allergic therapy.

The substance is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) as a systemic corticosteroid therapy for treatment of shock, anti-inflammatory and anti-allergy therapy. The identified alternatives on the current list are dexamethasone and prednisolone and the specific advantages captured are as follows: it has different clinical effects from the alternatives with more rapid onset, longer duration and greater efficacy; different mode of action from alternative (no appreciable mineralocorticoid activity).

It is worth noting that dexamethasone, which is listed as a current alternative, is included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with an MRL entry that includes *Equidae,* thus constituting an available satisfactory treatment alternative for which veterinary medicinal products are authorised for food-producing animals of the equine species. In addition, betamethasone, prednisolone and methylprednisolone are considered satisfactory alternatives for the same indications, and they are all listed in Table 1 of the Annex to Commission Regulation (EU) 37/2010; for prednisolone there are veterinary medicinal products authorised for food-producing animals of the equine species. All these can be used as systemic anti-inflammatory and anti-allergic therapy in *Equidae*, and from the available literature it cannot be supported that flumethasone brings added clinical benefit compared to these substances.

Inflammation and allergy, if untreated, may cause unacceptable suffering for the animal.

Flumethasone was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any flumethasone-containing veterinary medicinal product authorised for use in equine species. There are veterinary medicinal products authorised for use in species other than the equine (i.e. cat, dog).

The substance flumethasone is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering for the animal.

Gabapentin is an anti-convulsant acting at calcium channels, which are expressed in the spinal cord, thereby exhibiting analgesic properties (Fornasari, 2017). It is a structural analogue of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA). It was originally developed as an anti-epileptic for the treatment of certain types of seizures but is also used to treat neuropathic pain (Maneuf et al., 2006). It is an anti-convulsant medication that inhibits the release of excitatory neurotransmitters,

allowing for its use against pathologic neurotransmission such as that seen in neuropathic pain and seizure disorders (Kukkar et al., 2013).

Gabapentin is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for treatment of neuropathic pain. Alternatives identified in the current list include buprenorphine, fentanyl, morphine, and pethidine. Specific advantages captured in the current list are: gabapentin has a different mode and different site of action to alternative authorized substances; GABA-like substance which blocks calcium channels and inhibits formation of new synapses; it was previously considered to represent a novel treatment for neuropathic pain with evidence suggesting added clinical benefit in the management of pain related to neuropathy e.g. foot pain, laminitis and abdominal pain.

Two alternative active substances for controlling neuropathic pain are proposed to be kept in the list of essential substances, i.e. morphine and fentanyl. Gabapentin is not considered to bring added clinical benefit compared to these. In addition, alternatives for pain control are also listed in Table 1 of the Annex to Commission Regulation (EU) No 37/2010, e.g. butorphanol tartrate and meloxicam, with entries for *Equidae* and veterinary medicinal products authorised for food-producing animals of the equine species. Gabapentin therefore is also not considered to bring added clinical benefit.

Neuropathic pain, if untreated, is potentially life-threatening and may cause unacceptable suffering for the animal.

Gabapentin was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any gabapentin-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance gabapentin is not proposed to be qualified as essential, nor as bringing added clinical benefit for treatment of neuropathic pain; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering for the animal.

Ketorolac is a non-steroidal anti-inflammatory drug (NSAID). Since its use was approved in 1989 as an injectable analgesic, numerous studies conducted involving ketorolac have provided sound evidence of its efficacy via different routes of administration (Vadivelu et al., 2015).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), for the treatment of eye pain and inflammation (non-steroidal anti-inflammatory medicine) as eye drops for topical use. No alternatives are identified in the current list and the specific advantages captured are: the widest clinical experience with ketorolac compared to other potential candidates and less adverse effects in corneal wound healing.

In humans, ketorolac in the form of an ophthalmic solution is indicated for the treatment of ocular pain and inflammation following cataract surgery (McCormack, 2011). Scientific literature in horses is scarce; it's been suggested to be effective in the treatment of equine recurrent uveitis. The pharmacokinetics in horses after intravenous, intramuscular, and oral single-dose administration are described (Bianco et al, 2016), but the efficacy of systemic formulations in the eye (compared to that of topical formulations applied locally) remains to be discussed in the literature. Other systemic NSAIDs are listed in Table 1 of the Annex to Commission Regulation (EU) No 37/2010, with an entry for *Equidae*; however, the added benefit of a topical formulation is recognized for the treatment of eye pain and inflammation.

Eye pain and inflammation, if untreated might cause unacceptable suffering for the animal.

Ketorolac was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database retrieves at least one ketorolac-containing (as tromethamine) veterinary medicinal product authorised for use in equine species (non-food-producing horses). There are veterinary medicinal products authorised for use in species other than the equine (i.e. dog and cat).

The substance ketorolac is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: treatment of eye pain and inflammation. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering for the animal.

Morphine is an opioid agonist used for the relief of moderate to severe acute and chronic pain in both human and veterinary medicine. Morphine is an agonist of the μ and k receptors, whose activation results in analgesia. Morphine-like agonists act through the μ opioid receptors to cause pain relief, sedation, euphoria and respiratory depression. Morphine blocks the transmission of nociceptive signals, activates signalling by pain-modulating neurons to the spinal cord, and inhibits transmission from primary afferent nociceptors to dorsal horn sensory projection cells. With increasing doses, the degree of analgesia increases until an anaesthetic level is reached. It mainly interacts with μ-receptors, but also has a certain affinity to δ- und κ- receptors, which are distributed throughout the body mediating different effects (Nolan, 2000; Löscher, 2010; Pacifici, 2016; Baldo, 2018).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for analgesia. The identified alternatives in the current list include butorphanol, buprenorphine, pethidine and fentanyl, and specific advantages captured are as follows: full μ-agonist opioid analgesic; μ-receptor activity produces the best analgesia; used with sedatives for restraint, used for epidural anaesthesia; mid duration analgesic; morphine is the μ-opioid agonist with the best solubility characteristics for epidural administration; it provides long-acting analgesia with few systemic effects by this route; this technique is widely used in modern veterinary medicine for treating severe perioperative and chronic pain.

Morphine is more potent than other analgesics and displays clinical versatility, including its use in epidural procedures. It produces a significant analgesic effect in horses (López-Sanromán et al., 2022). When combined with alpha-2 agonists it can also be used to perform standing medical and surgical procedures which otherwise would require general anaesthesia (Muir, 2009).

The substance is not intended for the treatment of a specific condition. However, failure to achieve adequate levels of analgesia and/or anaesthesia could cause unacceptable suffering to the animal.

Morphine was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any morphine-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance morphine is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: analgesia. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering for the animal.

Pethidine (meperidine) has the same mechanism of action as morphine, acting as an agonist to the μ-opioid receptor (Baldo, 2018). It is the oldest synthetic opioid and belongs to the phenylpiperidine class (Latta et al., 2002; Buck, 2011). The onset of action is lightly more rapid than with morphine,

and the duration of action is slightly shorter. It can be used for the relief of most types of moderate to severe pain, including postoperative pain. Spasmolytic properties have also been described resulting from reducing smooth muscle action (Buck, 2011). However, there is now substantial evidence that this substance provides no greater analgesia or antispasmodic effect than other opioids (Buck, 2011).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for analgesia. The identified alternatives in the current list include butorphanol, buprenorphine, morphine and fentanyl, and the specific advantages captured are as follows: a μ-agonist opioid analgesic about 10 times less potent than morphine; short-acting opioid that has been proven to be effective to treat spasmodic colic in horses; only opioid with spasmolytic properties; more sedation and less potential for excitement than other opioids in horses.

It is noted that the substance butorphanol, which is listed as current alternative, is included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with a 'No MRL required' entry for *Equidae* and there are several veterinary medicinal products authorised for food-producing animals of the equine species. Butorphanol is indicated for relief of moderate to severe abdominal pain (e.g. associated with colic of gastrointestinal origin), for sedation after administration of certain alpha2-adrenoceptor agonists (e.g. detomidine or romifidine), and for therapeutic and diagnostic procedures such as minor standing surgery. In addition, the substance levomethadone, that is also included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with a 'No MRL required' status for *Equidae,* could serve as an alternative for which there are veterinary medicinal products authorised for an equivalent indication for food-producing animals of the equine species. Moreover, morphine and fentanyl are retained in the list of essential substances. Lastly, the advantages of pethidine as compared to other opiate being less potent and having antispasmodic effects do not appear to be substantiated anymore (Benner and Durham, 2011; Buck, 2011).

The substance is not intended for the treatment of a specific condition. However, failure to achieve adequate levels of analgesia could cause unacceptable suffering to the animal.

Pethidine was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any pethidine-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance pethidine is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering for the animal.

Triamcinolone acetonide is a synthetic corticosteroid, specifically a glucocorticoid. Corticosteroids exert potent anti-inflammatory and immunosuppressive effects through a number of mechanisms and can have both systemic and local effects. Most relevant in terms of treatment of joint disease, corticosteroids, when administered intra-articularly block the arachidonic acid cascade, limit capillary dilation and inhibit the release of several soluble inflammatory mediators (Wernecke et al., 2015; McIlwraith and Lattermann, 2019), thereby alleviating clinical symptoms such as pain and limiting harmful inflammatory processes that can lead to irreversible damage to joint structures. Triamcinolone acetonide is used in animals of the equine species to treat synovial inflammation and to manage the symptoms of osteoarthritis. Another indication for triamcinolone acetonide in equine species is suprachoroidal injection for treatment of recurrent uveitis (Gagnon et al., 2021).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), as intra-articular medication for treatment of degenerative joint disease and osteoarthritis. The identified alternative on the current list is methylprednisolone and the specific advantages are as follows: different cellular and biosynthetic effects from the alternative corticosteroid intra-articular medication methylprednisolone; triamcinolone is chondroprotective and promotes cartilage repair; more effective than systemic treatments (NSAIDs and chondroitin sulphate) and other (non-corticosteroid) intra-articular treatments for control of joint inflammation, pain and lameness in acute and chronic joint disease, especially degenerative joint disease and osteoarthritis; only effective non-surgical treatment for subchondral bone cysts.

While there is some inconsistency between studies, several reports suggest that intra-articular administration of methylprednisolone may have deleterious effects on cartilage health, particularly at higher or repeated doses (Trotter et al., 1991; Frisbie et al., 1998). Other studies have suggested a chondroprotective effect of triamcinolone acetonide when administered intra-articularly (Frisbie et al., 1997). As a result, intra-articular administration of triamcinolone acetonide is currently accepted as "best practice" for treatment of synovial inflammation (McIlwraith et al., 2019). This has the advantage of minimal risk of systemic adverse effects, as would need to be considered with administration of non-steroidal anti-inflammatories for example.

Joint inflammation and recurrent uveitis, if untreated, may cause unacceptable suffering for the animal.

Triamcinolone acetonide was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database retrieves at least two triamcinolone-acetonide-containing veterinary medicinal products authorised for use in equine species (non-food-producing horses). There are veterinary medicinal products authorised for use in species other than the equine (i.e. cat, dog).

The substance triamcinolone acetonide is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: for the treatment of joint inflammation. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering for the animal. Triamcinolone acetonide is also proposed for the treatment of recurrent uveitis in cases that are refractory to other treatments. Please refer to section 4.11 for further details.

Knowledge regarding analgesics in horses for this assessment was derived from textbooks, review articles, retrospective studies, and clinical trials in horses.

B. Considerations regarding consumer safety

Fentanyl is a lipophilic opiate producing pharmacological effect with a narrow margin between safe and toxic doses. It is authorised in the EU, as an injectable human medicinal product and veterinary medicinal product for dogs. In veterinary medicine, it is used primarily as parenteral analgesic in small animals, potentially useful in horses. The injectable and transdermal forms of the products are mainly used by veterinarians.

Fentanyl acts as a μ-opiate agonist with short duration of action when administered parenterally and is approximately 80 to 100 times more potent than morphine. Sublingual fentanyl appears to be effective in human patients with rapid-onset analgesia and short-acting duration. It is considered safe, and well tolerated (Hashemi et al., 2021).

Mu opioid receptors are found primarily in the pain regulating areas of the brain. They are thought to contribute to the analgesia, euphoria, respiratory depression, physical dependence, miosis, and hypothermic actions of opiates. Receptors for opiate analgesics are found in high concentrations in the limbic system, spinal cord, thalamus, hypothalamus, striatum, and midbrain. They are also found in tissues such as the gastrointestinal tract, urinary tract, and other smooth muscles (Plumb, 2015; Papich, 2020).

When used via a single intravenous dose, fentanyl has a relatively short duration of effect in most species studied. In humans, the elimination half-life is similar between fentanyl and morphine with 2 to 4 hours for fentanyl and 2 hours for morphine. This similarity in half-life is notable, despite fentanyl's faster onset, shorter duration of analgesic action, and higher potency compared to morphine (Comer and Cahill, 2019).

When administered to dogs as a single intravenous dose of 10 µg/kg, fentanyl rapidly distributes and exhibits a large volume of distribution of 5 l/kg. The terminal elimination half-life is approximately 45 minutes and total clearance is 78 ml/min/kg. Dogs administered a constant rate intravenous infusion of 10 µg/kg/hour were able to maintain blood levels around 1 ng/ml, assumed to be the therapeutic analgesic level, although not confirmed. In cats, the half-life after intravenous administration is approximately 2.5 hours and in horses about 49 minutes (Papich, 2021).

When transdermal fentanyl solution is applied to canine skin, solvent evaporation results in super-saturation of both a penetration enhancer and fentanyl. At the moment of drying (approximately 2-5 minutes following application), rapid dermal absorption and sequestration of fentanyl into the stratum corneum occurs. From the stratum corneum, fentanyl is then slowly absorbed in a period up to one week into the bloodstream. Pharmacokinetic values in individual dogs can vary considerably, but in a "typical" dog the time to reach a plasma level of 0.6 ng/ml (considered the minimum therapeutic analgesic plasma concentration in dogs) is 1.85 hours and 3.08 hours to reach 1 ng/ml; absorption lag-time averaged around 33 minutes. Peak plasma levels of 1.83 ng/ml occurred about 13.6 hours after administration. The terminal half-life was approximately 3 days. Mean plasma concentrations from days 0 to 4 were 1.32 ng/ml (Papich, 2021).

In horses, transdermal fentanyl is rapidly absorbed with therapeutic levels of 0.6-1 ng/ml achieved approximately 6 hours after application and persist for more than 48 hours. However, in about one-third of the horses in the study, plasma levels never reached 1 ng/ml (Papich, 2021).

Following intravenous administration of a single 2 mg dose of fentanyl to 6 horses, mean serum fentanyl concentrations declined rapidly and were below the LOQ of 0.25 ng/ml by 8 hours after administration. The mean volume of the central compartment in horses in this study was 0.11 l/kg; the volume of distribution at steady state was 0.68 l/kg. Elimination half-life was 130 minutes (Maxwell et al., 2003).

In humans, metabolism takes place in the liver and excretion is mainly through urine, with a limited amount through faeces. The half-life varies from 2 to 7 hours and may be prolonged to 15 hours in elderly patients or after repeated administration. In urine, only 0.4-6% of a dose is excreted as unchanged drug, whereas 69% is excreted as metabolites. Fentanyl is metabolised to a number of inactive metabolites. It is 99% N-dealkylated to norfentanyl by cytochrome P450, but other inactive metabolites have been identified; it can also be amide hydrolysed to form despropionylfentanyl, or alkyl hydroxylated to hydroxyfentanyl which is further N-dealkylated to hydroxynorfentanyl (De Priest et al., 2015).

The product information of human medicinal products mentions that the safe use in pregnancy has not been established, but fentanyl can cross the placenta in early pregnancy. It is also mentioned that

fentanyl is excreted in breast milk. Breast feeding is not recommended for 24 hours following fentanyl administration (Papich, 2021).

Allen et al. (2003) showed that fentanyl is genotoxic in the mouse lymphoma assay in the presence of S9 activation, but not in other tests for genotoxicity including the Ames Salmonella mutagenicity test, the primary rat hepatocyte UDS assay, the BALB/c-3T3 transformation test, the human lymphocyte chromosomal aberration assay and the in vitro CHO chromosomal aberration assay.

The product information of human medicinal products suggests that fentanyl was not associated with an increased incidence of tumours at subcutaneous doses up to 33 micrograms/kg/day in males or 100 micrograms/kg/day in females, which were the maximum tolerated doses for males and females, in a two-year carcinogenicity study in rats. Some tests on female rats showed reduced fertility as well as embryo mortality. These findings were related to maternal toxicity and not a direct effect of the drug on the developing embryo. There was no evidence of teratogenic effects. Fentanyl is not listed by the IARC.

Fentanyl is eliminated within hours or few days form the horse. The same observations are seen in humans and in species other than equines. Furthermore, information in humans show significant metabolism to inactive metabolites. Therefore, it can be accepted that fentanyl 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.

Ketorolac is currently authorized as a human and veterinary medicinal product for ocular use (eye drops). Ketorolac consists of two enantiomers (R- and S-form, as racemate) and its salt with trometamol (tromethamine) is generally used in medicinal products.

The kinetics of intravenous ketorolac in humans are characterized by a terminal half-life of 5.09 hours, low plasma clearance of 0.35 ml/min/kg and low tissue distribution; volume of distribution at steady state is 0.11 l/kg (Jung et al., 1988). Following oral administration, peak levels were rapidly attained within 0.9 hours and the systemic bioavailability was essentially complete (100%); its absorption is rapid, and the elimination half-life is approximately 6 hours (Jung et al., 1989).

The product information of human medicinal products suggests that following topical application of a 0.5% ketorolac ophthalmic solution, three times daily for 10 days in healthy volunteers, detectable plasma levels were observed in approximately 20% of the treated patients. These plasma levels comprised 4–8% of the mean steady-state plasma levels achieved with oral administration of ketorolac at a dose of 10 mg/kg bw four times a day.

In horses, kinetics of ketorolac, have been studied following intravenous, oral and intramuscular administration (Bianco et al., 2015). Following oral administration, the absorption is high with a bioavailability of about 90% and the elimination is fast, with a terminal half-life of 6 hours. No kinetic nor residue data in horses following ocular administration are found for ketorolac. The systemic absorption of the substance following topical (ocular) administration depends largely on the final formulation, and it is difficult to anticipate the bioavailability in horses following an ocular application of an unknown final formulation. However, it is noted that bioavailability following topical (ocular) application in humans is low.

The chemical dossier at ECHA provides information regarding its toxicity; ketorolac is acutely toxic if swallowed, but the dossier does not contain any further toxicological information (ECHA, 2024a) nor was relevant public literature found on repeated dosing, genotoxicity, toxicity to reproduction or carcinogenicity. The product information of human medicinal products suggests that ketorolac tromethamine was not carcinogenic in rats or mice, did not impair fertility in rats and was not

mutagenic in vitro in the Ames assay or in forward mutation assays; it did not result in an in vitro increase in unscheduled DNA or in an in vivo increase in chromosome breakage in mice. However, it did result in an increased incidence of chromosomal aberrations in Chinese hamster ovary cells.

Assuming a low bioavailability after ophthalmic administration to horses (similar to that of humans) and considering the known pharmacokinetic properties of the substance in horses and the apparent low toxicity, it can be accepted that ketorolac 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.

Morphine is an opioid agonist used for the relief of moderate to severe acute and chronic pain in both human and veterinary medicine.

In humans, morphine salts are well absorbed from the gastrointestinal tract but have poor oral bioavailability since they undergo extensive first-pass metabolism in the liver and gut. After subcutaneous or intramuscular injection, morphine is readily absorbed into the blood. The majority of morphine is conjugated with glucuronic acid in the liver and gut to produce morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). The latter is considered to contribute to the analgesic effect of morphine. Morphine is distributed throughout the body and crosses the blood-brain barrier less rapidly than other (more) lipid-soluble opioids. Mean plasma elimination half-life is about 2 hours. About 90% of total morphine is excreted in 24 hours with traces in urine for 48 hours or more (Sweetman, 2009).

The pharmacokinetics of morphine in the horse have been described in several reports. After intramuscular injections of 0.1 mg/kg, the apparent terminal half-life observed was approximately 1.5 hours, the volume of distribution per bioavailability was 4.5 l/kg and clearance per bioavailability was 35 ml/kg. Morphine concentrations were below the LOQ in less than or equal to 7 hours after administration in 74 horses (Devine et al., 2013).

Knych et al. (2014) identified 2 major metabolites, M3G and M6G following administration of four different doses of morphine to horses. Compared to humans, horses produced almost twice as much M3G but similar concentrations of M6G (Knych et al., 2014). The same metabolites were identified in another study (Hamamoto-Hardman et al., 2019). In this study, the terminal half-life of morphine following a single intravenous administration of 0.05 to 0.5 mg/kg ranged from 8.20 to 10.5 hours. The volume of distribution was 12.1 and 9.50 l/kg for the lowest and the highest dose, respectively, and the clearance was 24.3 ml/min/kg for the lowest dose and 33.54 ml/min/kg for the highest dose. Morphine was primarily eliminated as M3G and M6G; glucuronidation of morphine was rapid, with maximum plasma concentration for both metabolites occurring by 10 minutes. Urine concentrations of morphine, M3G and M6G after 72 hours of administering the highest dose of 0.5 mg/kg were 1.31, 50.7 and 1.16 ng/ml, respectively.

Another study reports that at 96 hours after administering 0.2 mg/kg intravenously, the urine concentrations of morphine and M6G were below the LOQ (0.25 ng/ml), while the concentrations of M3G were 4.01±0.64 ng/ml. In the same study, the terminal half-life observed was 12.5, 9.37 and 8.85 hours for oral doses of 0.2, 0.5 and 0.8 mg/kg, respectively, and it was 11.2 hours when an intravenous dose of 0.2 mg/kg was administered (Poth et al., 2023).

The mean terminal half-life for morphine following a single intravenous administration of 0.5 mg/kg to three adult horses was 10.43 hours, while the mean volume of distribution at steady state was 6.8 l/kg and the mean clearance was 30.36 ml/min/kg (Hamamoto-Hardman et al., 2022).

Regarding the toxicity of morphine, it can potentially be a lethal medication when not used properly. It causes a host of symptoms related to depression of the CNS. Severe respiratory depression is the most feared complication of morphine in cases of overdose.

Morphine is genotoxic only in vivo but most likely by a non-DNA reactive mode of action. Although carcinogenicity data for morphine itself are lacking, based on the lack of carcinogenicity of codeine which is extensively metabolised to morphine in rats, the CONTAM Panel concluded that morphine is unlikely to be carcinogenic (EFSA, 2011).

Despite the data variability observed in the available scientific literature, considering that it is eliminated within hours or few days from the horse and the low oral bioavailability reported, it can be accepted that morphine 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.

Triamcinolone acetonide is not a prodrug for triamcinolone and it has distinctly different corticosteroid pharmacodynamic properties. The acetate and acetonide esters alter water and lipid solubility, thereby delaying absorption and prolonging the duration of action. Acetonide esters are the least soluble corticosteroid and are considered to have intermediate to long duration of action following intra-articular or intramuscular administration (Soma et al., 2011). Triamcinolone acetonide is currently authorized in human medicinal products. It is authorised in veterinary medicines for dogs and cats.

In humans, the elimination half-life of triamcinolone acetonide is short at approximately 2 hours and the oral bioavailability averaged 23%. Carbon-14 ([^{14}C]) labeled triamcinolone acetonide was found to be systemically absorbed following oral administration in 6 healthy male subjects at a dose of 850 ± 4.92 µg of the parent compound. The metabolism and clearance of triamcinolone acetonide were extensive, with only a small fraction of the total plasma radioactivity being made up of triamcinolone acetonide. Little to no parent compound was detected in the plasma 24 hours after administration. Most of the urinary and fecally [^{14}C]-derived radioactivity was also excreted within 24 and 72 hours after administration, respectively. Mean plasma protein binding of triamcinolone acetonide was constant, predictable, and relatively low at 68%. Three principal metabolites of triamcinolone acetonide were profiled in plasma, urine, and faeces. These metabolites were identified as 6-hydroxy triamcinolone, 21-carboxylic acid triamcinolone acetonide, and 6-hydroxy-21-oic-triamcinolone acetonide. All three metabolites failed to show any concentration-dependent effects in anti-inflammatory models evaluating IL-5-sustained eosinophil viability and IgE-induced basophil histamine release (Argenti et al., 2000).

Following intravenous injection of triamcinolone acetonide at 5 mg/kg bw in human, the terminal elimination half-life was about 85 minutes with a total body clearance of 61.61 l/h. Comparison of the pharmacokinetic parameters of triamcinolone acetonide after intravenous administration of the high (10 mg/kg bw) and low (80 mg total) dose also showed nonlinearity. The difference in total body clearance, i.e. 45.2 l/h and 69.5 l/h respectively, was statistically significant ($p>0.05$) (Möllmann et al., 1985).

The pharmacokinetics of triamcinolone acetonide were studied in human after intravenous (2 mg), oral (5 mg) and inhaled (2 mg) administration. After intravenous administration, triamcinolone acetonide was eliminated with a total body clearance of 37 l/h and an elimination half-life of 2 hours. The oral bioavailability averaged 23%. The oral absorption was rapid achieving maximum triamcinolone acetonide levels of 10.5 ng/ml after 1 hour. After inhalation, bioavailability averaged 22% with maximum levels of 2.0 ng/ml observed after 2.1 hours (LOQ = 0.1 ng/mL) (Derendorf et al., 1995).

In horses, the elimination half-life of triamcinolone acetonide is about 6 hours, 1 day and 11 days following intravenous, intra-articular and intramuscular administration, respectively.

Twelve horses received a single intramuscular administration of triamcinolone acetonide (0.1 mg/kg bw), and after an appropriate washout period, the same horses received a single intra-articular triamcinolone acetonide administration (9 mg) into the right antebrachiocarpal joint. Maximum measured plasma triamcinolone acetonide concentrations were 0.996 ± 0.391 ng/ml at 13.2 hours and 1.27 ± 0.278 ng/ml at 6.5 hours for intramuscular and intra-articular administration, respectively. The plasma terminal half-life was 11.4 ± 6.53 days and 0.78 ± 1.00 days for intramuscular and intra-articular administration, respectively. Following intramuscular administration, triamcinolone acetonide was below the limit of detection (LOD = 0.05 ng/ml) by days 52 and 60 in plasma and urine, respectively. Following intra-articular administration triamcinolone acetonide was undetectable by day 7 in plasma and day 8 in urine. Triamcinolone acetonide was also undetectable in any of the joints sampled following intramuscular administration and remained above the limit of quantitation (LOQ = 0.1 ng/ml) for 21 days following intra-articular administration (Knych et al., 2013).

The plasma kinetics of triamcinolone acetonide was studied in 6 horses following intravenous, intra-articular and intramuscular administration at the dose of 0.04 mg/kg bw. The elimination half-life was about 6.1 hours, 23.8 hours and 150.2 hours following intravenous, intra-articular and intramuscular administration, respectively. Maximum plasma concentration following intra-articular administration was 2.0 ng/ml and was attained at 10 hours. Maximum plasma concentration following intramuscular administration was 0.34 ng/mL and was attained at 13.0 hours; concentration was still quantifiable (LOQ = 0.1 ng/mL) at 360 hours. (Soma et al., 2011)

Following intravenous administration of triamcinolone acetonide at 0.2 mg/kg bw in 5 horses, the elimination of triamcinolone acetonide had two components: a rapid and a slow phase. The half-life of the rapid phase was 83.5 minutes, and the half-life of the slow phase was 12.0 hours (French et al., 2000).

According to CLP notifications provided by companies to ECHA, triamcinolone acetonide is harmful if swallowed, may damage fertility or the unborn child, and causes damage to organs through prolonged or repeated exposure; no study data are provided (ECHA, 2024b). The substance is not registered at ECHA, i.e. further toxicological information is not available.

No genotoxicity and carcinogenicity studies are available.

Considering the low human oral bioavailability and the proposed route of administration in horses (i.e. intra-articular) it can be accepted that triamcinolone acetonide 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.

4.2.3. Assessment of new substances proposed to be added to the list in the stakeholder's survey

A. Considerations on the essentiality of the substance(s)

Bromfenac was mentioned (once) in the survey to stakeholders and it was suggested for addition to the list for treatment of uveitis and keratitis. In the responder's opinion, treatment with bromfenac results in less side effects than with steroids. The respondent further noted bromfenac's selectivity for cyclooxygenase (COX)-2 inhibition compared to ketorolac, which is listed in Regulation (EU) 1950/2006 but is primarily a COX-1 inhibitor and less potent than bromfenac. No scientific references were provided.

Bromfenac belongs to the class of non-steroidal anti-inflammatory drugs (NSAIDs). The anti-inflammatory activity of NSAIDs is mediated by inhibition of the COX enzyme, which catalyzes the rate-limiting step in the formation of prostaglandins in the arachidonic acid cascade (Waterbury et al., 2011). Its main indication is as an ophthalmic preparation topically applied and used to alleviate pain and inflammation after ocular surgery or in the management of uveitis (Waterbury et al., 2011; Allbaugh, 2017).

Alternative therapeutics identified for the treatment of ocular pain that are authorised for use in equine species include the systemically administered medications flunixin meglumine, ketoprofen and meloxicam. Other topical ophthalmic NSAID therapeutics would include diclofenac sodium and ketorolac, neither of which appear to be authorised for use in equine species. Ketorolac is proposed to be kept in the list of essential substances.

While topical corticosteroids could also be used to treat ocular pain and inflammation, these may be contra-indicated where there is corneal ulceration or calcific band keratopathy. Topical NSAIDs may be needed in addition to systemic medications to improve patient comfort (Allbaugh, 2017). A number of studies have also shown that the addition of topical NSAIDs resulted in less patient discomfort, reduced postoperative inflammation, prevention of miosis, and improvements in visual acuity in the early postoperative period compared to patients treated with topical steroids alone (Waterbury et al., 2011).

Uveitis or post operative ocular pain, if untreated, is potentially life-threatening and causes unacceptable suffering for the animal.

A search in the veterinary medicines database does not retrieve any bromfenac-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance bromfenac is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: treatment of uveitis and ocular inflammation. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering for the animal.

Methadone was mentioned (nine times) in the survey to stakeholders, and it was suggested for addition to the list as analgesic. Arguments were provided in support of this proposal, albeit no scientific references were provided, and are captured as follows: it has strong analgesic properties with less gastrointestinal side effects than other opioids; it acts as N-methyl-D-aspartate (NMDA) receptor antagonist in addition to its activity on μ-receptors; it has clinical versatility; it does not cause histamine release as compared to morphine; it is licensed for dogs which facilitates use via cascade and has better availability than other opioids. The responders further noted a regulatory disparity pertaining to differing authorisation statuses of methadone for horses within the EU.

The substances levomethadone i.e. the active enantiomer of methadone, and butorphanol are included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with 'No MRL required' status for *Equidae,* and there are veterinary medicinal products authorised for equivalent indication for food-producing animals of the equine species. Methadone is not considered to bring added clinical benefit in equine analgesia compared to these. In addition, alternatives (e.g. morphine) are proposed to be retained in the list.

The substance is not intended for the treatment of a specific condition. However, failure to achieve adequate levels of analgesia could cause unacceptable suffering to the animal.

A search in the veterinary medicines database does not retrieve any methadone-containing veterinary medicinal product authorised for use in equine species. There are veterinary medicinal products authorised for use in species other than the equine (i.e. dog and cat).

The substance methadone is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering for the animal.

Methocarbamol was mentioned (two times) in the survey to stakeholders, and it was suggested for addition to the list for treatment of muscle spasm and inflammation. More specifically, the responders noted its use in horses for treatment of painful musculoskeletal disorders, including muscle spasticity, physical overexertion myopathy or underlying pathological myopathy, pressure myopathy and tetanus. It was further stated that intravenous methocarbamol is approved by FDA as adjunctive therapy of acute inflammatory and traumatic conditions of skeletal muscle to reduce muscle spasm and promote skeletal muscle relaxation. The proposed indications were supported by textbooks and published peer-reviewed references (Booth, 1988; Muir et al., 1984; Muir et al., 1992; Papich, 2020; Plumb, 2018; Pruit, 2013; Riviere and Papich, 2017).

It is an aromatic glycerol ether, and its main indication is as a muscle relaxant. Its exact mechanism of action is unknown, but it is thought to act as CNS depressant (Knych et al., 2016). The potent skeletal muscle relaxation that it achieves may be due to a specific action on the internuncial neurons of the spinal cord to reduce acute skeletal muscle spasms without a concomitant alteration in muscle tone (Haussler, 2015). The main indication in horses is for the treatment of acute skeletal muscle inflammation and/or trauma, in particular that associated with exertional rhabdomyolysis (Rumpler et al., 2014; Knych et al., 2016).

Alternative therapies for the treatment of severe muscle inflammation include any non-steroidal anti-inflammatories such as flunixin (included in Table 1 of Commission Regulation (EU) No 37/2010 with an entry for *Equidae* and for which there are veterinary medicinal products authorised for food-producing equine species); however, more aggressive pain management strategies utilising a combination of treatments of differing modes of actions may be needed in severe cases (Haussler, 2015). Therefore, it is considered that in severe muscle conditions where non-steroidal anti-inflammatories alone are not sufficient to alleviate the animal's suffering, the addition of methocarbamol to treatment protocols gives additional benefit that cannot be achieved by alternative treatments that are authorised for food-producing animals of the equine species.

Acute muscle inflammation, if untreated, could cause unacceptable suffering to the animal.

A search in the veterinary medicines database does not retrieve any methocarbamol-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance methocarbamol is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: as part of treatment protocols in severe painful muscle spasms/muscle inflammation conditions. It is considered that the alternatives do not always yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering for the animal.

Phenylbutazone was mentioned (twenty-seven times) in the survey to stakeholders and it was suggested for addition to the list. Different indications related to pain management were mentioned,

including chronic treatment of lameness and laminitis. The responders suggested it is widely used, more potent compared to other NSAIDs, and provides longer effect. In addition, some mentioned it is widely used in geriatric equids, improving their health and welfare. References were provided, which are discussed below.

Phenylbutazone is one of the oldest nonsteroidal anti-inflammatory drugs (NSAIDs) used in veterinary medicine, second only to aspirin (Tobin et al., 1986). It is still commonly used in horses for treating pain and inflammation caused by musculoskeletal disorders and soft tissue injuries (Soma et al., 2012; Bowen et al., 2020; Jacobs et al., 2022), and considered as a reference analgesic, compared to other NSAIDs (Bowen et al., 2020).

The main effect of NSAIDs is the inhibition of cyclooxygenases (COXs), which convert polyunsaturated fatty acids into prostaglandins (PGs) during the inflammatory process (Blobaum and Marnett, 2007; Kynch, 2017). There are 2 forms of COX: constitutively expressed COX-1 and inducible COX-2 enzymes. Inhibition of the COX enzymes results in a reduced production of prostaglandins (PG) from their arachidonic acid precursor and clinically in a reduction of pain and inflammation. NSAIDs are classified as non-selective if they inhibit both forms (COX-1 + COX-2) at therapeutic concentrations or COX-2 selective if they primarily inhibit COX-2 forms at therapeutic concentrations.

Phenylbutazone is an enolic acid derivative of the pyrazolidine drug class and is metabolized into two main metabolites, oxyphenylbutazone and ɣ-hydroxyphenylbutazone. Phenylbutazone is highly protein-bound (98%), with a low volume of distribution (Vd; 0.17 L/kg), and therapeutic plasma concentrations are estimated to be between 1 and 4 µg/mL, using PK/PD modelling (Lees and Toutain, 2013). As an NSAID, phenylbutazone has anti-pyrexia, anti-inflammatory and analgesic properties. There are currently four NSAID alternative treatments in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 (i.e. firocoxib, flunixin, ketoprofen, and meloxicam) for which there are veterinary medicinal products authorised for food-producing equine species.

It is well documented that phenylbutazone provides effective analgesia for orthopaedic pain in horses (Flood and Stewart, 2022). The majority of comments from the survey concern the potential added advantage of phenylbutazone for various types of lameness in horses. Thus, the focus of this assessment was to explore the added clinical benefit of phenylbutazone for the treatment of lameness in horses.

To date, several studies have investigated the efficacy of phenylbutazone for various types of lameness in horses. These include comparative as well as cross-over studies, which have compared the analgesic effect of phenylbutazone against other marketed NSAIDs or placebo (see section 7.1 for a tabulated summary). Studies comparing phenylbutazone to a placebo or assessing phenylbutazone only were not considered further (i.e. Toutain et al., 1994; Mills et al., 1996; Hu et al., 2005; Foreman et al., 2008). For the remaining studies, differences in terms of study design, inclusion criteria, dosing, comparator products and evaluation of efficacy were noted.

Two studies investigating naturally occurring lameness were identified (Erkert et al., 2005; Keegan et al., 2008). These studies generally provide more robust results over experimental studies (i.e. induced lameness). However, these types of studies also introduce a higher number of confounding factors, such as those related to other comorbidity factors, chronicity of the lameness, etc., and thus typically require large samples sizes. Erkert et al. (2005) compared the effect of intravenous administration of 4.4 mg phenylbutazone/kg or 1.1 mg flunixin/kg, every 24 hours for four days, using 12 horses in a crossover design. Lameness was measured by means of a modified AAEP scale and force plate analysis. Non-significant differences in force plate values were observed between horses receiving phenylbutazone or flunixin. Keegan et al. (2008) studied phenylbutazone against the combination

phenylbutazone + flunixin in 29 horses suffering from naturally occurring lameness of heterogeneous origins. While this study design is not useful for determining any added clinical benefit of phenylbutazone over other NSAIDs, it is noteworthy that phenylbutazone alone improved lameness, but not in a statistically significant fashion. Furthermore, an unpublished European study was considered for this assessment. This was a clinical crossover, blinded study comparing three oral NSAIDs (flunixin, meloxicam, phenylbutazone) in a small group of horses with naturally occurring lameness over a short period of time.

Three experimental studies were further considered where lameness or synovitis is induced following recognized pain models, namely the adjustable heart bar shoe (HBS) and the SYN-LPS synovitis (Foreman et al., 2010; Foreman and Ruemmler, 2011; Banse and Cribb, 2017). Different scoring systems were used, i.e. subjective pain lameness scoring systems, like the AAEP scale, and objective lameness scoring systems, such as force plate or kinematic devices used on the treadmill. Foreman et al. (2010) could only report a slightly earlier decrease in lameness scores (AAEP scale) for phenylbutazone compared to flunixin. Foreman and Ruemmler (2011) also report an earlier decrease of lameness scores (AAEP scale) in phenylbutazone and phenylbutazone + flunixin groups compared to flunixin only. Banse and Cribb (2017) reported that while phenylbutazone was more effective than meloxicam at reducing pain in the HBS model at the oral doses used, meloxicam was more effective at reducing pain in the SYN model.

The apparent quicker onset of action after oral administration (from 20 minutes to more than one hour) observed in some studies and the apparent longer duration of effect observed was the basis for the discussion as to whether phenylbutazone could qualify for an added clinical benefit, compared to NSAIDs approved for food horses. The reported correlations are weak and consistent statistically significant differences were not noted; while studies using AAEP scoring systems did observe differences between phenylbutazone and other NSAID for lameness treatments, such differences were not observed using objective lameness scoring systems. Within the clinical setting, for a quicker onset of action a clinician has the option to use an intravenous formulation; thus, the clinical relevance of the observed earlier onset of effect is questioned. Also, for the apparent longer duration of effect, this is also questioned from the nature of the disease to treat, that would always require a treatment lasting as long as needed. Additionally, sufficient studies are not currently available direct comparing the efficacy of phenylbutazone to that of all the available authorised products (e.g. ketoprofen, firocoxib). Overall, and with due consideration of the large number of efficacious alternatives available for food-producing equine species, it was concluded that an added clinical benefit was not demonstrated with the currently available evidence.

Under field conditions, musculoskeletal diseases in horses are known to involve a combination of mechanical and inflammatory factors; therefore, clinical response to treatment may considerably vary depending on the nature of the disease, i.e. on the relative contributions of mechanical and inflammatory pain to the overall lameness, and on the specific pharmacodynamic and pharmacokinetic characteristics of the treatment. There is a tendency among clinical practitioners to believe that phenylbutazone is more effective than flunixin for the treatment of orthopaedic pain (Duz et al., 2019). While indeed some publications suggest that phenylbutazone appears to be the most effective analgesic for orthopaedic pain in comparison to COX-2 selective NSAIDs, it is difficult to compare studies when different dosages are used (Flood and Stewart, 2022). In addition, some other studies have found that phenylbutazone is not superior to flunixin for the treatment of musculoskeletal pain associated with joint disease or mechanical pain (Foreman et al., 2010; Bowen et al., 2020). There is not a consensus concerning consistent added benefit reported of phenylbutazone over other NSAIDs for orthopaedic conditions in horses.

Lameness, if untreated, can cause unacceptable suffering of the animal in some circumstances. A search in the veterinary medicines database does not retrieve any phenylbutazone-containing veterinary medicinal product authorised for use in equine species. There are veterinary medicinal products authorised for use in species other than the equine (i.e. cat and dog). As previously indicated, four other NSAIDs (firocoxib, flunixin, ketoprofen, and meloxicam) have an established MRL and are authorised for food-producing equine species.

The substance phenylbutazone is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering for the animal.

Pregabalin was mentioned (twice) in the survey to stakeholders and it was suggested for addition to the list for treatment of neuropathic pain. The responders listed gabapentin, buprenorphine, fentanyl, morphine and pethidine as alternatives and stated the specific advantages of pregabalin as follows: different mode and site of action from alternatives; GABA-like substance which blocks calcium channels and inhibits formation of new synapses; novel treatment for neuropathic pain with evidence suggesting added clinical benefit in the management of pain related to neuropathy, e.g. foot pain, laminitis, and abdominal pain. No scientific references were provided in relation to its efficacy; a study by Mullen et al. (2013) assessing the pharmacokinetics of pregabalin in horses was provided by one of the responders.

Pregabalin is used as an antiepileptic drug predominately in cats. It belongs to the group of anticonvulsants and is used to treat neuropathic pain in horses as an alternative to gabapentin, buprenorphine, fentanyl, morphine, and pethidine. Pregabalin, like gabapentin, is an analogue of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA). However, the effect is not mediated by binding to GABA receptors, but presumably by decreasing calcium influx in terminal nerve terminals, resulting in decreased presynaptic release of glutamate. An indirect reduction in norepinephrine release may also play a role. Morphine and fentanyl, which are listed as alternatives by the responders are proposed to be kept on the list of essential substances and can be used for controlling neuropathic pain. Pregabalin is not considered to bring added clinical benefit compared to these. In addition, alternatives for controlling neuropathic pain are also listed in Table 1 of the Annex to Commission Regulation (EU) No 37/2010, e.g. butorphanol tartrate and meloxicam, with entries for *Equidae* and veterinary medicinal products authorised for food-producing animals of the equine species. Pregabalin is also not considered to bring added clinical benefit compared to these.

Neuropathic pain, if untreated, is potentially life-threatening and may cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any pregabalin-containing veterinary medicinal product authorised for use in equine species. There are veterinary medicinal products authorised for use in species other than the equine (i.e. cat).

The substance pregabalin is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indication in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering for the animal.

Knowledge regarding analgesics in horses for this assessment was derived from textbooks, review articles, retrospective studies, and clinical trials in horses.

B. Considerations regarding consumer safety

Bromfenac is authorised in the EU, as ophthalmic human medicinal product topically applied and used to alleviate pain and inflammation after ocular surgery or in the management of uveitis. Is a non-steroidal anti-inflammatory drug (NSAID).

Skjodt and Davies (1999) studied the pharmacokinetics of orally administered bromfenac in humans. Peak plasma concentration was reached 0.5 hours after oral administration. Bromfenac binds extensively to plasma albumin. The area under the plasma concentration-time curve is linearly proportional to the dose for oral doses up to 150 mg. Only small amounts of bromfenac are eliminated unchanged, with the remaining drug being biotransformed into glucuronide metabolites which are excreted in urine and bile. Rapid elimination occurs in healthy individuals (half-life 0.5 to 4.0 h). Renal disease, hepatic disease and aging alter the disposition kinetics of bromfenac (Skjodt and Davies, 1999). The plasma concentration of bromfenac following ocular administration in humans is unknown (Drugbank, 2024). However, human product information indicates no quantifiable plasma concentrations following twice daily dosing with bromfenac eye drops.

In horses, the elimination half-life is 2 hours, and the excretion is in urine (20-50%) and bile (40-60%) (Bertone and Horspool, 2004).

Use of bromfenac in human ophthalmology is well established (Hoy, 2015; Sheppard, 2016; Macrì et al., 2017; Shankar et al., 2022). As reviewed by Sheppard (2016), the addition of a bromine atom to the chemical structure increases the molecule's lipophilicity, enhances penetration into ocular tissues, and increases the potency against COX-1 and COX-2 relative to other NSAIDs. Following a single ocular dose of bromfenac 0.09% in rabbits, bromfenac was detected within all ocular tissues, with the exception of the vitreous humor, after 24 hours. However, two studies in humans scheduled to undergo vitrectomy and who did not have vitreous haemorrhage found that bromfenac 0.09% and other assessed NSAIDs (ketorolac, nepafenac, indomethacin) penetrated into the vitreous cavity; in one of these studies bromfenac achieved vitreous levels sufficient to significantly reduce vitreous prostaglandin E2 (PGE2) concentrations. A formulation of bromfenac was later developed in which the pH was lowered (from 8.3 to 7.8) to increase the nonionized fraction of the drug and thus facilitate a reduction in dose to 0.07% while maintaining ocular bioavailability. A recent study in rabbits demonstrated that bromfenac 0.07% penetrated ocular tissues at similar levels to those observed with bromfenac 0.09% (Sheppard, 2016).

Studies in humans show topical eye administration of bromfenac is well tolerated. Information from human medicinal products identifies abnormal sensation in eye, corneal erosion, eye pruritus, eye pain, and eye redness as the most common adverse effects following treatment. Idiosyncratic hepatic toxicity in humans has been reported following oral administration (Skjodt and Davies, 1999; Jeon et al., 2024).

Bromfenac was negative in a standard battery of genotoxicity tests. Concerning carcinogenicity, tests were negative (up to the highest dose tested of 0.6 mg/kg/d orally for rats). Reprotoxicity of Bromfenac is also negative under the administration of 0.3 mg/kg/d orally (Bertone and Horspool, 2004). Information in human medicinal products supports these findings; non-clinical data reveal no special hazard for humans based on conventional studies of safety, pharmacology, repeated-dose toxicity, genotoxicity and carcinogenic potential. It is noted that 0.9 mg/kg/day in rats at oral doses

(900 times the recommended ophthalmic dose) caused embryofoetal lethality, increased neonatal mortality, and reduced postnatal growth. Pregnant rabbits treated orally with 7.5 mg/kg/day (7500 times the recommended ophthalmic dose) caused increased post-implantation loss.

Considering the low systemic availability after ocular use and fast plasma elimination, and the proposed use of the substance, it can be accepted that bromfenac 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.

Methocarbamol is a centrally acting skeletal muscle relaxant whose action may be due to general depressant effects on the CNS. It is commonly used orally in humans as an adjunct in the short-term symptomatic treatment of painful muscle spasm associated with musculoskeletal conditions.

Methocarbamol is rapidly and almost completely absorbed from the gastrointestinal tract after oral doses in humans. The onset of action is 30 minutes. It binds moderately to plasma proteins, from 46-50%. Its plasma half-life is reported to be about 1 to 2 hours. It is metabolized by dealkylation and hydroxylation and is excreted in urine primarily as the glucuronide and sulfate conjugates of its metabolites. A small amount is excreted in faeces. Methocarbamol exhibits plasma clearance rates ranging from 0.20 to 0.80 L/h/kg, and it is typically dosed every 6 hours based on observed clinical pharmacokinetics (Scriba, 2009; Sibrack, J., 2024).

Some pharmacokinetic data is available in horses. Methocarbamol is rapidly absorbed and extensively metabolized after oral administration. The extent of bioavailability was characterized by a median (range) of 54.4 (43.2-72.8%) and an oral clearance of 16.5 (13.0-20) ml/min/kg was observed. By 48 hours after the last oral dose the concentrations were below the LLOQ. The volume of distribution at steady state was 1.05 (±0.09) L/kg. The median (range) terminal half-life for the intravenous [2.96 (2.46-4.71) h] and oral [2.89 (2.21-4.88] h) routes were similar. The total systemic clearance after intravenous dose was 8.99 (6.68-10.8) ml/min/kg (Rumpler et al., 2014). In other study in horses, the median extent of bioavailability and the terminal half-life after single 50 and 100 mg/kg oral doses were 82.9 and 110% and 95.4 and 97.8 mins, respectively. After intravenous administration of 30 mg/kg, the terminal half-life ranged from 59.1 to 89.6 mins and the median total plasma clearance was 9.41 ml/min/kg. The median volume of distribution at steady state was 756 ml/kg (Muir et al., 1992). Methocarbamol was measured in serum and urine samples from four horses administered orally with 3 g twice daily for four days and 3 g via a stomach tube on the fifth day. The drug was detected (LOD = 0.2 µg/ml) in serum up to 6 hours in 3 horses and up to 3 hours in one horse. In urine, a mean concentration of 4.5 µg/ml was observed 121 hours after the treatment (Koupai-Abyazani et al., 1997). The elimination half-life was not significantly different between horses that received a single 15 g dose and those administered multiple 15 g oral doses followed by a single IV administration, suggesting that elimination is still first order following oral administration of doses ranging from 5-15 g. In this study the elimination half-lives ranged from 2.64 to 6.07 hours. Furthermore, in this study it is stated that in horseracing, methocarbamol (American Racing Commissioners International Class 4 substance) has a recommended withdrawal time of 48 h (Knych et al., 2016).

Methocarbamol is characterized by a favourable safety profile when administered either orally or in injectable form (Jung and Chae, 2019). However, little information is available from toxicity studies. From the information in human medicinal products, there have been very rare reports of foetal and congenital abnormalities following in utero exposure to methocarbamol. In vitro and in vivo examinations as to the genetic toxicology of methocarbamol did not reveal any mutagenic potential and long-term studies to evaluate the carcinogenic potential of methocarbamol have not been performed.

Considering its pharmacokinetic characteristics and the available toxicological information it can be accepted that methocarbamol 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.

4.2.4. Conclusion

Based on the above assessment and justifications, the following recommendations are proposed:

1. The following active substances, currently in Regulation (EU) 1950/2006 as amended by Regulation (EU) 122/2013, are proposed to be retained in the list, either without modification or with an amendment of the current entry as shown below:

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Fentanyl	multimodal approach for moderate to severe acute painful conditions	butorphanol, morphine	produces better analgesia than certain other opioids and can be used for very painful conditions; recognized value for use in multi-modal approaches
Ketorolac	treatment of eye pain and inflammation	systemic NSAID therapy (e.g. flunixin)	formulated for local application
Morphine	analgesia	butorphanol, fentanyl	more potent than other analgesics
Triamcinolone Acetonide	for the treatment of joint inflammation	methylprednisolone	less harmful effects on cartilage metabolism

2. The following active substances, currently in Regulation (EU) 1950/2006 as amended by Regulation (EU) 122/2013, are proposed to be removed from the list: buprenorphine, flumethasone, gabapentin, pethidine.

3. The following active substances, suggested for addition to the list in the survey to stakeholders, are proposed to be added to the list with an entry as shown below:

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Bromfenac	treatment of uveitis and ocular inflammation	systemic NSAIDs (e.g. flunixin); topical (ocular) ketorolac	topical NSAIDs may result in less patient discomfort, reduced postoperative inflammation, prevention of miosis, and improvements in visual acuity in the early postoperative period
Methocarbamol	as part of treatment protocols in severe painful muscle spasms/muscle inflammation conditions	systemic NSAIDs (e.g. flunixin)	potent skeletal muscle relaxation; specific action on the internuncial neurons of the spinal cord to reduce acute skeletal muscle spasms without a concomitant alteration in muscle tone

4. The following active substances, suggested for addition to the list in the survey to stakeholders, are not proposed for inclusion: methadone, phenylbutazone, pregabalin.

4.3. Antimicrobials

4.3.1. Overview

	Active substance (ATCvet code)
Substances in Regulation (EU) 1950/2006 **proposed to be retained**, with or without amendment of the current entry	Acyclovir (QD06BB03); Amikacin (QJ01GB06); Azithromycin (QJ01FA10); Miconazole (QD01AC02); Nystatin (QD01AA01); Ofloxacin (QS01AE01); Polymyxin B (QS01AA18)
Substances in Regulation (EU) 1950/2006 **proposed to be removed**	Griseofulvin (QD01BA01); Idoxuridine (QS01AD01); Isometamidium (QP51DX04); Ketoconazole (QJ02AB02); Ponazuril (QP51BC04); Pyrimethamine (QP51BX56); Rifampicin (QJ04AB02); Ticarcillin(QJ01CA13)
Substances from stakeholders' survey **proposed for inclusion**	Amphotericin B (QJ02AA01); Clarithromycin (QJ01FA09); Fusidic acid (QS01AA13); Ganciclovir (QJ05AB06; QS01AD09); Moxifloxacin (QS01AE07); Valacyclovir (QJ05AB11); Voriconazole (QJ02AC03)
Substances from stakeholders' survey **not proposed for inclusion**	Buparvaquone (QP51EX03); Ciprofloxacin (QS03AA07); Fluconazole (QJ02AC01); Rifamycin (QS01AA16); Tenoic acid (no ATCvet code identified); Tobramycin (QS01AA12)

4.3.2. Review of the existing entries in Regulation (EU) 1950/2006, as amended by Regulation (EU) 122/2013, considering the survey results

A. Considerations on the essentiality of the substance(s)

i. Antibiotics

Amikacin is a semi-synthetic aminoglycoside antibiotic used in the treatment of Gram-negative infections.

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for treatment of septic arthritis. The identified alternatives in the current list are gentamicin or other aminoglycosides and the specific advantage of amikacin is that it is better tolerated in foals.

Similar to other aminoglycosides, amikacin is not appreciably absorbed following oral (or intrauterine) administration, and is thus administered via the intravenous route or intramuscularly, when intravenous access is not available (Sizar et al., 2023). Aminoglycosides must penetrate into bacteria to assert their bacteriocidal effect. This is initiated by an oxygen-dependent interaction between the antibiotic cations and the negatively charged ions of the bacterial membrane lipopolysaccharides. Once inside the bacterial cell, aminoglycosides bind to the 30S ribosomal subunit and cause a misreading of the genetic code, interrupting normal bacterial protein synthesis. This leads to changes in cell membrane permeability, resulting in additional antibiotic uptake, further cell disruption, and ultimately, cell death. Amikacin is unaffected by several bacterial enzymes that inactivate other aminoglycosides. Optimal antibacterial activity of amikacin can be described as concentration-dependent, with a target maximum plasma concentration (C_{max}) of 8 to 10 times the MIC generally recommended for

bactericidal effect and to minimize selection of resistant isolates. For isolates with intermediate susceptibility, a target plasma amikacin concentration of ≥53 to 60 µg/mL or 50 to 60 µg/mL at 30 minutes or a concentration of ≥40 µg/mL at one hour post-administration has been recommended (Paegelow et al., 2024). Amikacin is categorised as a critically important antimicrobial for human health by the World Health Organization (WHO). It is frequently administered intravenous, subcutaneous, intramuscular, by intra-articular injection, nebulization or by local venous or intraosseous perfusion in many species.

Septic arthritis is the purulent invasion of a joint by an infectious agent. Septic arthritis can be a sequela to septicemia in foals or trauma to the joint (puncture wound), or iatrogenic introduction after intraarticular injection or surgery. Risk factors for establishing a bacterial infection include devitalised tissue, foreign material, virulence and number of bacteria, as well as local or systemic immune compromise of the patient. Main clinical signs of septic arthritis include joint effusion and lameness. The degree of lameness can vary depending on size, age and type of the horse, duration of infection, as well as pathogenicity and virulence of the infecting organism. The definitive diagnosis of septic arthritis is confirmed by cytological and microbiological analysis of synovial fluid from an aseptically performed arthrocentesis. Other diagnostic imaging techniques are also commonly used.

Gentamicin and ceftiofur are included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with MRL entries for all mammalian food producing species, and there are veterinary medicinal products authorised for septic arthritis for food-producing animals of the equine species. However, it is still considered that amikacin brings added clinical benefit compared to these treatment options for treating septicemia in horses and foals with a better safety profile for the target animal.

Septicemia, if untreated, may be life-threatening and can cause unacceptable suffering of the animal (i.e. permanent damage to the joint).

Amikacin was mentioned (five times) in the survey to stakeholders, proposing the entry be modified to indicate the substance is not to be used as a preventative.

A search in the veterinary medicines database retrieves at least one amikacin-containing veterinary medicinal product authorised for use in equine species (non-food-producing horses).

The substance amikacin is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: for the treatment of septicemia in horses and foals. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Azithromycin is a 15-member ring macrolide antibiotic. The 15-membered ring macrolides are termed azalides as they have a nitrogen atom in the lactone ring. Macrolides inhibit protein synthesis by reversibly binding to 50S subunits of the ribosome. They inhibit the transpeptidation and translocation process, causing premature detachment of incomplete polypeptide chains. Macrolides are generally bacteriostatic drugs, but they may be bactericidal at high concentrations and against a low inoculum of some highly susceptible bacteria.

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for treatment of *Rhodococcus equi* infections. The identified alternative on the current list is erythromycin and the specific advantage is that it is standard treatment in combination with rifampicin, and better tolerated in foals than erythromycin.

Rhodococcus equi infection in foals is commonly characterised as a multifocal pyogranulomatous pneumonia with abscess formation; extrapulmonary infections are also common (Yamshchikov et al.,

2010; Giguère et al., 2011a). Foals are typically infected from birth (Chaffin et al., 2003; Cohen et al., 2013), whereby the insidious onset of bronchopneumonia does not result in the onset of clinical signs until typically between 1 to 5 months of age (Cohen et al., 2013). Control of foal rhodococcosis is challenging due to the lack of an effective vaccine and relies on antimicrobial therapy and other control measures. The ability of *R. equi* to survive and replicate within macrophages is the basis for its pathogenicity. Based on thoracic ultrasound, foals with rhodococcal pneumonia can be characterised into three groups: subclinical rhodococcal pneumonia, as the most common type, with peripheral pulmonary consolidation or abscessation without manifesting clinical signs; mild-to-moderate clinical signs of rhodococcal pneumonia in which less than 50% of lung is involved and no evidence of other body sites infected; severe rhodococcal pneumonia in which more than 50% of lung is involved and respiratory distress and other body sites could be infected.

Although many antimicrobials are active in vitro against *R. equi*, this in vitro activity does not correlate with in vivo antimicrobial efficacy since antimicrobials must penetrate three sites to above minimum inhibitory concentrations (MIC) for *R. equi*: (1) lungs; (2) the encapsulated pyogranulomatous abscess/es; (3) the intracellular location of *R. equi* within alveolar macrophages. Treatments for *R. equi* infections are prolonged, with several weeks to months required to achieve complete recovery (Giguère et al., 2011b; Giguère et al., 2012).

R. equi infections in foals can also be treated with other macrolide antibiotics i.e. clarithromycin, erythromycin, gamithromycin, tulathromycin, or tetracyclines. Erythromycin and doxycycline are included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with MRL entries for all food producing species, including *Equidae.* Gamithromycin and tulathromycin are also included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with MRL entries for food producing species other than *Equidae.* Furthermore, clarithromycin is recommended for the list of essential substances. All the above-mentioned substances constitute as satisfactory alternatives. These alternatives can also be used as monotherapy for *R. equi*. Hyperimmune plasma can be used as a preventative (Kahn et al., 2023) and gallium maltolate, a semimetal compound with antimicrobial activity, has also been described as an alternative treatment for *R. equi* in foals (Cohen et al., 2015).

Azithromycin brings added clinical benefit in cases of *R. equi* infections in foals that can be resolved as monotherapy or in combination with doxycycline only. Azithromycin is more active than erythromycin against gram-negative bacteria and has a considerably lengthened half-life relative to erythromycin. Oral bioavailability of azithromycin is approximately 50% in foals. Serum elimination half-life is 20 hours in foals. The extensive tissue distribution of azithromycin appears to result from its concentration within macrophages and neutrophils. The half-life of azithromycin in foal neutrophils is 49 hours. Bronchoalveolar cells and pulmonary epithelial lining fluid concentrations in foals are 15- to 170-fold and 1- to 16-fold higher than concurrent serum concentrations, respectively (Jacks et al., 2003). Azithromycin is categorized as a highly important critical antimicrobial for human health by the World Health Organization (WHO).

Azithromycin was assessed in six randomised blinded and double-blinded controlled clinical trials (RCTs) for efficacy in mild-to-moderate cases of *R. equi* pneumonia, with good results in 4 RCTs. This includes as monotherapy or in combination with either rifampicin or doxycycline (Venner et al., 2012; Venner et al., 2013a; Venner et al., 2013b; Hildebrand et al., 2015; Rutenberg et al., 2017; Wetzig et al., 2020). Pharmacokinetic studies in foals have shown that azithromycin as monotherapy maintains parent drug concentrations in serum, pulmonary epithelial lining fluid, and broncho-alveolar cells higher than the MIC90 for *R. equi* for the entire dosing interval (Suarez-Mier et al., 2007).

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.

Azithromycin was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any azithromycin-containing veterinary medicinal product authorised for use in equine species. There are veterinary medicinal products authorised for use in species other than the equine (i.e. dog).

The substance azithromycin is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: to treat *Rhodococcus equi* infections. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Ofloxacin is an antibiotic from the group of fluoroquinolones (gyrase inhibitors). The broad spectrum of activity includes facultative anaerobes, aerobes and other micro-organisms such as chlamydia, especially gram-negative bacteria. It is used for treatment of external eye infections caused by gram-positive and gram-negative micro-organisms sensitive to ofloxacin, such as conjunctivitis, keratitis (corneal ulcers), blepharitis and blepharoconjunctivitis and dacryocystitis; it is also used in the prophylaxis of pre- and post-operative infections and, generally, for the treatment of wounds of the eye and surrounding structures.

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), for the treatment of eye infections resistant to commonly used ophthalmic antibiotic treatments. No alternatives are identified in the current list and the specific advantage captured is the widest clinical experience with ofloxacin compared to other potential candidates. It also has a good penetration of the entire cornea up to the anterior chamber of the eye. It is also stated that ofloxacin should only be used as a reserve antibiotic in individual cases.

Its effective use in human medicine for the treatment of bacterial keratitis is well known (Chalder et al., 2020; Pearce et al., 2023); ofloxacin provides enhanced clinical experience compared to other antibiotic drugs and has proven to penetrate the entire cornea up to the anterior chamber of the eye. While published scientific evidence in horses is scarce, evidence of other antibiotics within the same class is available (Barnett et al., 2004; Mishra et al., 2015). Expertise within the expert group preparing this scientific advice confirmed the clinical relevance of ofloxacin for the proposed indication. In addition, as there is no cross resistance between fluoroquinolones and other classes of antibiotics, it may be of clinical value when other antibiotics are no longer effective (Varshney et al., 2014). Moxifloxacin is an alternative treatment proposed for addition to the list.

Bacterial eye infections, if untreated, might cause unacceptable suffering of the animal.

Ofloxacin was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database retrieves at least two ofloxacin-containing veterinary medicinal products authorized for use in equine species (non-food-producing horses) containing ofloxacin for ocular use.

The substance ofloxacin is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: treatment of external eye infections caused by gram-positive and gram-negative micro-organisms

susceptible to ofloxacin. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Polymyxin B is a polypeptide (polycationic) antibiotic belonging to the polymyxin class.

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for the systemic treatment for endotoxaemia associated with severe colic and other gastrointestinal diseases. The identified alternatives in the current list are flunixin and bismuth subsalicylate, and the specific advantages listed are its different mode of action (endotoxin binding agent) compared to other systemic alternatives (flunixin), and its early acting in the endotoxin-induced cascade; a different mechanism of binding, different route of administration and different site of action compared to oral alternatives (bismuth); and that it aids in the prevention of initiation of the inflammatory cascade induced by binding endotoxin and preventing binding to Toll-like receptors.

Polymyxins disrupt the outer bacterial cell membrane of certain Gram-negative bacteria, by interacting with the negatively charged phosphate groups of lipid A subunits of lipopolysaccharide, leading to a displacement of cations (mainly Mg2+ and Ca2+) from the outer membrane and resulting in cell leakage and bacterial death. Polymyxins are active particularly against Gram-negative bacteria such as most *Enterobacterales* (e.g. *E. coli*, *Klebsiella* spp., *Salmonella* spp.), *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, including those displaying carbapenem resistance. Some Gram-negative bacteria are inherently resistant to polymyxins, such as *Serratia*, *Stenotrophomonas* and *Proteus* spp. Polymyxins are categorized as HPCIA (Highest Priority Critically Important Antimicrobials) for human medicine in the WHO list of Medically important antimicrobials (WHO, 2024).

One of the uses of polymyxin B in equine medicine is for a non-antibacterial purpose at subtherapeutic doses for the treatment of systemic inflammatory response syndrome (SIRS) due to endotoxemia in horses and foals (Morresey and MacKay, 2006). SIRS associated with endotoxemia remains a leading cause of morbidity and mortality in both neonatal and adult equids (Mercer et al., 2023). Endotoxin is a lipopolysaccharide (LPS) consisting of three main structural components: the hydrophobic lipid A, a core oligosaccharide, and the hydrophilic antigen O. While endotoxemia specifically refers to circulating endotoxin (a cell wall component of Gram-negative bacteria), the term has commonly been used more broadly as a clinical description of horses experiencing SIRS, secondary to sepsis.

Polymyxin B binds to bacterial toxins and therefore reduces activation of the pro-inflammatory cascade. The most prominent pro-inflammatory cytokines in equine SIRS are TNF-α, IL-1β, and IL-6, all of which are also regulators of innate immunity (Tadros and Frank, 2012). Increases in these pro-inflammatory cytokines has been positively correlated with the clinical signs of SIRS in horses, including an increase in body temperature, increase in heart rate, and decrease in total white blood cell count, as well as an increase in mortality rate in horses with strangulating or inflammatory intestinal disorders (Morris et al., 1990, 1991). Since prostaglandins have been shown to affect the formation of pro-inflammatory cytokines, such as IL-6, and IL-1β has been shown to induce mRNA expression of COX-2, then COX-inhibitors (i.e. non-steroidal anti-inflammatories drugs - NSAIDs) remain central to the treatment of SIRS associated with endotoxemia (Mercer et al., 2023).

Horses and humans share a pronounced sensitivity to the systemic effects of endotoxemia. Endotoxins (free-floating) are produced within the equine gastrointestinal tract and absorbed systemically, secondary to a gastrointestinal disease or due to a Gram-negative bacterial infection. Sub-therapeutic polymyxin B doses for SIRS in horses varies between 5000 and 10,000 IU/kg IV every 8 to 12 hours (Barton et al., 2004; Morresey and MacKay, 2006; Werners, 2017). Doses up to 25 000 IU/kg/day are needed to reach true antimicrobial effects in humans, compared to much lower dose for anti-endotoxic effects in horses. There is limited evidence that prophylaxis with polymyxin B improves survival

associated with SIRS (Barton et al., 2004). However, polymyxin B is typically given to horses and foals when there are already the full clinical manifestations of SIRS, for which no clinical trials have been published. Once SIRS is triggered by endotoxemia, with associated clinical signs, polymyxin B is unlikely to be clinically effective since it has no direct effect on SIRS.

Also, administering polymyxin B intravenously to horses results in serious adverse effects (Schwarz et al., 2013; van Spijk et al., 2022). A study showed neurotoxicosis in healthy horses receiving 7 doses of polymyxin B 6000 IU/kg IV q12h. Mild-to-moderate ataxia was reversible after cessation of therapy. Number of polymyxin B doses and co-administration of gentamicin increased the severity of ataxia and nephrotoxicity (van Spijk et al., 2022). Conversely, polymyxin B is not used in human medicine for endotoxemia due to serious adverse effects.

Use of sub-therapeutic doses of polymyxins has resulted in global transmissible polymyxin resistance in animal and human populations. Plasmid-mediated polymyxin-resistant genes have been identified in horses (Börjesson et al., 2020). Therefore, equine clinicians must consider replacing polymyxin use as much as possible (Isgren, 2021).

Alternatives to subtherapeutic, low dose polymyxin B for SIRS include hydration fluids, NSAIDs or intravenous lidocaine (Peiró et al., 2010). From a multicentre, blinded, randomised clinical trial comparing the use of flunixin meglumine or firocoxib in horses with small intestinal strangulating obstruction, Ziegler et al. (2019) found that firocoxib significantly reduced a major biomarker of endotoxaemia more compared with flunixin meglumine, while continuing to provide similar levels of pain control. Flunixin, firocoxib (and bismuth subsalicylate), are included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 and there are veterinary medicinal products authorised for food-producing animal s of the equine species; it is considered that polymyxin B does not add clinical value for the treatment of endotoxemia in horses compared to these alternative treatments.

However, polymyxin B was mentioned (nine times) in the survey to stakeholders and it was proposed that its entry be modified allowing it for local treatment of bacterial eye infections, bacterial keratitis and corneal ulcers. The specific advantages listed by the responders included that it constitutes an effective alternative to systemic treatments.

A review of the literature indeed shows a number of references to polymyxin B as a topical ophthalmic preparation for the treatment of bacterial keratitis (Nation, 2018; Chalder et al., 2020; Vercruysse et al., 2022; Foote et al., 2023). While ofloxacin and moxifloxacin are recommended for the list for similar indications, it is considered that polymyxin B adds clinical benefit as an alternative treatment.

Bacterial keratitis, if untreated, is not life-threatening but may cause unacceptable suffering of the animal.

A search in the veterinary medicines database retrieves a polymyxin B-containing veterinary medicinal product authorised for use in equine species (non-food-producing horses). There are veterinary medicinal products authorised for use in species other than the equine (e.g. dog and cat).

The substance polymyxin B is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indications: for the treatment of bacterial keratitis, topical use. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Rifampicin is part of the rifamycin group of antimicrobials and further part of the ansamycin macrocyclic antibiotic class. The name rifampin is used in the North America, whereas it is called rifampicin in Europe and Australia.

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for the treatment of *Rhodococcus equi* infections. No alternatives are identified, and the specific advantage listed is that it is the treatment of choice, in combination with erythromycin or azithromycin.

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). Rifamycins are World Health Organisation (WHO) critically important antimicrobials (CIAs) in human medicine for the first-line treatment of mycobacterial infections (tuberculosis, leprosy and mycobacterium avium complex - MAC). All rifamycins are further listed on the WHO AWaRe Watch group (WHO, 2023).

Rifampicin enters bacteria and forms stable complexes with the β-subunit of bacterial DNA-dependent RNA polymerase, while not affecting mammalian polymerase (Frank, 1990). The degree of antibacterial activity of rifampicin is related to the binding affinity to the β-subunit of prokaryotic DNA-dependent RNA polymerase (Wehrli et al., 1968). This binding results in inactivation of the enzymes and inhibition of RNA synthesis by preventing chain initiation (Papich and Riviere, 2009).

In foals given 10 mg/kg of rifampicin orally, the drug had a mean serum half-life of 17.5 hours. This is significantly longer than that found in adult horses (4.2 ± 1.2 hours) administered the same dose orally, with food (Burrows et al., 1985; Papich and Riviere, 2009). After short-term rifampicin administration in foals, Peters et al. (2012) found in vivo that the deacetylated, more-polar metabolite accumulated 1.4- to 6-fold in pulmonary epithelial lining fluid (PELF) and 8- to 60-fold in broncho-alveolar (BAL) cells, accounting for approximately 2% of the total drug exposure in PELF but 25% in BAL cells (Peters et al., 2012). Orally given rifampin was slowly absorbed (T_{max}, range: 2.5–8.0 h) and eliminated with apparent half-lives of ~6–8 h. Trough concentrations in PELF and BAL cells were 1.01 $\pm$ 0.20 µg/mL and 1.25 $\pm$ 0.29 µg/mL, respectively, after 10 mg/kg body weight rifampicin and 2.71 $\pm$ 1.25 µg/mL and 3.09 $\pm$ 1.63 µg/mL, respectively, after 20 mg/kg body weight rifampicin (Berlin et al., 2017).

Rhodococcus equi infections in foals are characterized as a multifocal pyogranulomatous pneumonia, with abscess formation, but extrapulmonary infections are also common (Yamshchikov et al., 2010; Giguère et al., 2011b). *Rhodococcus* foals are typically infected from birth (Chaffen et al., 2003; Cohen et al., 2013), whereby the insidious onset of bronchopneumonia does not result in the onset of clinical signs until typically between 1 to 5 months of age (Cohen et al., 2013). Control of foal rhodococcosis is challenging due to the lack of an effective vaccine and relies on antimicrobial therapy and other control measures. The ability of *R. equi* to survive and replicate in macrophages is the basis for its pathogenicity.

R. equi pneumonia is a common reason for critically important antimicrobial use in foals. Culture/susceptibility is performed rarely, in favour of thoracic ultrasound. The popularity of thoracic ultrasound in foals has resulted in substantial increases in the misuse of critically important antimicrobials, for prevention and treatment. Based on thoracic ultrasound, foals with rhodococcal pneumonia can be characterised into three groups:

- Subclinical rhodococcal pneumonia: most common type (peripheral pulmonary consolidation or abscessation without manifesting clinical signs);
- Mild-to-Moderate clinical signs of rhodococcal pneumonia: <50% lung involvement (no evidence of other body sites infected);

- Severe rhodococcal pneumonia: >50% lung involvement (respiratory distress and other body sites could be infected).

Although many antimicrobials are active in vitro against *Rhodococcus equi*, this in vitro activity does not correlate with in vivo antimicrobial efficacy (Jacks et al., 2003), since antimicrobials must penetrate three sites to above MICs for *R. equi* (i.e. 1., lungs; 2., encapsulated pyogranulomatous abscess/es; 3., intracellular location of *R. equi* within alveolar macrophages). Antimicrobial treatments are not only applied to clinically affected animals but also to prevent outbreaks and presumptive cases identified by thoracic ultrasonography (Venner et al., 2013). Treatments for *Rhodococcus equi* infections are prolonged, with several weeks to months required to achieve complete recovery (Giguère et al., 2011a; Giguère et al., 2012).

A tradition developed in the 1980's whereby the combination of a macrolide (e.g. erythromycin, clarithromycin, or azithromycin) with rifampicin became the standard international approach for foals infected with *R. equi* (Giguère et al., 2011b), or more recently doxycycline. However, these recommendations (consensus) were published before the publications of blinded and double-blinded randomised clinical trial (RCTs) results, as well as peer-reviewed studies about antagonistic drug interactions between rifampicin and other co-medications in foals. Now a wealth of published studies conducted in foals is available that all lead to the same conclusions that rifampicin combination therapy is not consistent with good antimicrobial stewardship. This includes three types of peer-reviewed published studies in foals:

- Pharmacokinetic/pharmacodynamic studies showing strong negative antagonistic in vivo drug interactions between macrolides and rifampicin that prevent either drug from reaching the target site of infection (alveolar macrophages) at concentrations for *R. equi* MICs.
- Blinded and double-blinded controlled RCTs in foals investigating monotherapy versus rifampicin combination therapy against a placebo group.
- Observation studies investigating antimicrobial resistance in *R. equi* as a consequence of rifampicin combination therapies.

Rifampicin's in vivo interactions with body systems and other drugs is exceedingly complex. Original traditional use of rifampicin combinations in foals did not appreciate the impact of metabolic pathways affected by rifampicin and its role in drug interactions. For rifampicin-treated foals, the important PK/PD sites are the intestine, the liver and the bronchial and alveolar epithelial cells as well as PELF.

Rifampicin acts as a perpetrator drug for many co-administered drugs, causing clinically relevant drug interactions via metabolic enzyme induction (Asaumi et al., 2018). Rifampicin binds to the nuclear pregnane X receptor (PXR) and the constitutive androstane receptor (CAR). PXR and CAR in turn activate a set of target genes, including phase I enzymes (e.g. Cytochrome P450s enzymes - CYP), phase II enzymes including UDP-glucuronosyltransferases (UGTs), glutathione-S transferases (GSTs) and the phase III detoxification systems, including efflux carriers of the ATP-binding cassette (ABC) transporter family including P-glycoprotein (ABCB1) and multidrug resistance-associated protein 2 (MRP2) (ABCC2) transporters and uptake carriers including the organic anion transporters (OATs) (Chang et al., 2017). The PK consequences of enzyme induction by rifampicin depends on the physical location of the enzymes. They include decreased or negligible bioavailability for orally administered drugs, increased hepatic clearance or accelerated formation of reactive metabolites.

Rifampicin can induce the metabolic enzymes and transport proteins involved in its own disposition (auto-induction), as well as the accelerated metabolism of other drugs (e.g. macrolides). CYP enzyme induction is typically not seen with less than 5 days of therapy, but once induction occurs, the increase in enzyme activity may last for more than 2 weeks after discontinuation of treatment (Burrows et al.,

1992). Berlin et al. (2016) showed that foal in vivo CYP3A4 induction was increased more than 10-fold under the influence of rifampicin, resulting in decreased rifampicin systemic exposure by ~60% after chronic oral treatment (Berlin et al., 2017). Therefore, over the course of treatment, decreasing concentrations of the parent drug (rifampicin, macrolides) are available to reach the target site (alveolar macrophages) of infection. The main metabolites of rifampicin (e.g. 25-o-desacetylrifampin) and macrolide metabolites (e.g. 14-hydroxyclarithromycin for clarithromycin) are bioactive. However, there is no information in terms of MICs for these metabolites against *R. equi*, and thus it is unclear if metabolites of rifampicin and macrolide contribute to the clinical efficacy. Also, the main metabolite (25-o-desacetylrifampin) is more water soluble than rifampicin and thus less likely to penetrate pulmonary abscesses and intracellular regions of alveolar macrophages.

The most important drug interactions are between rifampicin and macrolides. Macrolides move against a steep concentration gradient from plasma via the PELF into bronchoalveolar cells to reach concentrations many times the concurrent plasma concentrations. Macrolide concentrations into PELF and bronchoalveolar cells are influenced by CYP3A4 and the drug transporters, P-glycoprotein (ABCB1), ABCC2 and OATPs, which all can be modulated and/or up-regulated via the nuclear PXR, by rifampicin. Rifampicin increases intestinal expression of P-glycoprotein transporters, reducing the oral bioavailability of macrolides that are P-glycoprotein substrates.

The magnitude of drug interactions depends on the route, duration, and timing of rifampicin, making it difficult to predict drug interactions. For example, when clarithromycin is administered concurrently with rifampicin, both in oral dosage regimens, the oral bioavailability of clarithromycin was decreased by up to 90% to plasma concentrations below the MIC of *R. equi* (Peters et al., 2011; Peters et al., 2012). The decrease in bioavailability of clarithromycin was primarily due to rifampicin induction of intestinal P-glycoprotein as well as a minor reduction due to an unknown modulation in clarithromycin uptake (Berlin et al., 2016). In foals, concurrent administration of rifampicin with tulathromycin (IM) resulted in significantly lower plasma concentrations and lower pulmonary accumulation of tulathromycin (Venner et al., 2010). There was a tendency for lower concentrations in the PELF and significantly lower concentrations in bronchoalveolar cells compared to monotherapy. Conversely, concurrent administration of rifampicin with the azalide, gamithromycin, significantly increased plasma concentrations of gamithromycin, likely due to rifampicin inhibition of hepatic uptake and biliary excretion leading to a reduction in the total body clearance of gamithromycin (Berlin et al., 2018). However, gamithromycin reduced plasma concentrations of rifampicin by lowering intestinal absorption of rifampicin via inhibition of an as yet unknown uptake mechanism.

While specific clinical studies have not examined all drug interactions between rifampicin and other macrolides, erythromycin has high affinity for CPY3A4, is a substrate of P-glycoprotein, and is considered similar to clarithromycin in the risk of clinically significant negative drug interactions when administered concurrently with rifampicin.

Based on the results of six randomised clinical trials (RCTs), with a negative control group (placebo), the following conclusions can be retained:

- A notable 'placebo' affect (self-cure of up to 88%) has been identified for foals with subclinical bronchopneumonia on *R. equi* endemic farms, with a median ultrasound abscess size of 10-15 cm; antimicrobial therapy does not significantly accelerate lesion resolution relative to the placebo group (Venner et al., 2012). Thus, antimicrobial therapy in subclinical cases is not justified and only clinical/ultrasound monitoring should be done.
- Mild-to-moderate *R. equi* bronchopneumonia cases (ultrasound abscess size > 10-15 cm) warrant antimicrobial therapy; RCTs confirm that monotherapy with macrolides perform equally as well

(non-inferior) as rifampicin concomitant therapies (Hildebrand et al., 2015; Rutenberg et al., 2017; Wetzig et al., 2020;). The use of rifampicin concomitant therapies provided no added clinical advantage for the treatment of these cases.

- Foals with severe *R. equi* bronchopneumonia require antimicrobial therapy but have a poor prognosis and have not been evaluated in RCTs. If combination antimicrobials are considered necessary then azithromycin/doxycycline or clarithromycin/doxycycline has been evaluated and considered synergistic (Giguère et al., 2012; Erol et al., 2022).

As previously indicated, it is considered necessary (prudent use) to always use rifampicin in combination with a synergistic antimicrobial to limit the development of antimicrobial resistance. Rifampicin resistance arises quickly and easily in *R. equi*, even within one treatment period, typically from single-point substitution/s of the chromosomal *rpoB* gene (Fines et al., 2001).

Rifampicin-resistant *R. equi* are well known in equine medicine. The prevalence of *R. equi* isolates resistant to both macrolides and rifampicin has significantly increased since 2007 and is of concern for both human and animal health (Huber et al., 2018, 2019). Overuse of macrolide/rifampicin combinations in foals resulted in widespread emergence of multi-drug resistant *R. equi* (Alvarez-Narvaez et al., 2020; Alvarez-Narvaez et al., 2021), with up to 40% of foals yielding isolates highly resistant to macrolides and rifampicin as a result of mass antimicrobial treatment (Burton et al., 2013; Álvarez-Narváez et al., 2019).

Increasing rates of AMR in *R. equi* were also recognised from the EMA scientific advice under Article 107(6) of Regulation (EU) 2019/6 for establishment of a list of antimicrobials which shall not be used in accordance with Art. 112, 113 and 114 or which shall only be used in accordance with these articles subject to certain conditions, where rifampicin was not recommended to be used for prophylaxis of *R. equi* infection (EMA/CVMP/151584/2021)[31].

Overall, it can be concluded based on the evidence available, that macrolide-rifampicin combinations do not penetrate all three target sites to reach therapeutic *R. equi* MIC concentrations for both antimicrobials, during the entire treatment course (1., lungs; 2., encapsulated pyogranulomatous abscess/es; 3., intracellular location of *R. equi* within alveolar macrophages). Macrolide-rifampicin concomitant therapies are not synergistic in vivo. Pharmacokinetic studies revealed negative drug interactions that contribute to increasing rates of *R. equi* resistance to rifampicin and macrolides.

Results of six randomized clinical trials confirm that rifampicin combination therapy does neither offer a significant nor added clinical advantage over monotherapy with macrolides or doxycycline in foals with mild to moderate pulmonary *R. equi* infections (Venner et al., 2012, 2013a, 2013b; Hildebrand et al., 2015; Rutenberg et al., 2017; Wetzig et al., 2020). Given increasing evidence of negative drug interactions with significant impact on therapeutic concentrations of macrolides and the rapid emergence of resistance to rifampicin, the use of this critically important human drug should no longer be recommended for use in foals with *R. equi* infections.

For treatment of clinical *R. equi* infections in foals, alternative antibiotic treatments are available based on clinical experience as well as the results of randomized blinded and double-blinded controlled clinical trials (RCTs). This includes monotherapy with various macrolides or doxycycline as described in published RCTs (Venner et al., 2012, 2013a, 2013b; Hildebrand et al., 2015; Rutenberg et al., 2017; Wetzig et al., 2020). Pharmacokinetic studies in foals have shown that clinical doses maintain monotherapy parent drug concentrations in serum, pulmonary epithelial lining fluid, and broncho-

[31] Scientific advice under Art.107(6) of Reg.(EU)2019/6 for establishment of a list of antimicrobials which shall not be used in accordance with Art. 112, 113 and 114 or which shall only be used in accordance with these articles subject to certain conditions (EMA/CVMP/151584/2021) (accessible via this link).

alveolar cells higher than the MIC90 for *R. equi* for the entire dosing interval; this includes clarithromycin (Womble et al., 2006; Suarez-Mier et al., 2007), azithromycin (Suarez-Mier et al., 2007), tulathromycin (Venner et al., 2010), doxycycline (Womble et al., 2007).

Rhodococcus equi infections in foals can also be treated with various macrolide antibiotics (e.g. azithromycin, clarithromycin, erythromycin, gamithromycin, or tulathromycin), or tetracyclines (doxycycline, minocycline). Erythromycin, gamithromycin, tulathromycin and doxycycline are included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with an MRL entry; there are registered VMPs in Europe for food-producing animal species containing these substances. These alternatives can also be used as monotherapy for *R. equi*. If an alternative combination therapy is warranted, then azithromycin/clarithromycin is considered to be synergistic with doxycycline/minocycline. Azithromycin and clarithromycin are proposed to be retained in the list. One randomised controlled clinical trial evaluated azithromycin-doxycycline combination on *R. equi* endemic farms, including 81 mild-moderate clinically affected foals, and found an efficacy rate of 98.7% (Wetzig et al., 2020). An in vitro study found the clarithromycin-doxycycline/minocycline combinations to be synergistic against *R. equi* (Erol et al., 2021).

Hyperimmune plasma can be used as a preventative for *R. equi* (Kahn et al., 2023). Gallium maltolate (a semimetal compound with antimicrobial activity) has also been described as an alternative treatment for *R. equi* in foals (Cohen et al., 2015).

Rhodococcus equi infections, if untreated, are not always life-threatening, except for the severe form of the disease; it may pose a risk for public health since *R. equi* is considered as zoonotic (Yamshchikov et al., 2010). *Rhodococcus equi* infections, if untreated, may cause unacceptable suffering of the animal, only for the severe form of the disease.

Rifampicin was mentioned (six times) in the survey to stakeholders. It was proposed (five times) that a limitation of use to a maximum of 15 days be added; it was proposed (one time) that the substance be removed from the list since the combination of rifampin with a macrolide had proven to be antagonistic, i.e. rifampin reduces the oral bioavailability of the macrolide to subtherapeutic concentrations.

A search in the veterinary medicines database does not retrieve any rifampicin-containing veterinary medicinal product authorised for use in equine species.

The substance rifampicin is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Ticarcillin is a carboxypenicillin as a semisynthetic antibiotic with a broad spectrum of bactericidal activity against many gram-positive and gram-negative aerobic and anaerobic bacteria.

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for treatment of *Klebsiella* spp. infections. No alternatives are identified in the current list, and the specific advantage is that it is a specific antibiotic for *Klebsiella* spp. infections.

As stated in the mandate received from the European Commission on the 9 February 2023, *substances listed in the Annex to Commission Implementing Regulation (EU) 2022/1255 designating antimicrobials or groups of antimicrobials reserved for human treatment of certain infections in humans, should not*

be considered. Ticarcillin is listed in the annex of the afore-mentioned Commission Implementing Regulation as substances reserved for human-use only.

Thus, the substance ticarcillin cannot be considered for inclusion in the list.

Ticarcillin was mentioned (five times) in the survey to stakeholders and it was proposed to be removed for the reason stated above.

Knowledge regarding antibiotics in horses/foals for this assessment was derived from textbooks, review articles, retrospective studies and clinical trials; clinical trials were available for all indications for treatment.

ii. Antifungals

Griseofulvin is a benzofuran cyclohexene fungistatic antibiotic that inhibits mitosis by disorganizing the spindle microtubules and may also interfere with cytoplasmic microtubules. Griseofulvin is also reported to inhibit fungal RNA and DNA synthesis.

Griseofulvin is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), for the systemic treatment of ringworm. No alternatives are identified in the current list and the specific advantage captured is that griseofulvin given orally has good activity against trichophyton, microsporum, and epidermophyton.

Griseofulvin binds to keratin in keratin precursor cells and only reaches the site of action when old hair or skin is replaced. It takes days to weeks of therapy for griseofulvin to distribute to these tissues, particularly to the site of infection. Virtually all dermatophytes of animal origin are inhibited by griseofulvin concentrations of 0.2-0.5 µg/ml. Other hyphal fungi, yeasts, dimorphic fungi, and bacteria are unaffected by griseofulvin. 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. Absorption, after oral administration, depends greatly on particle size and diet (e.g. high-fat diet). It is unclear as to bioavailability characteristics in horses. Griseofulvin is metabolized by the liver to an inactive metabolite. Less than 1% of the drug is excreted unchanged in the urine, and most of the drug is excreted in feces.

Most cases of ringworm are superficial skin infections that can be treated with topical medications (e.g. disinfectants or antifungals other than griseofulvin). Only in very rare cases could a systemic antifungal agent be indicated. In general, ringworm is not a life-threatening condition. Some ringworm causing fungal species are zoonotic, but not considered a public health threat (Moskaluk and VandeWoude, 2022). Enilconazole is listed in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with a 'No MRL required' status for *Equidae*, and there are veterinary medicinal products authorised for food-producing animals of the equine species, thus available for the treatment of ringworm infections in food-producing horses; this alternative does yield equally satisfactory results in terms of successfully treating the animal and avoiding unnecessary suffering for the animal.

Griseofulvin was mentioned (once) in the survey to stakeholders, and it was proposed to remove it from the list since due to its teratogenicity potential and known to be associated with aplastic anemia. Its teratogenic effect has been noted for all species, and it is generally recommended that griseofulvin should neither be given to any pregnant animal nor animals that will enter the human food chain (Schutte and Van de Ingh, 1997).

Ringworm, if untreated, does not cause unacceptable suffering for the animal.

A search in the veterinary medicines database retrieves a griseofulvin-containing veterinary medicinal product authorised for use in equine species (non-food-producing horses).

The substance griseofulvin is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Ketoconazole belongs to a large group of synthetic agents that are effective in the topical treatment of dermatophyte infections and superficial forms of candidiasis: the azoles. Ketoconazole was developed in the late 1970s with the advantage of a broad antifungal spectrum, the option of oral administration, and relatively low toxicity. Ketoconazole (and miconazole) have the common antifungal action of inhibiting 14α-demethylase, a cytochrome P450-dependent enzyme responsible for the demethylation of lanosterol to ergosterol. Ergosterol is the principal sterol in fungal cell membranes just like cholesterol is the principal sterol in mammalian cells. Inhibition of 14α-demethylase results in the accumulation of various methylated sterols and the depletion of ergosterol with subsequent disruption of cell membrane structure and function.

Ketoconazole is a poorly water-soluble, highly lipophilic, weak dibasic compound, and generally fungistatic against a wide range of fungi including dermatophytes, yeasts, and dimorphic fungi. Administration of ketoconazole orally to adult horses at a dose of 30 mg/kg does not result in detectable serum concentrations. Administration of the same dose in 0.2 N HCl resulted in peak serum concentrations of 3.7 µg/ml and a bioavailability of only 23% (Prades et al., 1989). Ketoconazole is also available for topical antifungal therapy, though it is less active in vitro than clotrimazole, itraconazole, or miconazole. Ketoconazole may be embryotoxic and teratogenic and should not be given to pregnant animals.

Ketoconazole is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for the systemic treatment of fungal pneumonia and guttural pouch mycosis; no alternatives are identified, and the specific advantage captured is the wide clinical experience with ketoconazole that shows good efficacy compared to other potential candidates.

The use of systemic ketoconazole for guttural pouch mycosis represents a misunderstanding of the disease pathogenesis. In most cases clinical signs are not appreciated (e.g. epistaxis) until the fungal lesion has eroded a major artery associated with the equine guttural pouches (e.g. the internal carotid or maxillary artery). Thus, the treatment-of-choice is surgical occlusion of the affected artery. Topical antifungals are administered, occasionally, via endoscope onto the fungal plaque as an adjunctive therapy, but most cases will completely resolve with just surgical occlusion of the affected artery. For the adjunctive therapy, enilconazole is an adequate alternative listed in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with a 'No MRL required' status for *Equidae* and veterinary medicinal products authorised for food-producing animals of the equine species. Other (topical) antifungals are retained in the list.

Fungal pneumonia does occur in horses, but it is mostly described in the USA and it's unclear to what extent it represents an issue in the EU. Fungal pneumonia in horses, as a primary condition, is very uncommon but can be secondary to an immunosuppressive state (e.g. a chronic or debilitating illness). Due to the rarity of fungal infections in horses, efficacy of treatment protocols has not been thoroughly studied (Higgins and Pusterla, 2006). Cases in horses have been described due to the following agents: *Coccidioides immitis*, *Blastomyces dermatitidis*, *Histoplasma capsulatum*, *Cryptococcus*

neoformans, *Cryptococcus gattii*, Aspergillus species, and *Pneumocystis carinii* (now called *Pneumocystis jiroveci*). *Pneumocystis carinii* is known in Europe where the treatment of choice is trimethoprim-sulfonamide. This combination is authorised for use in food-producing equine species. In addition, the substance amphotericin B is proposed to be included in the list for the treatment of fungal pneumonia. Ketoconazole is a potent immunosuppressive agent, suppressing T-lymphocyte proliferation, and as such does not represent an adequate clinical choice for a serious condition such as fungal pneumonia.

Guttural pouch mycosis is a life-threatening condition that, if untreated, causes unacceptable suffering of the animal. Fungal pneumonia, if untreated, causes unacceptable suffering of the animal and it is potentially life-threatening. While some causative organisms are considered zoonotic, it is not considered a significant public health risk.

Ketoconazole was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any ketoconazole-containing veterinary medicinal product authorised for use in equine species. There are veterinary medicinal products authorised for use in species other than the equine (e.g. dog and cat).

The substance ketoconazole is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Miconazole also belongs to the azoles group. Miconazole is an effective topical antifungal agent that has (as ketoconazole) the common antifungal action of inhibiting 14α-demethylase, a cytochrome P450-dependent enzyme responsible for the demethylation of lanosterol to ergosterol. Ergosterol is the principal sterol in fungal cell membranes just like cholesterol is the principal sterol in mammalian cells. Inhibition of 14α-demethylase results in the accumulation of various methylated sterols and the depletion of ergosterol with subsequent disruption of cell membrane structure and function.

Miconazole is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for the treatment of fungal infections of the eye; no alternatives are identified, and the specific advantage captured is its topical use of the affected eye, with wider antifungal activity and/or lesser irritation than other antifungal agents.

Miconazole has proven useful for topical treatment of dermatophyte, candida, *Aspergillus* spp., and *Malassezia* infections. It is commonly used topically for the treatment of keratomycosis in horses. Fungal keratitis with or without corneal ulceration is well described in horses. This typically occurs following corneal trauma. Fungal organisms initially colonise the area of exposed stroma and can incite secondary inflammation (i.e. uveitis). *Aspergillus*, *Fusarium*, *Cylindrocarpon*, *Curvularia*, *Penicillium*, *Cystodendron*, *Mortierella wolfii*, yeasts, and molds are known causes of ulcerative and nonulcerative keratomycosis in horses (Brooks, 2008). Different treatment alternatives are available for treatment of keratomycosis in non-food-producing horses, as described in the literature (e.g. natamycin, fluconazole, econazole, voriconazole, clotrimazole or itraconazole; diluted povidone iodine (1:50) can also be used for equine keratomycosis; Galera and Brooks, 2012); however, options for food-producing horses are limited. Natamycin is listed in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with a 'No MRL required' status in *Equidae*. Clotrimazole, which has a similar activity, is the active substance included in veterinary medicinal products authorised for use in non-food-producing horses (clotrimazole is not listed in Table 1). Voriconazole is proposed for addition to the list. However,

when compared to miconazole, it is considered that these alternatives do not yield equally satisfactory results. Surgical options are also available, which include a keratectomy with a conjunctival graft or a full thickness corneal graft.

Fungal infection of the eye, if untreated, may cause unacceptable suffering of the animal.

Miconazole was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any miconazole-containing veterinary medicinal product authorised for use in equine species. There are veterinary medicinal products authorised for use in species other than the equine (e.g. dog and cat).

The substance miconazole is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: for the treatment of fungal infection of the eye. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Nystatin is a polyene antibiotic that disorganizes the membrane of fungi, occupying ergosterol-binding sites and altering membrane permeability, such that intracellular ions leak-out from the cell. The drug is effective against *Candida, Malassezia, Cryptococcus*, and some dermatophytes. Several *Candida* species other than *C. albicans* are resistant. Nystatin is used clinically as a topical, broad-spectrum antifungal drug.

Nystatin is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), for the treatment of yeast infections for eyes and genital tract. No alternatives are identified in the current list and the specific advantage captured is its specific activity against yeast infections.

Yeast infections of the equine eye, while very uncommon, have been described. Yeast is also part of normal microflora of the genital tract of mares that can generate opportunistic infections (e.g. endometritis). Common genera present include *Candida spp*., *Cryptococcus spp*., *Saccharomyces spp*., *Geotrichum spp*., *Rhodotorula spp*., *Malassezia spp*., *Trichosporon spp*., *Kluyveromyces spp*. and *Sporothrix spp*. (Azarvandi et al., 2017). As regards availability of alternative treatments for eyes, while the alternative treatments listed under miconazole could be considered, nystatin brings added clinical benefit compared to these for the treatment of yeast infections; similarly, for the treatment of yeast infections of the genital tract, nystatin brings added clinical benefit compared to other substances proposed for addition to the list: amphotericin B.

Fungal and yeast infection of the eye, if untreated, may cause unacceptable suffering of the animal.

Nystatin was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any nystatin-containing veterinary medicinal product authorised for use in equine species. There are veterinary medicinal products authorised for use in species other than the equine (i.e. dog and cat).

The substance nystatin is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: for the treatment of fungal and yeast infections of the eye. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Knowledge regarding antifungals in horses for this assessment was derived from textbooks, review articles, and retrospective studies. No clinical trials could be identified for antifungal use in horses.

iii. Antiprotozoals

Isometamidium and **pyrimethamine** are listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), for treatment of equine protozoal myeloencephalitis. Listed as alternatives to each, the specific advantage of isometamidium is for refractory cases to treatment with pyrimethamine, whereas for pyrimethamine the advantage listed is a 75% success rate when used in conjunction with sulfadiazine-sulfonamide.

Isometamidium, pyrimethamine and ponazuril (see below) are in the current list for the treatment of equine protozoal myeloencephalitis (EPM thereafter). It is well established that EPM can be caused by either *Sarcocystis neurona* or *Neospora hughesi*, although the vast majority of cases are from *S. neurona*. It should be noted that EPM due to *Sarcocystis neurona* is not a disease in Europe because the definitive host is not present. The opossum *Didelphis virginiana* is the definitive host for *S. neurona* in North America (as well other South American opossums). *S. neurona* is not transmitted horizontally between horses, nor can it be transmitted to horses from non-equine intermediate hosts. *Sarcocystis neurona* in Europe appears to be restricted to those horses that return or are imported from North America (Howe et al., 2014; MacKay and Howe, 2022).

The complete life cycle of *N. hughesi* is unknown, so all mode(s) of transmission of this parasite to horses remain poorly understood. Canids are a definitive host for the related species *Neospora caninum*, but it has not been established that dogs or wild canids are a definitive host for *N. hughesi*. Several studies indicate that *N. hughesi* can be transmitted transplacentally in horses (Reed et al., 2016). It is unclear if *Neospora hughesi* is a cause of EPM in Europe.

Isometamidium is a phenanthridine aromatic amidine with a narrow therapeutic index that has traditionally been used to treat *Trypanosoma* spp. infections (Reed and Saville, 1996). Use of isometamidium for the treatment of EPM is no longer listed according to a recent consensus statement on EPM (Reed at al., 2016). Pyrimethamine belongs to the group of antifolate drugs, and in addition to antiprotozoal effects, it exerts a strong proapoptotic activity (PubChem, 2024). Pyrimethamine is typically administered together with sulfonamides, that act synergistically by interfering with folic acid metabolism and biosynthesis of purine and pyrimidine nucleotides necessary for the parasite's survival. One of the pharmacokinetic characteristics is that steady-state CSF concentrations of pyrimethamine can be obtained after 4–6 hours after a single administration. Additionally, pyrimethamine is concentrated in CNS tissue relative to plasma. The success rate with the pyrimethamine-sulfonamide combination treatment is estimated to be 60% to 70% and the relapse rate to be 10% (Reed and Saville, 1996).

A recent consensus statement on EPM recommends that anticoccidial drugs should be used to control infection; diclazuril, ponazuril, or sulfadiazine/pyrimethamine combinations (among others) are listed as treatments of choice. Additional medical and supportive treatment should be provided based on the severity of neurologic deficits and complications arising from them (Reed et al., 2016).

Alternative treatments are available in Europe for food-producing animals. Both diclazuril and toltrazuril are available as marketed VMPs, and these are very similar molecules to ponazuril. Both are in Table 1 of the Annex to Commission Regulation (EU) No 37/2010: toltrazuril has MRLs established for all mammalian food-producing species, and diclazuril has a 'no MRL required' status for all ruminants and porcine. Diclazuril is currently recommended for the treatment of EPM (Reed et al., 2016), and toltrazuril had been previously recommended (Dirikolu et al., 2013). Also, the combination of sulfonamides and pyrimethamine is very similar to the combination of trimethoprim and

sulfonamides, which is authorised in several VMPs in Europe for use in food-producing horses. Horses with EPM treated with trimethoprim-sulfadiazine have reportedly had substantial improvements in neurologic signs, although according to results of one study, 2 of 3 treated horses had relapses after the medication was discontinued, suggesting incomplete removal of parasites from the CNS (MacKay, 1997). Nonsteroidal anti-inflammatory drugs such as flunixin meglumine (listed in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 and with VMPs authorised for food-producing equine species) are frequently given to moderately or severely affected horses during the first 3-7 days of antiprotozoal treatment and in an attempt to prevent worsening of neurologic deficits during the early antiprotozoal treatment. Corticosteroids (i.e. dexamethasone, also listed in Table 1) are indicated too.

Equine protozoal myeloencephalitis, if untreated, can be life-threatening due to severe neurologic abnormalities and causes unacceptable suffering of the animal.

Neither isometamidium nor pyrimethamine were mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any isometamidium-nor pyrimethamine-containing veterinary medicinal product authorised for use in equine species. There are pyrimethamine-containing veterinary medicinal products authorised for use in species other than the equine (e.g. hamster, rabbit, homing pigeon).

The substances isometamidium and pyrimethamine are not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and they do not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Ponazuril is a member of the benzeneacetonitrile group of compounds, as a broad-spectrum anticoccidial substance, related to the herbicide atrazine and thought to target the parasite's apicoplast organelle. In horses, pharmacokinetic studies have established that therapeutic steady-state concentrations of ponazuril are achieved by day 2 to 7 (Reed and Saville, 1996).

Ponazuril is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), for equine protozoal myelitis (*Sarcocystis neurona*) treatment. Isometamidium and pyrimethamine are the alternatives identified in the current list, and the specific advantages are as follows: different mode of action compared to other authorised substances, useful as alternative therapy when disease refractory to other treatments; reduced incidence of side effects (diarrhoea) compared to pyrimethamine/sulphonamide treatments; increased clinical efficacy compared to isometamidium and pyrimethamine.

As indicated above, alternative treatments are available in Europe for food-producing animals. Both diclazuril and toltrazuril are available with marketed VMPs, and these are very similar molecules to ponazuril. Both are in Table 1 of the Annex to Commission Regulation (EU) No 37/2010: toltrazuril has MRLs established for all mammalian food-producing species, and diclazuril has a 'no MRL required' status for all ruminants and porcine. Diclazuril is currently recommended for the treatment of EPM (Reed et al., 2016), and toltrazuril had been previously recommended (Dirikolu et al., 2013).

Equine protozoal myelitis, if untreated, can be life-threatening due to severe neurologic abnormalities and causes unacceptable suffering of the animal.

Ponazuril was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any ponazuril-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance ponazuril is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Knowledge regarding antiprotozoals in horses for this assessment was derived from textbooks, review articles, and retrospective studies. Clinical trials were available for all antiprotozoals for the treatment of EPM, including alternatives, except isometamidium.

iv. Antivirals

Acyclovir is a nucleoside analogue that inhibits the action of viral DNA polymerase and DNA replication of different herpesvirus. Due to the action of viral thymidine kinase, it is converted into acyclovir monophosphate, which in turn is converted to acyclovir diphosphate by guanylate kinase (King, 1988; O'Brien and Campoli-Richards, 1989). The latter is converted into acyclovir triphosphate by nucleoside diphosphate kinase, pyruvate kinase, creatine kinase, phosphoglycerate kinase, succinyl-CoA synthetase, phosphoenolpyruvate carboxykinase and adenylosuccinate synthetase (O'Brien and Campoli-Richards, 1989; Miller and Miller, 1982). Acyclovir triphosphate has a higher affinity for viral DNA polymerase than cellular DNA polymerase and incorporates into the DNA where the missing 2' and 3' carbons causes DNA chain termination (King, 1988). It also competes for viral DNA polymerase so that other bases cannot bind to it and thus inactivates this enzyme (King, 1988).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), for treatment of ocular ulcers (antiviral) and topical use. Idoxuridine is identified as its alternative and the specific advantage captured is that both, acyclovir and idoxuridine, have been shown to be equally effective in the treatment of ulcerative herpetic keratitis.

In equines, herpesvirus-2 (EHV-2) can be found; it is commonly associated with keratoconjunctivitis, causing punctate corneal lesions which may lead to viral keratitis and/or ocular ulceration (Brooks et al., 2000; Borchers et al., 2006). Even if its role as primary pathogen remains unclear, since e.g. EHV-2 can also be isolated from healthy eyes, herpes virus is the only known viral pathogen associated with certain eye diseases in Europe (Borchers et al., 2006). Thus, the substance merits consideration for the treatment of ocular ulcers; while idoxuridine and ganciclovir could constitute alternative treatments, acyclovir is generally the first-choice medication due to its efficacy in the treatment of ulcerative herpetic keratitis (Balderson et al., 2015). Gancyclovir is an alternative treatment proposed for addition to the list.

Ocular ulcers, if untreated, might cause unacceptable suffering of the animal.

Acyclovir was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any acyclovir-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance acyclovir is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: for the treatment of herpetic ocular ulcers. It is considered that the alternatives do not yield equally

satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Idoxuridine is a nucleoside analogue of deoxyuridine that inhibits viral DNA synthesis (Reuveni et al., 1991). It acts inhibiting viral replication by substituting itself for thymidine in the viral DNA. This in turn inhibits thymidylate phosphorylase and viral DNA polymerases from functioning properly and thus blocks the reproduction of the virus (Reuveni et al., 1991; Mills, 2017). Idoxuridine has also been described as less effective and potentially more toxic when compared to other drugs that are delivered topically, e.g. acyclovir (Balderson et al., 2015).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for treatment of ocular ulcers (antiviral) and topical use. In this case, acyclovir is identified as its alternative and the specific advantage captured is the same as indicated for acyclovir (equally effective in the treatment of ulcerative herpetic keratitis).

Indeed, idoxuridine was found to be less effective and potentially more toxic when compared to acyclovir in a meta-analysis conducted by Balderson et al. (2015). Data on its use in horses is scarce, probably also due to the fact that the substance seems not to be available, neither as human nor veterinary medicinal product. Alternatives proposed to be retained (acyclovir) or added (ganciclovir) to the list do yield better results.

Ocular ulcers, if untreated, might cause unacceptable suffering of the animal.

Idoxuridine was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any idoxuridine-containing veterinary medicinal product authorised for use in equine species. In fact, there seems to be no authorised veterinary or human medicinal product containing this substance in the EU (according to a search ran by ATC code in the MRI and VMRI product indexes[32]).

The substance idoxuridine is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Knowledge regarding antivirals in horses for this assessment was derived from textbooks and review articles.

B. Considerations regarding consumer safety

i. Antibiotics

Amikacin is an antibiotic belonging to the aminoglycoside group. There is relatively limited information available relevant for consumer safety assessment.

Amikacin, as other aminoglycosides, is poorly absorbed from the gastrointestinal tract, but are rapidly absorbed after intramuscular injection. Amikacin cannot be delivered orally probably due to efflux of drug by P-glycoprotein pump in the brush border of intestine (Jagannath et al., 1999; Scriba, 2009; El-Say et al., 2024). Elimination of aminoglycosides after parenteral administration occurs almost entirely

[32] Mutual Recognition Information product index (MRI, for human medicinal products) and Veterinary Mutual Recognition Information product index (VMRI, for veterinary medicinal products)

by glomerular filtration. In humans with normal renal function, elimination rates can be highly variable depending on the type of aminoglycoside. Patients with decreased renal function experience significantly prolonged half-lives of aminoglycosides (Plumb, 2015). However, early studies in humans had already indicated that amikacin seems to be less nephrotoxic compared to other aminoglycosides (i.e. gentamicin and tobramicin) from pathology data, where amikacin induced significantly lower lysosomal overloading and no loss of phospholipase A1 activity (De Broe et al., 1984).

In horses, a recent study investigated foals receiving 3 amikacin treatment protocols: (1) IV-only (25 mg/kg q24h IV), (2) concurrent IV and IA (16.7 mg/kg q24h IV and 8.3 mg/kg q24h into 1 tarsocrural joint), and (3) IA-only (8.3 mg/kg q24h into 1 tarsocrural joint) (Paegelow et al., 2024). In this study there were no signs of accumulation over the 3 days of treatment as the AUC values were essentially the same after the first, second and third dose (Paegelow et al., 2024).

Pinto et al. (2011) had already studied pharmacokinetics of amikacin in horse plasma and selected body fluids of healthy individuals after a single intravenous dose of 10 mg amikacin/kg bw. Some reported from this study are (mean±SD): extrapolated plasma concentration for the elimination phase 67.8±7.44 µg/ml, area under the curve 139±34.0 µg*h/ml, elimination half-life 1.34±0.408 h, total body clearance 1.25±0.281 ml/min/kg bw; and mean residence time (MRT) 1.81±0.561 h. At 24 h, the plasma concentration of amikacin for all horses was below the minimum detectable concentration for the assay; C_{max} in synovial and peritoneal fluid were 19.7±7.14 µg/ml and 21.4±4.39 µg/ml and time to maximum concentration 65±12.2 min and 115±12.2 min, respectively. Amikacin in the interstitial fluid reached a mean peak concentration of 12.7±5.34 µg/ml and after 24 h the mean concentration was 3.31±1.69 µg/ml.

Bucki et al. (2004) also reported pharmacokinetic values following once-daily 25 mg/kg amikacin (intravenous bolus) in healthy foals and hospitalized equine neonates. Median half-life, clearance, and volume of distribution of amikacin in healthy 2- to 3-day-old foals were 5.07 hours (4.86-5.45 hours), 1.82 mL/min/kg (1.35-1.97 mL/min/kg), and 0.785 L/kg (0.638-0.862 L/kg), respectively. Statistically significant (P <.05) decreases in area under the curve (14% decrease), mean residence time (19% decrease), and C24h plasma amikacin concentrations (29% decrease) occurred between days 2–3 and 10–11. Plasma amikacin concentrations in healthy foals at 0.5 hours (C05h) were significantly higher (P= .02) than those of hospitalized foals. Sepsis, prematurity, and hypoxemia did not alter amikacin concentrations. The proportion of foals with C05h >40 µg/mL was significantly higher (P <.0001) in hospitalized foals receiving a dose of amikacin at 25 mg/kg (22/24 or 92%) than in foals receiving a dose at 21 mg/kg (9/25 or 36%), whereas no difference was found in the proportion of foals with C24h concentrations ≥3 µg/mL between the 2 groups.

In an old study, Orsini et al. (1985) reported the t1/2 of amikacin was 1.44, 1.57 and 1.14 h for the 4.4, 6.6 and 11.0 mg/kg doses tested, respectively.

Plumb (2015) notes the substance is mainly distributed in the extracellular fluid and the bioavailability after extravascular injection (either intramuscular or subcutaneous) is greater than 90%. The approximate elimination half-lives for amikacin are reported to be 5 hours in foals and 1.14-2.3 hours in adult horses (Plumb, 2015).

With regards risks during pregnancy, in humans, the FDA categorizes amikacin as category C for use during pregnancy (Animal studies have shown an adverse effect on the foetus, but there are no adequate studies in humans; or there are no animal reproduction studies and no adequate studies in humans). Aminoglycosides are excreted in milk; amounts in milk are unlikely to be of significant concern after the first few days of life of the newborn. Amikacin is not listed in the IARC database.

No standard residue data are available for amikacin in horses. Orsini et al. (1996) studied and reported concentrations of amikacin in endometrial tissue and plasma in mares in oestrus after intrauterine infusion of 1.0 or 2.0 g once a day for 3 consecutive days, and after 9.7 or 14.5 mg/kg bw injected intramuscularly once a day for 3 consecutive days. In serum, no amikacin was detected at the infusion dose of 1.0 g dose; at the infusion dose of 2.0 g once a day, very low levels of serum amikacin were detected up to 4 h post-infusion. Following intramuscular administration, serum values were not detected at 24 hours with either dose. Endometrial tissue concentrations of amikacin are also reported. Following intrauterine infusion, amikacin concentration peaked at 1 h and remained at or above the MIC for 8 h at the 1.0 g dose (range, 1.85 to 36.43 µg/g) and for 24 h at the 2.0 g dose (range, 3.23 to 67.68 µg/g). Increasing the dose from 1.0 to 2.0 g resulted in proportionally increased levels of amikacin in the endometrium. At 24 hours values had decreased notably to a maximum detected value of 1.9±0.4 µg/g. Following intramuscular injection, endometrial concentrations peaked at 1 h and remained at or above the MICs for 8 h for both the 9.7 mg/kg bw dose (range, 1.73 to 21.75 µg/g) and the 14.5 mg/kg bw dose (range, 2.9 to 21.0 µg/g). At 24 hours amikacin values in endometrial tissue were not detected.

There's amikacin published residue data from species other than horses. Abdel Aziz et al. (2022) studied the depletion of amikacin residues in fresh, boiled and frozen rabbit's tissues using High Performance Liquid Chromatography (HPLC). Fifteen rabbits were injected with an intramuscular dose of 15 mg amikacin/kg bw for 7 successive days. Samples were obtained from kidneys, liver and breast muscle on the 1st, 3rd, 5th, 7th, and 10th day after the last dose. Results revealed that residues of amikacin in the fresh kidneys, liver and breast muscle were 14.2± 0.57 µg/g, 9.02± 0.45µg/g, and 8.23± 0.18 µg/g, respectively in the first day post-treatment. The residues' level had declined to 0.19 ± 0.02 µg/g in kidney and not detected in liver and breast muscle on the 10th day post treatment.

Aboubakr et al. (2016) reported the disposition kinetics and serum availability of amikacin in broiler chickens after single intravenous and intramuscular administrations of 10 mg/kg body weight. Amikacin was detected in liver and kidney for 5 days following a single intramuscular injection and not detectable from the 6th day onwards.

The disposition of residues of gentamicin and amikacin in rabbits had been previously studied by Kornguth and Kunin (1977). Rabbits were injected intramuscularly with 15 mg/kg gentamicin or amikacin, and the antibiotic levels in tissues were determined 20 h after either a single or multiple injections. Data showed that kidney was the major site of antibiotic deposition and that the drug levels increased after multiple injections. After a single injection, amikacin was found only in kidney and in urine; gentamicin concentrations in kidney and urine were higher than those of amikacin. After seven consecutive injections, the kidney levels of both antibiotics were not significantly different; however, again higher amounts of gentamicin were present in the other tissues. Amikacin at equal doses tended to accumulate less than gentamicin in kidney after a single injection, and in other tissues both after single and multiple injections.

No standard residue depletion study is available for horses. There is some short-term residue data available from three different species, even if in non-standard edible tissues, that could indicate that no residues are to be expected after six-months. The pharmacokinetic profile of amikacin suggests a poor absorption from the gastrointestinal tract, and in horses it also supports a fast elimination of the drug from the animal. Therefore, with the overall data available it can be accepted that the substance 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.

Azithromycin is a macrolide antibiotic, useful for treating *Rhodococcus* infections in foals. While azithromycin's pharmacokinetics support oral dosing in adult horses, concerns for potential antimicrobial-associated enterocolitis require further studies (Plumb, 2015; Papich, 2021).

Azithromycin is a broad-spectrum macrolide antibiotic with a long half-life and a high degree of tissue penetration. In humans, bioavailability of azithromycin is 37% following oral administration. Absorption is not affected by food. Macrolide absorption in the intestines is mediated by P-glycoprotein (ABCB1) efflux transporters, which are known to be encoded by the ABCB1 gene. Azithromycin is highly stable at a low pH, giving it a longer serum half-life and increasing its concentrations in tissues compared to erythromycin. Lung tissue has high concentrations following oral administration (Fohner et al., 2017).

The product information for human medicinal products reports a terminal plasma elimination half-life of 2 to 4 days. In elderly volunteers (more than 65 years old), higher AUC values (29%) were always observed after a 5-day course of treatment than in younger volunteers (less than 45 years). However, these differences are not considered to be clinically relevant. Approximately 12% of an intravenously administered dose is excreted unchanged in the urine over a period of 3 days; the major proportion in the first 24 hours. Concentrations of up to 237 µg/ml azithromycin, 2 days after a 5-day course of treatment, have been found in human bile, together with 10 metabolites formed by n- and o-demethylation, by hydroxylation of the desosamine and aglycone rings, and by splitting of the cladinose conjugate.

The pharmacokinetics of azithromycin have been described in cats and dogs. In dogs, the drug has excellent bioavailability after oral administration (97%). Tissue concentrations apparently do not mirror those in the serum after multiple doses, and tissue half-lives in the dogs may be up to 90 hours. Greater than 50% of an oral dose is excreted unchanged in the bile. In cats, oral bioavailability is 58%. Tissue half-lives are less than in dogs and range from 13 hours in adipose tissue to 72 hours in cardiac muscle. As with dogs, cats excrete the majority of a given dose in the bile.

In foals, azithromycin is variably absorbed after oral administration with a mean systemic bioavailability ranging from 40-60%. The effect of food on absorption, if any, is not clear. It has a very high volume of distribution of 11.6-18.6 l/kg. Elimination half-life is approximately 20-26 hours. The drug concentrates in bronchoalveolar cells and pulmonary epithelial fluid. Elimination half-life in polymorphonuclear neutrophils (PMNs) is about 2 days.

In adult horses after intragastric administration (tablets suspended in 500 ml of water), oral bioavailability averaged 45% with peak levels occurring about an hour after dosing. Plasma elimination half-life after intravenous dosing was approximately 18 hours. Plasma concentrations after a single intragastric dose remained above the MIC90 for 6-12 hours for beta-hemolytic streptococci, *Pasteurella* spp. and *Staphylococcus* spp. For these bacteria, intracellular concentrations of azithromycin in alveolar macrophages were above MIC90 for at least 48 hours and in neutrophils for at least 120 hours after a dose. When compared to erythromycin, azithromycin has better absorption characteristics, longer tissue half-lives, and higher concentrations in tissues and white blood cells. Azithromycin achieves high concentrations in bronchial secretions and has excellent ocular penetration (Leclere et al., 2012; Plumb, 2015; Papich, 2021).

The product information of human medicinal products states that there has been no evidence of a potential for genetic and chromosome mutations in in vivo and in vitro test models. No teratogenic effects were observed in embryotoxicity studies in rats after oral administration of azithromycin. In rats, azithromycin dosages of 100 and 200 mg/kg bw/day led to mild retardations in foetal ossification and in maternal weight gain. In peri- and postnatal studies in rats, mild retardations following

treatment with 50 mg/kg/day azithromycin and above were observed. Azithromycin is not listed by the IARC.

Considering the relatively quick elimination described in horses and the low toxicity reported for the substance, it can be accepted that azithromycin 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.

Ofloxacin is a fluoroquinolone antibiotic. Its active isomer, levofloxacin, is currently authorised as human medicinal product for topical administration both to the eye and ear.

In humans, the oral bioavailability is high, and maximum concentration is reached within 1 to 2 hours; the elimination half-life is also fast, around 6 to 7 hours (Fish and Chow, 1997). Its metabolite levofloxacin exhibits a rapid and wide tissue distribution including lung, skin, urinary tract, prostate and other soft tissues and body fluids (Langtry and Lamb, 1998). Approximately 80% of levofloxacin is eliminated as unchanged drug in the urine; minimal metabolism occurs with the formation of pharmacologically inactive metabolites (Fish and Chow, 1997).

In horses, kinetics of levofloxacin, are studied following intravenous and intramuscular administration (Goudah et al., 2008). Following intramuscular administration, the absorption is high with a bioavailability about 92%. The elimination is fast with elimination half-life of 3 hours. Neither kinetic nor residue data for ofloxacin/levofloxacin were found following ocular administration to horses.

The product information of human medicinal products suggests, from non-clinical data, that no special hazard for humans based on repeated dose toxicity, carcinogenicity, reproductive and developmental toxicity studies is to be expected. Levofloxacin did not affect fertility or reproductive performance in rats; foetal delayed maturation was observed as a result of maternal toxicity. There was no mutagenic induction in bacterial or mammalian cells, but it did induce chromosome aberrations in Chinese hamster lung cells in vitro; these effects can be attributed to inhibition of topoisomerase II. No genotoxic potential was shown in in vivo tests. In common with other fluoroquinolones, levofloxacin showed effects on cartilage in rats and dogs; these findings were more marked in young animals. Levofloxacin is not listed by IARC.

Considering the available data on its pharmacokinetics in humans and horses, and the apparent low toxicity, it can be accepted that ofloxacin 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.

Polymyxin B (as sulphate) belongs to the group of polypeptide antibiotics isolated from bacteria. It acts mainly against Gram-negative bacilli bacteria, e.g. infections with enterobacteria, and Pseudomonas, by binding to the phospholipid membrane and rupturing the bacterial cytoplasmic membrane. The application in horse is topically to the eye (ocular). Polymyxins are very soluble in water.

Regarding human pharmacokinetics, the data is scarce and primarily modelled data exists (Avedissian et al., 2019). The oral application of polymyxin B is known to result in practically no absorption of the substance (Avedissian et al., 2019). The plasma half-life of polymyxin B is about 6 hours in humans (intramuscular route) (VIDAL Compendium). Polymyxin B is not changed by metabolic processes (Zavascki et al., 2008; Avedissian et al., 2019). The plasma half-life in patients with normal renal function is 6 to 7 hours. Polymyxin B half-life is also stated to be approximately 9–11.5 hours in ill patients (Avedissian et al., 2019). Pharmacokinetic parameters of polymyxin B in critically ill patients were a volume of distribution of 71-194 ml/kg, a plasma protein binding of 78-90%, and a total body clearance of 0.27-0.81 ml/min/kg (Zavascki et al., 2008). These high values of volume of distribution and low body clearance reflect the high degree of tissue binding.

According to information from veterinary medicinal products, polymyxins are practically not absorbed after oral (~1%) or topical administration. Polymyxins are highly bound to plasma protein when administered intravenously (up to 75%). Polymyxin B binds also to negatively charged phospholipids in tissues due to its free amino acid groups. After repeated doses, polymyxin accumulates in tissues with four to five times higher concentrations than in serum at peak concentrations. After treatment it remains in tissues for at least five to seven days. Also, low recovery due to tissue binding is observed. Löscher et al. (2014) confirms that the tissue mobility is low. Polymyxin B is excreted renally mainly by glomerular filtration and the plasma half-lives of the polymyxins are 3 to 6 hours. The elimination half-life of an antibiotic belonging to the same class, colistin, is as well 4 to 6 hours for intravenous and intramuscular routes in the calf (Renard et al., 1991).

According to information from veterinary medicinal products, two oral sub-chronic toxicity tests according to OECD were conducted for rats and for beagles each at dose-levels of 3, 10 and 30 mg/kg bw/d. In rats a NOEL at 3 mg/kg/day was established. Higher doses induced slight signs of nephrotoxicity and had general negative effects on health. In beagles a NOEL was established at 10 mg/kg/day as at higher doses slight signs of nephrotoxicity and severe hypersalivation and other effects were observed.

No standard genotoxicity (or carcinogenicity) assays with polymyxin B are publicly available. One publicly available study (no information on GLP or guideline) investigated the molecular mechanisms underlying the nephrotoxicity in clinical use in humans (Yun et al., 2018, including erratum). They observed slightly increased formation of micronuclei and abnormal mitotic events in vitro in immortalized human proximal tubular cells as well as an increase γH2AX foci (indicative of double-stranded breaks) in vitro and in mice treated subcutaneously with polymyxin B. Due to the exposure route in the in vivo study and the nature of the substance, the relevance of these results with regard to consumer safety are questionable and are considered supporting information.

Also, from information in veterinary medicinal products it is known that no mutagenicity, fertility impairment or teratogenicity was found in studies carried out. For polymyxin B, in vitro and in vivo mutagenicity tests were conducted and polymyxin was considered as non-mutagenic in the bacterial reverse mutation test with Salmonella typhimurium. Also, polymyxin B did not induce chromosomal damage in an oral bone marrow micronucleus test in mice. In a study of embryo-foetal toxicity, fertility and post-natal development by oral administration in rats the conclusion was that 10 mg/kg was a NOEL for paternal/maternal toxicity and for peri- and post-natal development. In the 20/30 mg/kg/day group a slightly lower body weight gain was noted in male and female parents and a reduction in growth and a higher rate of mortality of pups. There were no effects on fertility and embryo-foetal development at any dose-level.

In humans, given the low absorption following topical application, polymyxin B is considered to have low side effects in infants. Polymyxins are not absorbed from the gastrointestinal tract following oral administration except in the neonates. At therapeutic doses, polymyxin B is regarded to be less nephrotoxic than colistin (Avedissian et al., 2019). Neurotoxicity is another side effect of polymyxin B. Polymyxin B side effects are reversible but persist for many days after treatment due to the strong tissue binding.

No publicly available residue depletion data were found for polymyxin B administrated in horses or in closely related species of the *Equidae* family or in other species. Tissue binding is known to persist for several days, but no data for longer periods has been collected. According to information from veterinary medicinal products, tissue distribution was examined after oral 10 mg/kg and intravenous 1 mg/kg radioactivity administration to rats and 7500 IU/mg radioactivity intravenous administration in calves. In the rats, blood sampling and finally dissection was conducted at 1, 2, 4 and 7 days after

treatment. After oral administration the absorption was below 1% and after intravenous administration the test substance distributed throughout the body and a slow elimination was observed. In the calves study the tissue concentrations were examined at 4, 8, 12, 24 and 48 hours after treatment. The concentration in muscle was a 15-20 times greater total amount than in kidneys and 3-8 times greater than in the liver. After 48h more than 30% of the intravenous dose was still in the tissues.

Considering the low systemic availability after ocular application, the short plasma elimination half-life when systemically available and the strong tissue binding so far not described persisting for more than several days on the one hand, and the low oral absorption in humans on the other hand, it can be accepted that ocular administered polymyxin B 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.

ii. Antifungals

Miconazole is an azole antifungal effective in the topical treatment of dermatophyte infections and superficial forms of candidiasis. It is currently authorized as human medicinal product for topical use.

In human the oral bioavailability is low (25- 30 %). About 90% of miconazole is bound to plasma protein. Miconazole is extensively metabolised, less than 1 % being excreted in the urine unchanged. The elimination half-life is long (20 – 25 hours) (Heel et al., 1980).

Data from the product information of human medicinal products suggest that plasma concentrations were below the limit of quantification in healthy volunteers following a single-dose application of 50 mg of miconazole to the buccal mucosa; measurable plasma concentrations ranged from 0.5 to 0.83 mcg/ml. Most of the absorbed miconazole is metabolised in the liver; there are no active metabolites. Miconazole is toxic when administered by intravenous route, and it is therefore limited to topical ophthalmic and dermatologic formulations (Colitz et al., 2007). When administered intravenously at individual doses ranging from 10 to 12 mg/kg bw, the short-term peak serum levels were 5 to 13 mg/l, declining to 2-5 mg/l in approximately 30 mins, and further decrease to 0.5 mg/l after 12 hours. Miconazole does not diffuse into the CSF to therapeutic concentrations (Plempel, 1979).

No kinetic data in horses was found.

The product information of human medicinal products suggests no specific hazard for humans based on repeated dose toxicity, genotoxicity, and reproductive toxicity studies. Teratogenic effects have not occurred in animal studies. Fetotoxicity at high oral doses has been observed; the relevance of this to humans is uncertain; caution should be given in pregnant women. Following topical administration, particular attention must be paid to the release of residue from the application site.

Miconazole is not listed by the IARC.

Considering the limited human oral bioavailability (25-30%), the extensive metabolism and the apparent low toxicity, it can be accepted that miconazole 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.

Nystatin is a polyene antibiotic clinically used as a topical, broad-spectrum antifungal drug. Nystatin is currently authorized in human and veterinary medicines for topical uses. The use of nystatin is limited to topical use due to systemic toxicity (Riviere and Papich, 2018).

Nystatin is not absorbed well from the gastrointestinal tract (Riviere and Papich, 2018). In fact, the product information of human medicinal products mentions negligible absorption from the gastrointestinal tract; no pharmacokinetic data is available. No animal studies have been performed to evaluate reproductive toxicity, carcinogenicity, mutagenicity or effects on fertility.

No kinetic data in humans and horses was found.

Despite the very limited data available, considering that nystatin is not absorbed by oral route, it can be accepted that it 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.

iii. Antiprotozoals

Not warranted since none of the above is considered essential.

iv. Antivirals

Acyclovir is a nucleoside analogue that inhibits the action of viral DNA polymerase and DNA replication of different herpesviruses. It is currently authorised as human medicinal products for oral, intravenous and topical administration both to the skin and eye.

Its pharmacokinetics are well described in the scientific literature (Gnann et al., 1983; Laskin, 1983; Wagstaff et al., 1994) which correspond to a two-compartment open model. Absorption of oral acyclovir in humans is slow and variable, with a bioavailability of 15 to 30% and mean peak plasma concentrations achieved between 1.5 to 2.5 hours post administration. The main metabolite, 9-carboxymethoxymethyl guanine, is pharmacologically inactive; a minor metabolite, 8-hydroxy-9-(2-hydroxyethoxymethyl) guanine, represents less than 0.2% of the given dose. The parent compound and its metabolites are excreted by the kidney, via glomerular filtration and tubular secretion. The elimination half-life in adults with normal renal function is 2 to 3 hours, extending to about 20 hours in patients with end-stage renal failure; the mean total clearance is 15.6 l/h/1.73 m^2. In infants aged ≥1 year, the pharmacokinetics of acyclovir are generally comparable with those of adults.

From metabolism studies in mice and rats, high-performance liquid chromatography showed that 94% and 95% of the urinary radioactivity corresponded to unchanged acyclovir. Minor urinary metabolites were identified as 9-carboxymethoxymethyl guanine and 8-hydroxy-9-(2-hydroxy-ethoxymethyl) guanine (Miranda et al., 1981). A study performed in rabbits showed elimination-phase half-lives (t ½ beta) for plasma acyclovir of 0.8 and 2.2 hours (Good and Miranda, 1982).

Acyclovir has undergone extensive preclinical testing for both in vivo and in vitro systems to determine its potential for toxicity. Data from these studies demonstrate little toxicity, although intravenous infusion of large doses have been described to cause crystallization in the renal tubules and rarely, tubular necrosis in humans (IARC, 2000). It is considered as not classifiable as to its carcinogenicity to humans (group 3 IARC).

Minimal systemic absorption following ophthalmic administration has been reported.

Considering the proposed use of the substance (i.e. topically applied to the eye), the pharmacokinetic characteristics described above and its low toxicity, it can be accepted that acyclovir 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.

4.3.3. Assessment of new substances proposed to be added to the list in the stakeholder's survey

A. Considerations on the essentiality of the substance(s)

i. Antibiotics

Ciprofloxacin was mentioned (eight times) in the survey to stakeholders and it was suggested for addition to the list for ophthalmic treatment. No specific indication was mentioned, and no scientific references were provided.

It is a second-generation fluoroquinolone with bactericidal and concentration-dependent activity against many gram-negative and gram-positive bacteria and is active in both stationery and growth phases of bacterial replication (Budde and McCluskey, 2023). Ciprofloxacin acts by targeting bacterial alpha subunits of DNA gyrase (Pietsch et al., 2017), which prevents bacterial DNA from supercoiling and thus DNA replication (LeBel, 1988). It binds to bacterial DNA gyrase with 100 times the affinity of mammalian DNA gyrase being the most potent gyrase inhibitor among the fluoroquinolones (Dowling et al., 1995; Varshney et al., 2014). As there is no cross resistance between fluoroquinolones and other classes of antibiotics it may be of clinical value when other antibiotics are no longer effective (Varshney et al., 2014). Due to its lipophilic properties, ciprofloxacin accumulates in phagocytic cells such as macrophages, polymorphonuclear leukocytes, or neutrophilic granulocytes, which are part of chronic inflammatory responses (DeManuelle et al., 1998). Its most important pharmacokinetic feature from a clinical perspective is the excellent penetration into almost all tissues and intercellular compartments (Grayson et al., 2018).

Ciprofloxacin has been described to successfully cure superficial or mid-stromal bacterial abscesses when applied for 1-4 weeks in horses (Barnett et al., 2004). In 2 horses, eye infections were treated with ciprofloxacin in combination with systemic antibiotic administration which decreased the severity of eye infection (Mishra et al., 2015). It has a good activity against many gram-negative bacilli and cocci, including most species and strains of *Pseudomonas aeruginosa, Klebsiella spp., E. coli, Enterobacter, Campylobacter, Shigella, Salmonella, Aeromonas, Haemophilus, Proteus, Yersinia, Serratia*, and *Vibrio spp.* (Budde and McCluskey, 2023). In humans, topical ciprofloxacin achieves reasonable penetration into corneal tissue (Price et al., 1995). Ofloxacin and moxifloxacin, which are recommended for the list of essential substances, constitutes an alternative treatment for bacterial eye infection in equine species. Ofloxacin provides enhanced clinical experience compared to other antibiotic drugs and has proven to penetrate the entire cornea up to the anterior chamber of the eye. Ciprofloxacin is not considered to bring added clinical benefit compared to ofloxacin.

Bacterial eye infections, if untreated, can cause unacceptable suffering of the animal (i.e. permanent eye damage) and be life-threatening in severe cases.

A search in the veterinary medicines database does not retrieve any ciprofloxacin-containing veterinary medicinal products authorised for use in equine species.

The substance ciprofloxacin is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indication in animals of the equine species; it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Clarithromycin was mentioned (eight times) in the survey to stakeholders and it was suggested for addition to the list for treatment of *Rhodococcus equi* infections in foals. It was stated that clarithromycin is the drug of choice for treating *R. equi* infections in foals when used in combination with rifampin. It has better efficacy and fewer side effects compared to other macrolides. It was further stated that erythromycin and azithromycin are alternative treatment options. The proposed indication and further statements were supported by published peer-reviewed references (Giguère et al., 2004; Muscatello et al., 2007; Giguère, 2017; Erol et al., 2021).

Clarithromycin is a 14-member ring macrolide antibiotic. The 14-member ring group contains compounds of natural origin (erythromycin, oleandomycin) and semisynthetic derivatives (clarithromycin, roxithromycin, dirithromycin). Clarithromycin, a 6-o-methyl derivative of erythromycin is approximately twice as active as erythromycin against bacteria on a weight basis, with a half-life about twice as that of erythromycin. Macrolides inhibit protein synthesis by reversibly binding to 50S subunits of the ribosome. They inhibit the transpeptidation and translocation process, causing premature detachment of incomplete polypeptide chains. Macrolides are generally bacteriostatic drugs, but they may be bactericidal at high concentrations and against a low inoculum of some highly susceptible bacteria.

Rhodococcus equi infections in foals are commonly characterised as a multifocal pyogranulomatous pneumonia with abscess formation; extrapulmonary infections are also common (Yamshchikov et al., 2010; Giguère et al., 2011a). *Rhodococcus* foals are typically infected from birth (Chaffin et al., 2003; Cohen et al., 2013), whereby the insidious onset of bronchopneumonia does not result in the onset of clinical signs until typically between 1 to 5 months of age (Cohen et al., 2013). Control of foal rhodococcosis is challenging due to the lack of an effective vaccine and relies on antimicrobial therapy and other control measures. The ability of *R. equi* to survive and replicate in macrophages is the basis for its pathogenicity. Based on thoracic ultrasound, foals with rhodococcal pneumonia can be characterised into three groups: subclinical rhodococcal pneumonia with most common type peripheral pulmonary consolidation or abscessation without manifesting clinical signs; mild-to-moderate clinical signs of rhodococcal pneumonia in which less than 50% of lung is involved and no evidence of other body sites infected; severe rhodococcal pneumonia in which more than 50% of lung is involved and respiratory distress and other body sites could be infected.

Although many antimicrobials are active in vitro against *R. equi*, this in vitro activity does not correlate with in vivo antimicrobial efficacy since antimicrobials must penetrate three sites to above minimum inhibitory concentrations (MIC) for *R. equi*: (1) lungs; (2) the encapsulated pyogranulomatous abscess/es; (3) the intracellular location of *R. equi* within alveolar macrophages. Treatments for *R. equi* infections are prolonged, with several weeks to months required to achieve complete recovery (Giguère et al., 2011b; Giguère et al., 2012).

R. equi infections in foals can also be treated with other macrolide antibiotics i.e. azithromycin, erythromycin, gamithromycin, tulathromycin, or the tetracycline doxycycline. Erythromycin and doxycycline are included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with MRL entries for all food producing species, including *Equidae.* Gamithromycin and tulathromycin are also included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with MRL entries for food producing species other than *Equidae.* Furthermore, azithromycin is retained in the list of essential substances. All the above-mentioned substances constitute as satisfactory alternatives. These alternatives can also be used as monotherapy for *R. equi*. Hyperimmune plasma can be used as a preventative (Kahn et al., 2023) and gallium maltolate, a semimetal compound with antimicrobial activity, has also been described as an alternative treatment for *R. equi* in foals (Cohen et al., 2015). It is considered that clarithromycin brings added clinical benefit in the treatment of *Rhodococcus equi* infections.

Historically, combination treatment with erythromycin, or other macrolide, and rifampicin has been the standard treatment for *R. equi* in foals. Clarithromycin is more active against *R. equi* in vitro than erythromycin or azithromycin (Jacks et al., 2003). In addition, in a retrospective study of examined and treated foals, the combination of clarithromycin and rifampin was found to be better compared to the combination of azithromycin and rifampin or erythromycin and rifampin for the treatment of foals

with pneumonia caused by *R. equi* (Giguére et al., 2004). No randomized clinical trials have assessed clarithromycin for *R. equi* in foals.

However, when clarithromycin is administered concurrently with rifampicin, both in oral dosage regimens, the oral bioavailability of clarithromycin was decreased by up to 90% to plasma concentrations below the MIC of *R. equi* (Peters et al., 2011; Peters et al., 2012). The decrease in bioavailability of clarithromycin was primarily due to rifampicin induction of intestinal P-glycoprotein as well as a minor reduction due to an unknown modulation in clarithromycin uptake (Berlin et al., 2016).

Clarithromycin, as monotherapy, has an elimination half-life of less than 6 hours, a clearance of 1.2 l/h/kg, and a high volume of distribution of approximately 10 l/kg, indicating that it accumulates in certain tissues or organs. Oral bioavailability is close to 60%. In foals, clarithromycin achieves considerably greater concentrations in pulmonary epithelial lining fluid and alveolar macrophages than either erythromycin or azithromycin. However, the half-life of clarithromycin at these sites is much shorter than that of azithromycin (Suarez-Mier et al., 2007). Clarithromycin is categorized as a highly important critical antimicrobial for human health by the World Health Organization (WHO).

Rhodococcus equi infections, if untreated, may only 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.

A search in the veterinary medicines database does not retrieve any clarithromycin-containing veterinary medicinal product authorised for use in equine species. There are veterinary medicinal products authorised for use in species other than the equine (i.e. dog).

The substance clarithromycin is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: to treat *Rhodococcus equi* infections. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Fusidic acid was mentioned (ten times) in the survey to stakeholders and it was suggested for addition to the list. It is cited as commonly used for infection of the eye/conjunctivitis as a first-line treatment. No scientific references were provided.

Fusidic acid, also known as *sodium fusidate,* is an amphoteric and lipophilic topical antimicrobial isolated from the fermentation broth of *Fusidium coccineum* and *Mucor ramannianus* (Vanderhaeghe et al., 1965; O'Neil et al., 2001; Dowling, 2006). Owing to its resemblance to prednisolone, fusidic acid is said to belong to its own class of antibiotics, the steroid antibiotics (Grayson et al., 2018). It is mainly bacteriostatic (Bobadilla-González et al., 2009), but also bactericidal at higher concentrations (Saijonmaa-Koulumies et al., 1998). The mechanism of action of fusidic acid is to interfere with bacterial protein synthesis, specifically by preventing the translocation of the elongation factor G (EF-G) from the ribosome (Vardanyan and Hruby, 2016). Binding of fusidic acid to the elongation factor G stabilizes the ribosome-G-GDP complex thereby inhibiting binding of aminoacyl t-RNA to the A site of the ribosome (Dowling, 2006). This way, chain elongation is prevented bringing bacterial growth to a halt (Chopra, 1976). Optimum efficiency is at a pH of 6.0 (Stahlmann and Lode, 2005).

The most significant feature of fusidic acid is its high degree of activity against *Staphylococcus aureus*, including beta-lactamase producing and methicillin-resistant strains (Grayson et al., 2018). Gram-negative bacteria are usually resistant (Grayson et al., 2018). Among topical antibiotics, fusidic acid along with fluoroquinolones have the best intra-corneal and intra-cameral penetration (Robert and Tassy, 2000). Fusidic acid has been described to be the primary choice in superficial, uncomplicated

corneal ulcers and acute conjunctivitis in horses (Medicine, Agricultural and Food Sciences, 2019). While alternatives against Gram-positive topical eye infections are recommended for the list (i.e. ofloxacin and moxifloxacin), fusidic acid brings added clinical benefit compared to them.

Bacterial eye infections, if untreated, can cause unacceptable suffering of the animal (i.e. permanent damage to the eye).

A search in the veterinary medicines database does not retrieve any fusidic acid-containing veterinary medicinal product authorised for use in equine species. There are veterinary medicinal products authorised for use in species other than the equine (i.e. dog, cat, rabbit).

The substance fusidic acid is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: topical treatment of eye infections caused by gram-positive bacteria susceptible to fusidic acid. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Moxifloxacin was mentioned (once) in the survey to stakeholders and it was suggested for addition to the list for treatment of septic keratitis in cases in which ofloxacin is inadequate or not available. No scientific references were provided.

It is a fourth-generation fluoroquinolone and acts as a gyrase inhibitor. It is administered either orally as a tablet, via eye drops, or intravenously in the form of an infusion or syringe. It has strong activity against gram-positive, gram-negative, atypical, and anaerobic bacteria. Consequently, the drug is used in a wide spectrum of infections. The substance has an advantageous pharmacological profile including high bioavailability of more than 90%, large volume of distribution, long half-life (6–12 hours), and no dose adjustment required for kidney disease or moderate hepatic impairment (Campbell, 2022). Moxifloxacin blocks the rapid-component delayed-rectifier potassium channel in the heart, and thus prolongs the QTc interval by 6 minutes after oral administration and 12 minutes after intravenous administration. Moxifloxacin carries a greater risk of QT interval prolongation than ciprofloxacin, levofloxacin, and ofloxacin. It appears to have a low propensity for causing phototoxic and central nervous system excitatory effects (Scholar, 2008).

Moxifloxacin has been used in small animals for treatment of infections refractory to other drugs, including skin infections, pneumonia, and soft tissue infections. The spectrum of activity includes gram-positive cocci and anaerobic bacteria that may be resistant to other quinolones (Papich, 2016). Moxifloxacin exhibited favorable pharmacokinetic parameters in the horse with rapid absorption, a high peak serum concentration, large AUC, long elimination half-life, and persistence of high intracellular concentrations in alveolar cells (Gardner et al., 2004).

Studies have shown that moxifloxacin can reach therapeutic concentrations in tears and corneal tissue and intact epithelium in horses (Westermeyer et al., 2011). Moxifloxacin was better able to penetrate healthy equine corneas and reach measurable aqueous humor concentrations than was ciprofloxacin, indicating a greater value in the treatment of deep corneal or intraocular bacterial infections caused by susceptible organisms (Clode et al., 2010). Compared to other fluoroquinolones, moxifloxacin might be more effective against bacteria that have developed resistance to this class of antibiotics. It possibly provides additional benefits to horses for topical treatment of external eye infections caused by gram-positive cocci, gram-negative, atypical and anaerobic bacteria such as *P. aeruginosa* species.

Bacterial eye infections, if untreated, can cause unacceptable suffering of the animal (i.e. permanent damage to the eye).

A search in the veterinary medicines database does not retrieve any moxifloxacin-containing veterinary medicinal product authorised for use in equine species.

The substance moxifloxacin is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: topical treatment of external eye infections caused by gram-positive cocci, gram-negative, atypical and anaerobic bacteria such as *P. aeruginosa* species. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal or ensuring the safety of those treating the animal.

Rifamycin sodium was mentioned (fourteen times) in the survey to stakeholders and it was suggested for addition to the list for local treatment in bacterial eye infections, bacterial keratitis and corneal ulcers. Specific advantage stated is that it allows for local treatment of specific bacterial eye infections, (e.g. gram-negative infections) for topical use, allowing to reduce doses required for systemic treatment and help combat antibiotic resistance. No scientific references were provided.

It is part of the rifamycin group of antimicrobials and further part of the ansamycin macrocyclic antibiotic class. Rifamycin sodium is described in the Ph.Eur. monograph 0432 and corresponds to the sodium salt of rifamycin SV, obtained through a chemical transformation of rifamycin B. There are actually seven molecules known as rifamycins, each characterized by different letters based on their chemical structure: rifamycin A, B, C, D, E, S, and SV, all isolated from the fermentation culture of the bacterium *A. mediterranei*. For example, rifampicin is a semi-synthetic compound derived from rifamycin B.

Rifamycin enters bacteria and forms stable complexes with the β-subunit of bacterial DNA-dependent RNA polymerase, while not affecting mammalian polymerase. This binding results in inactivation of the enzymes and inhibition of RNA synthesis by preventing chain initiation (Papich and Riviere, 2009). Rifamycin is expected to be a broad-spectrum, concentration or time-dependent, bactericidal and/or bacteriostatic antibiotic, with activity against gram-positive and facultative anaerobic organisms. The dosage of rifamycin as eye drops is 2 drops 4 times daily as a solution containing 100,000 IU/10 ml, i.e. 11.27 mg/ml. If 2 drops are equivalent to 0.1 ml, the corresponding amount is 1.13 mg, 4 times daily, i.e. 4.52 mg/600kg/day, or 7.5 µg/kg. Rifamycins are World Health Organisation (WHO) critically important antimicrobials (CIAs) in human medicine for the first-line treatment of mycobacterial infections (tuberculosis, leprosy and mycobacterium avium complex (MAC)). All rifamycins are further listed on the WHO AWaRe Watch group.

Topical treatments are generally considered less likely to select for antimicrobial-resistant mutants compared to systemic treatments; it is noted that no rifamycin systemic formulation is available. Well established experiences with other antibiotics of the Rifamycin class (e.g. rifampicin) has shown that rifamycin resistance involves mutations in a limited number of highly conserved amino acids of the RNA polymerase β-subunit (RNAP) encoded by the chromosomal rpoB gene, thereby reducing binding affinity and dictating the level of antimicrobial resistance (Fines et al., 2001). Rifamycin resistance arises quickly and easily, even within one treatment period, typically from single-point substitutions. Thus, it is always considered prudent use to always use rifamycins in combination with a synergistic antimicrobial to limit the development of antimicrobial resistance. Rifampicin-resistant *R. equi* are well known in equine medicine, but other resistance monitoring in horses is not part of any EU surveillance programs.

No specific need for rifamycin sodium has been identified in equine ophthalmology. Multi-resistant bacterial pathogens involved in equine eye infections have not been identified, whereby alternative antimicrobials available are still effective (Foote et al., 2023). Alternatives for topical treatment of

bacterial eye infections are recommended for the list, including fusidic acid, moxifloxacin and ofloxacin. Rifamycin is not considered to bring added clinical benefit in the treatment of bacterial eye infections compared these alternatives.

Bacterial eye infections, if untreated, can cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any rifamycin sodium -containing veterinary medicinal product authorised for use in equine species. Topical eye formulation containing rifamycin as a monosubstance is authorised as human medicinal product.

The substance rifamycin sodium is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indication in animals of the equine species; it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Tenoic acid (or thenoic acid) was mentioned (sixteen times) in the survey to stakeholders and it was suggested for addition to the list as injectable antiseptic and antimicrobial adjuvant for systemic use in pulmonary infections. The clinical benefit stated is that it is the only known product being antiseptic, expectorant and trophic working in synergy or even increasing the activity of the antibiotic in respiratory treatment.

In terms of organic chemistry, in the scientific literature thenoic acid is defined as either of two isomeric crystalline acids C4H3SCOOH made from thiophene, also known as thiophene-carboxylic acid. An opinion from the French Agency for Food, Environmental and Occupational Health and Safety (ANSES) defines thenoic acid as a lithium salt, known as lithium thenoate (derivative of thiophene). This substance is described to have a stimulant effect on the cells of the pulmonary lining, producing mucus, and on the cells of the nasal and bronchial mucous membranes. It has an antiseptic effect through direct antimicrobial activity and through mechanical clearing with an increase in natural secretions. Therefore, it is assumed that thenoic acid/tenoic acid from the survey refers to lithium thenoate.

The only scientific reference provided by the responders is the study by Scicluna et al. (2013). This is a conference abstract describing a clinical study in 15 adult horses (5.9 ± 3.77 years old) examined for respiratory problems, including blood samples (cell count), respiratory endoscopy (pharynx and trachea secretions scores 0-3) and tracheal wash (TW). Horses were divided into 3 groups, L=lithium thenoate (initial dose of 3.8 g followed by 2.9 g), AB=antibiotic (gentamicin 6.6 mg/kg), ABL=lithium thenoate and gentamicin) and treated intravenously for 3 days before being re-examined. Secretions scores decreased in pharynx (-0,4) and trachea (-0,8) in ABL group. TW analysis showed a decrease in total cells population with ABL (-25%), in neutrophils with L and ABL (-30 and -35%), in macrophages with L (-80%) and in epithelial cells with ABL (-50%). Bacteria counts decreased in L, AB, ABL groups (- 60%, -25%, -80%). Less fungal elements were found after L treatment. The author concludes an antiseptic and mucolytic effect of lithium theonate in horses with respiratory disease, and a synergic/potentiation action with gentamicin when used simultaneously.

There is no supporting full publication of these results in a peer-reviewed scientific journal, and a literature search could not reveal any other supporting publications. There is not enough detail from this conference abstract to conclude on the clinical effect(s) of lithium thenoate. For example, it is unclear as to which respiratory disease was diagnosed, white blood cell (WBC) counts on TW is not normally performed in lieu of broncho-alveolar cell counts, it is unclear as to which bacteria were identified and how bacterial counts were performed. Therefore, the proposed clinical effect(s) of lithium thenoate i.e. antiseptic, expectorant, trophic, antimicrobial adjuvant/synergy have not been

supported/confirmed from the peer-reviewed scientific literature. Alternatives for horses to support lower airway disease (e.g. expectorant, decreased mucus, bronchodilation) include dembrexine and clenbuterol both of which are included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with 'No MRL required' and MRL entries for *Equidae,* respectively, thus qualifying as satisfactory alternatives for which there are veterinary medicinal products authorised for food-producing animals of the equine species. In addition, ambroxol is recommended for the list as a surfactant stimulant.

Pulmonary infections, if untreated, may be life-threatening in their severe form. It can cause unacceptable suffering of the animal.

The substance thenoic acid/tenoic acid is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indication in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Tobramycin was mentioned (two times) in the survey to stakeholders and it was suggested for addition to the list for treatment of severe septic keratitis, when indicated by sensitivity testing. No scientific references were provided.

Tobramycin belongs to the aminoglycoside group. It has a bactericidal effect by inhibiting bacterial protein biosynthesis. It binds to the 30S subunit of the ribosomes and thus prevents initiation. It is a broad-spectrum antibiotic, particularly effective against aerobic, gram-negative pathogens such as Pseudomonas aeruginosa and Staphylococcus spp. Tobramycin is hardly absorbed after oral administration. A possible benefit of oral administration is only for intestinal decontamination. Good bioavailability is achieved with parenteral or local application; intrathecal administration is also possible. Tobramycin is not biotransformed and is predominantly eliminated renally with an elimination half-life of about 2 to 3 hours.

In equine medicine, tobramycin is used for the treatment of severe septic keratitis, bacterial ulcerative keratitis and septic ocular surface diseases (Keller and Hendrix, 2005; Scotty et al., 2008; Czerwinski et al., 2012; Leigue et al., 2016; Vercruysse et al., 2022) when indicated by sensitivity testing. It is further used in the treatment of synovial or bone infections via intra-articular or regional limb perfusion (Taintor et al., 2006; Rubio-Martinez et al., 2012; Newman et al., 2013).

From this group of substances, gentamicin and neomycin are included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with MRL entries for all mammalian food-producing species, and there are veterinary medicinal products as eye formulation containing these substances. Kanamycin is also included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with MRL entries for all food-producing species except fin fish. Therefore, all three substances qualify as satisfactory alternatives. Tobramycin is not considered to bring added clinical benefit compared to these. Alternatives are retained in the list for the treatment of bacterial eye infections.

Bacterial eye infections, if untreated, can cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any tobramycin-containing veterinary medicinal product authorised for use in equine species. There are veterinary medicinal products authorised for use in species other than the equine (i.e. dog, cat).

The substance tobramycin is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indication in animals of the equine species; satisfactory alternative

treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Knowledge regarding antibiotics in horses/foals for this assessment was derived from textbooks, review articles, retrospective studies and clinical trials; clinical trials were available for all indications for treatment.

ii. Antifungals

Amphotericin B was mentioned (once) in the survey to stakeholders and it was suggested for addition to the list for the treatment of severe fungal keratitis, used as subconjunctival injections or topically. No references were provided in support of this proposal.

Amphotericin B is a broad-spectrum, polyene antifungal agent. Amphotericin B was the mainstay of systemic antifungal treatment for many years, until the authorization of the azole antifungal drugs. Amphotericin B is still the drug of choice for systemic treatment of filamentous fungal or dimorphic fungal infections, due to its fungicidal activity. Lipid formulations of amphotericin B are generally used to reduce nephrotoxicity and include a liposomal preparation and a lipid complex. Amphotericin B usefulness is limited by its toxicity and inability to distribute to many tissues; however, penetration into CSF is increased in the presence of meningitis and with use of the liposomal formulation.

Polyene's antifungals mechanism of action is to bind with ergosterol within the fungal cell membrane, increasing its permeability and causing leakage of potassium ions and essential small organic molecules from the fungal cell. Also, the oxidation of amphotericin B destabilizes the fungal membrane by generating free radicals. This induces oxidative stress, causing fungal cell death. Amphotericin B binds cholesterol in mammalian cell membranes less avidly, but this still makes it the most toxic of the clinically useful systemic antifungal drugs.

Isolates with an MIC ≤1 µg/ml are considered susceptible. Intrinsic resistance to amphotericin B is common in Aspergillus spp., Scedosporium spp., *Trichosporon*, *Candida auris*, and *C. lusitaniae* (Mandell et al., 2019). The underlying mechanism is not known (Posch et al., 2018). Dermatophytes and strains of *Pseudoallescheria boydii* are often intrinsically resistant to amphotericin B. Acquired resistance can occur due to reductions of ergosterol content of the fungal cell membrane, resulting from mutations in ergosterol biosynthesis genes (ERG) or increased production of reactive-oxidant scavengers (Jensen et al., 2015).

Amphotericin B is poorly absorbed orally (<5%), so parenteral (intravenous) administration is required. Its distribution is limited to extracellular fluid compartments. The metabolic pathways are unknown but it exhibits biphasic elimination. The drug is thought to bind to plasma or cellular lipoproteins and is released slowly from these sites. Approximately 5% of the injected dose is excreted by the kidneys and continues to be excreted in the urine of humans for several weeks after cessation of therapy. Systemic absorption from the lungs following aerosol administration is poor so this route has been used successfully for the treatment of pulmonary aspergillosis. Experiences from human medicine reveal that the pharmacokinetics of amphotericin B differ according to the formulation. Peak plasma concentrations after administration of the liposomal formulation are higher than those achieved with conventional amphotericin B. In contrast, peak plasma concentrations after administration of the lipid complex or colloidal formulations are lower due to more rapid distribution of the drug to tissues.

The lipid complexes, but not the liposomal or conventional amphotericin B, are concentrated and accumulate within lung tissue (Matot and Pizov, 2000).

Renal toxicity inevitably accompanies treatment with micellar (conventional) amphotericin B. Toxicity is attributed to renal vasoconstriction and subsequent reduction in glomerular filtration rate. The drug may also have a direct toxic effect. Early reversible nephrotoxicosis occurs with each daily dose, but permanent nephrotoxicosis is related to the total cumulative dose. Other adverse effects include thrombophlebitis at the injection site and hypokalemia, with resulting cardiac arrhythmias, sweating, nausea, malaise, and depression.

For horses, amphotericin B is not suitable for the local treatment of mycotic keratitis because of its poor activity against some filamentous fungi and its locally irritating properties. There are several reports of intralesional or systemic use of amphotericin B in horses. A wide range of dosages and administration protocols have been used for systemic administration. Successful treatment of pulmonary cryptococcosis was reported with daily infusions of amphotericin B at 0.5 mg/kg for a month. Administration of amphotericin B via intravenous regional limb perfusion was effective for treating pythiosis of the distal limbs in horses (Dória et al., 2012), caused by *Hyphomyces destruens* (aka. phycomycosis, swamp cancer, Florida horse leeches, Gulf Coast fungus).

Fungal pneumonia, if untreated, could be life-threatening and causes unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any amphotericin-B-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance amphotericin B is proposed to be qualified as essential because no satisfactory alternative treatments are authorised for food-producing animals of the equine species for the following indication: for the systemic treatment of fungal pneumonia.

Fluconazole was mentioned (once) in the survey to stakeholders and it was suggested for addition to the list for the systemic treatment of fungal keratitis when voriconazole is not available. No references were provided in support of this proposal, though it was indicated that several studies regarding pharmacokinetics are available.

Fluconazole is a triazole antifungal and a specific inhibitor of the fungal enzyme, lanosterol 14-alpha-demthylase. This inhibition prevents the conversion of fungal cell lanosterol to the membrane lipid ergosterol. Fluconazole possesses the narrowest spectrum of activity of all the azole antifungals available for systemic use and it is generally considered to be a fungistatic agent.

Fluconazole is available in both oral and intravenous formulations although it is used almost exclusively orally in veterinary medicine. In animals, fluconazole is the drug of choice for cryptococcal infections, for local, or systemic treatment of candidal infections, and for the treatment of coccidiosis. It is active against most Candida spp., as well as dimorphic fungi; it is ineffective against Aspergillus and Fusarium spp. Organisms with MIC ≤8 µg/ml are regarded as susceptible, with MIC 16-32 µg/ml as intermediate, and ≥64 µg/ml resistant. *Pichia kudriavzeveii* (*Candida krusei*) is intrinsically resistant to fluconazole and many Nakaseomyces (Candida) glabrata isolates may exhibit resistance. In contrast to other azoles, fluconazole is water soluble and weakly protein bound. Oral absorption is unaffected by gastric pH. Therefore, it is well absorbed after oral administration and because of its low molecular weight, water solubility, and lack of protein binding, distributes widely to tissues. Its ability to reach high concentrations (50–90% of serum) within CSF is a particular advantage for treating yeast (e.g. Cryptococcus) meningitis. It is excreted unchanged in the urine. Fluconazole's lack of significant hepatic metabolism allows for linear elimination kinetics. Plasma elimination half-life in horses is 40

hours (Latimer et al., 2001). Oral bioavailability is 100% for horses. Systemic treatment of fungal keratitis would not be the preferred clinical approach, nor would fluconazole be the drug of choice. Alternatives are proposed for inclusion in the list for the treatment of fungal keratitis, i.e. miconazole and voriconazole, which are considered better alternatives. In case systemic treatment is required, amphotericin B would be the treatment alternative.

Fungal keratitis, if untreated, generally does not cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any fluconazole-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance fluconazole is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Voriconazole was mentioned (three times) in the survey to stakeholders and it was suggested for addition to the list for the topical and systemic treatment of fungal keratitis. The responders indicated it has better spectrum of action, absorption, and penetration than fluconazole, and that no alternatives are available. A reference was provided (Mora-Pereira et al., 2020).

Voriconazole is a triazole antifungal structurally related to fluconazole. Voriconazole is active against a wide spectrum of medically important fungi, including dermatophytes, opportunistic yeasts (Candida spp., *Cryptococcus neoformans*), opportunistic filamentous fungi (Aspergillus spp., Fusarium spp.), and dimorphic fungi. Isolates with an MIC ≤1 µg/ml are considered susceptible. In contrast to fluconazole, voriconazole is active against most *Pichia kudriavzeveii* (*Candida krusei*) and most *Nakaseomyces* (Candida) *glabrata* isolates. Voriconazole exerts time-dependent fungicidal activity against Aspergillus spp., in vitro.

Voriconazole is available as oral and intravenous formulations. It is extensively metabolized by the liver and, unlike fluconazole and amphotericin B, does not depend on renal function for excretion. Unlike itraconazole, voriconazole is not dependent on gastric acid for absorption and the drug is entirely absorbed in horses after oral administration (Davis et al., 2006; Colitz et al., 2007). Voriconazole has excellent tissue penetration and distributes widely into body fluids (Passler et al., 2010). A pharmacokinetic study in horses looked at 10 mg/kg IV and PO. Volume of distribution was 1.6 l/kg and oral bioavailability was 95%, with tissue concentrations approximately 50% of plasma concentrations. Adverse effects and drug interactions are similar to those reported with other triazoles. Experience with the use of voriconazole in domestic animal species is limited.

In both people and horses, voriconazole has become the first choice of treatment for keratomycosis due to its broad spectrum of activity, good corneal penetration, and a low minimum inhibitory concentration (MIC) for common fungi implicated in this disease, such as Aspergillus spp., and Fusarium spp., when compared to other azole drugs (Mora-Pereira et al., 2020). In the ranges of MICs for filamentous and yeast organisms, most isolates are < 0.5 µg/mL, therefore antifungal therapies are expected to have MICs of unbound drug above this value to be considered of clinical efficacy. It is thus supported that voriconazole offers a clinical advantage since it can be used topically and systemically for fungal keratitis, with better spectrum of action, absorption, and penetration than other azole drugs (e.g. miconazole, proposed for inclusion in the list).

Fungal keratitis, if untreated, may cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any voriconazole-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance voriconazole is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: treatment of fungal keratitis, topical and systemic use. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Knowledge regarding antifungals in horses for this assessment was derived from textbooks, review articles, and retrospective studies. No clinical trials could be identified for antifungal use in horses.

iii. Antiprotozoals

Buparvaquone was mentioned (twice) in the survey to stakeholders and it was suggested for addition to the list for the treatment of equine piroplasmosis. It is presented as an alternative treatment for equine piroplasmosis caused by sensitive *T. equi* isolates in horses that do not respond adequately to imidocarb, or for the treatment of persistent cases that do not respond to other chemotherapeutic options.

Buparvaquone is a hydroxynaphthoquinone antiprotozoal drug related to atovaquone and parvaquone. Hydroxynaphthoquinones are thought to selectively block protozoan mitochondrial electron transport, resulting in inhibition of pyrimidine and ATP synthesis (Wise et al., 2013). Buparvaquone resistance appears to be associated with parasite mutations at the mitochondrial cytochrome b quinone Q binding site.

Equine piroplasmosis is a tick-borne protozoal disease of horses, mules, donkeys and zebra (Wise et at., 2013). The aetiological agents are blood parasites named *Theileria equi* and *Babesia caballi*. *B. caballi* immediately infects erythrocytes, whereas *T. equi* infects peripheral blood mononuclear cells. Approximately fourteen species of Ixodid ticks have been identified as transstadial vectors of *B. caballi* and *T. equi*, while eight of these species are also able to transmit *B. caballi* infections transovarially. Infected animals may remain carriers of these blood parasites for long periods and act as sources of infection for tick vectors. These parasites are also easily spread by blood contaminated instruments. The clinical signs of equine piroplasmosis are often non-specific (mild inappetence, poor performance, loss in body condition), and can easily be confused with other conditions. Piroplasmosis can occur in peracute, acute and chronic forms. The acute cases are more common, characterised by fever (usually >40°C), reduced appetite and malaise, elevated respiratory and pulse rates, congestion of mucous membranes, and faecal balls that are smaller and drier than normal (mild colic). Clinical signs in subacute cases are similar. In addition, affected animals show loss of weight, and fever is sometimes intermittent. The mucous membranes vary from pale pink to pink, or pale yellow to bright yellow. Petechiae or ecchymoses may also be visible on the mucous membranes. Mild edematous swelling of the distal part of the limbs sometimes occurs. Chronic cases usually present non-specific clinical signs, but the spleen can be enlarged on rectal examination. A rare peracute form where horses are found either dead or moribund has been reported (Wise et at., 2013). There are no vaccines available. Equine piroplasmosis has not been shown to be zoonotic .

T. equi infections are more typically difficult to treat than *B. caballi* infections. Numerous drugs have been reported to have variable efficacy in inhibiting *T. equi* and *B. caballi* both in cell culture and in vivo, making the literature difficult to interpret (Wise et al., 2013). Historically, it was reported that *B.*

caballi infection was self-limiting with clearance noted after several years, but there are several exceptions.

Buparvaquone is used outside the European Union for the treatment of bovine theileriosis. Some older studies have investigated buparvaquone in horses. The efficacy of buparvaquone in eliminating *T. equi* of European origin was investigated in carrier horses and experimentally infected splenectomized ponies (Zaugg and Lane, 1989, 1992). When administered at 2.5 mg/kg body weight, IM, 4 times at 96-hour intervals, buparvaquone was effective in eliminating *T. equi* carrier infection in one horse. Buparvaquone administered at 4 to 6 mg/kg IV and/or IM was therapeutically effective in 4 of 5 acute *T. equi* infections in splenectomized ponies. However, the treated ponies became carriers. Another study investigated buparvaquone in eliminating infection by *T. equi* of European origin in carrier horses and in splenectomized horses with experimentally induced acute infection. When administered at 5 mg/kg body weight, IV, 4 times at 48-hour intervals, buparvaquone caused a rapid decrease in parasitemia. However, secondary and tertiary recrudescent parasitemias invariably regress with the establishment of the carrier state. Buparvaquone, at the dose tested, had transient therapeutic efficacy against acute *T. equi* infection in splenectomized horses, but was not capable of clearing the carrier infection by itself.

In another study, 30 draught male horses (4-12 years old) infected with equine babesiosis were divided randomly into three groups: 1) treated with imidocarb dipropionate intramusculary at a dose rate of 4 mg/kg bw repeated after 48 h; 2) treated with diminazine aceturate intramusculary at dose rate of 3.5 mg/kg bw repeated after 48 h; 3) treated with buparvaquone intramusculary at dose rate of 6 mg/kg bw repeated after 48 h. Results showed that imidocarb was more effective in the treatment of equine babesiosis in comparison with diminazine and buparvaquone (Al-Mola and Al-Saad, 2006). Dodiya et al. (2022) investigated nine horses positively infected with equine piroplasmosis and treated by buparvaquone (4 mg/kg bw IM), along with flunixin meglumine, vitamin B1, B2 & B6 injection, butaphosphan for 5 days, and rehydration (Dextrose normal saline, Ringer's lactate and Intalyte), plasma expander and amino acids. All horses responded well to treatment.

Imidocarb dipropionate has shown considerable efficacy, both in the elimination of *T. equi* and *B. caballi* parasites during the chronic stage of the infection and is currently the drug of choice for the treatment of equine piroplasmosis. Imidocarb is included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 and has established MRLs in Europe. The adverse reactions seen as result of the anticholinesterase activity of imidocarb, including sweating, signs of agitation, colic, and diarrhoea, are typically transient and rarely life-threatening (Wise et al, 2013). Adverse reactions can be prevented with administering other substances (glycopyrrolate, atropine, or n-butylscopolamine). Imidocarb may cause a dose-dependent hepatotoxicity and nephrotoxicity (Donnellan and Marais, 2009), leading to periportal hepatic necrosis and renal tubular necrosis. Donkeys and mules are exquisitely sensitive to imidocarb, therefore its use in these species is not recommended.

Other alternative substances for the treatment of equine piroplasmosis are reported in the literature (diminazene aceturate, diaminazene diaceturate, amicarbilade isothionate, euflavine, arteminsin derivatives, buparvaquone, atovaquone and ponazuril; Rashid et al., 2008; Wise et al., 2013) but none of these can considered an alternative within the meaning of the current scientific advice. Oxytetracycline, however has shown to be effective against *T. equi*, though not against *B. caballi* (Zobba et al., 2008). Oxytetracycline is listed in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with MRLs for all food-producing species and veterinary medicinal products authorised for food-producing equine species. Aside from antiprotozoal drugs, acutely infected horses often require supportive care including intravenous fluids, nonsteroidal anti-inflammatory drugs, pain management, and blood transfusions.

Equine piroplasmosis, if untreated, can be life-threatening and causes unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any buparvaquone-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance buparvaquone is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Knowledge regarding antiprotozoals in horses for this assessment was derived from textbooks, review articles, and retrospective studies.

iv. Antivirals

Ganciclovir was mentioned (twice) in the survey to stakeholders and it was suggested for addition to the list for the treatment of equine herpes viral keratitis caused by EHV-2 or EHV-5 (gamma-herpesviruses), and for the treatment of equine coital rash caused by equine alpha-herpesvirus 3 (EHV-3).

Ganciclovir is a synthetic 2'-deoxyguanosine nucleoside analogue, which functions as a competitive inhibitor with deoxyguanosine triphosphate (dGTP), used by the DNA polymerase of viruses for replication. The virostatic activity of ganciclovir is due to inhibition of viral DNA synthesis through: (1) competitive inhibition of deoxyguanosine triphosphate incorporation into DNA via DNA polymerase, and (2) ganciclovir triphosphate incorporation into viral DNA causing DNA termination or severely limiting elongation of viral DNA. It is available in a variety of different formulations (intravenous, oral, and eye gel).

The equine herpes viruses (EHV) are a diverse group. The traditional pathogenic types of equine herpes virus are described as EHV-1, EHV-2, EHV-3, EHV-4, and EHV-5; vaccines are available for some virus-types.

Equine herpesvirus 1 (EHV-1) infections can cause a range of disease in horses, including respiratory disease, abortion, neonatal foal death, and neurologic diseases. Initially, EHV-1 infects the respiratory epithelium, followed by spread to respiratory lymph nodes. The disease is typically self-limiting, but in some cases, resulting leukocyte-associated viremia facilitates spread of the virus to other tissues such as the uterus or central nervous system (CNS) (Edington et al., 1986). Neurologic signs are believed to result from endothelial damage within the CNS, causing ischemic necrosis of the spinal cord. Research into different EHV-1 strains has revealed that a single point mutation within the DNA polymerase is strongly associated with the development of neurologic disease (Goodman et al., 2007). No antiviral treatment against EHV-1 for horses is marketed. Ganciclovir has been shown to be effective against abortigenic and neuropathogenic strains of EHV-1 in vitro (Garré et al., 2007).

Equine herpesvirus 2 (EHV-2) is a slowly growing, cell-associated gamma-herpesvirus. This virus is widespread throughout the equine population, commonly associated with eye-associated viral keratitis. Although its role as a pathogen is controversial, some authors have reported its association with upper respiratory tract disease, inappetence, lymphadenopathy, immunosuppression, keratoconjunctivitis, general malaise and poor performance (Garré et al., 2007).

Equine coital rash or equine coital exanthema (ECE) from EHV-3 is a highly contagious venereal disease characterized by the formation of papules, vesicles, pustules, and ulcers on the external genitalia of mares and stallions. However, signs of systemic illness (fever, anorexia, and pain) are rare. EHV-3 remains in a latent state after successful infection, and there are latently infected animals in which the virus is reactivated and is generally re-excreted subclinically. Since no vaccines or antiviral therapies are available, prevention consists of clinical examination of mares and stallions before mating or semen collection and resting from breeding activities when lesions are present. However, this does not identify subclinically infected animals. Ganciclovir is considered the most potent compound known to reduce EHV-3 replication (Vissani et al., 2020).

Equine alpha-herpesvirus 4 (EHV-4) is a common cause of rhinopneumonitis in horses. It is the most important viral cause of respiratory infection in foals.

Equine herpesvirus 5 (EHV-5) is associated with equine multinodular pulmonary fibrosis (EMPF). EMPF is a chronic, progressive, interstitial lung disease of adult horses. The disease is characterized histologically by marked interstitial fibrosis and mixed inflammatory cell infiltration of the lungs. The exact pathogenesis and predisposing factors currently remain elusive, but the prognosis is typically poor. However, equine herpesvirus-5 has been detected in the lungs of most PFMS cases described in the literature, suggesting an etiologic link. Furthermore, pulmonary fibrosis has been experimentally induced with EHV-5 isolated from the lungs of horses with EMPF and inoculated endoscopically into the accessory lung lobe of clinically normally horses (Williams, 2013). Since vaccination may not prevent the disease, antiherpetic therapies are used for both prophylaxis and for the treatment of viral disease.

Different studies support the efficacy of ganciclovir for the treatment of various equine herpes viruses. In a study comparing the in vitro efficacy of various antiviral drugs against EHV-1, ganciclovir was shown to be the most potent inhibitor of the virus (Garré et al., 2007). Ganciclovir at 0.05 µg/ml shows the best overall inhibitory activity in vitro against EHV-3. However, in vivo efficacy against EHV-1 of ganciclovir, or its prodrug valganciclovir has not been reported. For EHV-1, treatment is typically unnecessary since the disease is self-limiting. However, in cases of EHV-1 associated viremia then systemic antiviral treatments are desirable for neurologic cases. EHV-2 and EHV-3 are best treated locally, where systemic treatment is not necessary. EHV-4 is typically self-limiting, and EHV-5 may require systemic antiviral treatments.

Thieulent et al. (2022) investigated the protection induced by valganciclovir, the prodrug of ganciclovir, in Welsh mountain ponies experimentally infected with an EHV-1 ORF30-C2254 strain. Four ponies were administered valganciclovir immediately prior to experimental EHV-1 infection, while another four ponies received a placebo. Treatment consisted in 6.5 mg/kg body weight of valganciclovir administered orally three times the first day and twice daily for 13 days. Clinical signs of disease, virus shedding and viraemia were measured for up to 3 weeks. Severity of the cumulative clinical score and viral shedding were significantly reduced in the treated group compared with the control group. Viraemia was significantly reduced in the treated group when compared with the control group. Oral administration of valganciclovir induced no noticeable side effects but reduced clinical signs of disease, infectious virus shedding and viraemia in ponies experimentally infected with the EHV-1 C2254 variant.

Vissani et al. (2020) investigated ganciclovir for ECM in a double-blind completely randomized study design. Twenty mares were randomly divided into five groups (three treated with ganciclovir with different regimen of doses, one treated with a placebo, and one nontreated). Mares were experimentally infected with EHV-3 on day 0. Mares experimentally infected with EHV-3 and treated with ganciclovir twice a day for 13 days showed reduced levels and duration of viral excretion and less severe lesions. The viral excretion period was reduced from 18 to nine days compared with the untreated groups.

There is only one case report of successful treatment of horse with EHV-5 associated EMPF with intravenous ganciclovir (Romero et al., 2019).

Equine herpes viral keratitis, if untreated, can cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any ganciclovir-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance ganciclovir is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: to treat cases of equine herpes virus infection associated with complications, topical use. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Valacyclovir was mentioned (five times) in the survey to stakeholders and it was suggested for addition to the list for the treatment of equine herpes virus type 1 (EHV-1) and treatment of EHV-3 and EHV-5. It was indicated by the respondents that valacyclovir treatment significantly decreased viral replication and signs of disease in EHV-1-infected horses.

Valacyclovir is the L-valilester prodrug of acyclovir that is almost completely converted (to acyclovir and valine) by the enzyme valacyclovir hydrolase. Primary antiherpetic drugs, developed for routine and long-term systemic use in humans, generally have poor oral bioavailability. Valacyclovir, as a prodrug, enhances the enteral absorption of acyclovir through the activity of dipeptide transporters on the apical surface of enterocytes. After oral absorption, valacyclovir undergoes extensive first-pass intestinal and/or hepatic metabolism (hydrolysis) to produce acyclovir and L-valine. Acyclovir is a purine (guanine) nucleoside analogue, which inhibits DNA synthesis and blocks viral DNA replication. Acyclovir has a time-dependent pharmacokinetic-pharmacodynamic pattern with trough acyclovir plasma concentrations most predictive of acyclovir efficacy (Maxwell et al., 2017). Acyclovir is proposed for addition to the list.

Once absorbed, valacyclovir is rapidly hydrolysed to its active form, acyclovir, such that plasma concentrations of the prodrug are very low or absent in horses. Although valacyclovir is not found in equine plasma, acyclovir appears rapidly, with peak concentrations occurring 45-60 minutes after oral administration of valacyclovir and an absorption half-life of 0.5-0.7 hours. Oral absorption of valacyclovir in horses is considerably better (41–60%) than that of acyclovir and was not substantially changed at doses between 5 g and 15 g (8–34 mg/kg) valacyclovir (Maxwell et al., 2008). As valacyclovir absorption is a saturable process, higher doses of valacyclovir may produce lower than expected systemic acyclovir concentrations.

Valacyclovir administration decreases virus shedding and viraemia, compared with findings for control horses (Maxwell et al., 2017). Rectal temperatures and clinical disease scores in horses receiving valacyclovir prophylactically for 2 weeks were lower than those in control horses. The severity of ataxia, but not the risk of ataxia, decreased with the administration of valacyclovir. Viremia decreased when steady-state trough acyclovir plasma concentrations were > 0.8 µg/mL, supporting time-dependent activity of acyclovir.

Valacyclovir administration has shown promise in reducing viral shedding, pyrexia, clinical disease, and neurologic severity in experimentally infected horses when given prophylactically or early in the disease course (Garré et al., 2009; Maxwell et al., 2017). However, as an EHV-1 outbreak may not be recognized until some horses are in imminent danger of developing neurological signs, then more potent and predictable antiviral drugs may be necessary. Also, several cases of horses with EHV-1 myeloencephalopathy exhibit major neurologic symptoms where the horse cannot accept oral

medication. From the survey, valacyclovir is recommended for treatment of equine herpes virus infections (EHV-3 included). Oral administration to obtain systemic antiviral concentrations are not necessary for EHV-3. The use of the antiviral prodrug valacyclovir has been described in 1 horse with EMPF that was reported to be clinically healthy 2 years after treatment. A study by Easton-Jones et al. (2018) found valacyclovir treatment was insufficient to alter EHV-5 viral kinetics in horses with EMPF, in horses receiving 10 days of PO administered valacyclovir (loading dose 30 mg/kg, maintenance dose 20 mg/kg). While acyclovir is retained in the list, valacyclovir offers a better pharmacokinetic profile and a different route of administration thus adding clinical benefit.

Equine herpes viral infections, if untreated, can cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any valacyclovir-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance valacyclovir is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: to treat cases of equine herpes virus infections, oral use. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Knowledge regarding antivirals in horses for this assessment was derived from textbooks, review articles, case-studies, and prospective studies.

B. Considerations regarding consumer safety

i. Antibiotics

Clarithromycin is a macrolide derived from erythromycin with similar actions and uses. It is used orally or parenterally in humans. As a macrolide, it is classified under category C 'caution' in the 'Categorisation of antibiotics in the European Union' (EMA/CVMP/CHMP/682198/2017). Macrolides are used in horses for treatment of pneumonia and extrapulmonary conditions caused by *Rhodococcus equi*.

Clarithromycin is rapidly absorbed from the human gastrointestinal tract and undergoes first-pass metabolism; the bioavailability of the parent drug is about 52-55%. The pharmacokinetics are non-linear and dose dependent; high doses may produce disproportionate increases in peak concentrations of the parent drug, due to saturation of the metabolic pathways. Its principal active metabolite is 14-hydroxyclarithromycin. Both the parent compound and the metabolite are widely distributed. The mean apparent volume of distribution of clarithromycin in adults ranges from 191 to 306 L, and plasma protein binding has been reported to be from 42 to 72%. Clarithromycin has extensive diffusion into saliva, sputum, lung tissue, epithelial lining fluid, tonsils, nasal mucosa and middle ear fluid. In respiratory tract tissues and fluids, clarithromycin concentrations are 3- to 30-fold higher than plasma concentrations. It is extensively metabolized in the liver and excreted in faeces via the bile. At least 8 metabolites have been recovered; while 20 to 40% is excreted in the urine as unchanged drug, the main and other metabolites are also excreted in the urine accounting for 10 to 15% of the dose. The mean elimination half-life of clarithromycin was increased from 2.27 to 5.98 hours at doses of 100 and 1200 mg, respectively, and over the same dose range, the mean elimination half-life of 14-hydroxyclarithromycin increased from 2.4 to 9.19 hours (Rodvold, 1999; Scriba, 2009).

Pharmacokinetics of clarithromycin have also been studied in foals. After a single intravenous bolus of 7.5 mg/kg, the terminal elimination half-life was 5.4 hours, the clearance was 1.27 ± 0.25 L/h/kg and

the apparent volume of distribution was 10.4 ± 2.1 L/kg. The mean oral bioavailability was 57.3% after intragastric administration (Womble et al., 2006). In foals, macrolides have an extraordinary capacity to accumulate in different lung tissue compartments. They show a rapid and extensive distribution and long persistence in pulmonary epithelial lining fluid (PELF) and bronchoalveolar lavage (BAL) cells from foals. Many physicochemical factors may favour the accumulation of macrolides in the lung, including low molecular weight and a large degree of dissociation at plasma pH. The data available suggest that clarithromycin has the maximum capacity to accumulate in PELF in comparison with azithromycin, tilmicosin and gamithromycin. Macrolides are eliminated by bile and urine. Clarithromycin is metabolized to 14-hydroxy-clarithromycin in foals (Villarino and Martín-Jiménez, 2012).

Regarding toxicity, only limited data are available concerning the effect of clarithromycin on the human foetus when used in pregnancy. Animal studies have shown that clarithromycin can induce foetal loss in rabbits and monkeys when used at different doses (from very low to high). Studies in mice revealed a variable incidence of cleft palate with oral dosages of 500-1000 mg/kg daily during gestation days 6-15. Teratogenicity studies in rats with dosages up to 160 mg/kg/day administered during the period of major organogenesis, and in rabbits at oral dosages up to 125 mg/kg/day, did not demonstrate evidence of teratogenicity. Other studies in rats demonstrated a low incidence of cardiovascular anomalies at a clarithromycin dosage of 150 mg/kg/day (American Society of Health-System Pharmacists, 2012). In women, Andersen et al. (2013) found an increased risk of miscarriage when redeeming a prescription of clarithromycin in the first trimester of pregnancy but did not find an association between exposure to clarithromycin and major congenital malformations. This is supported by other authors (Fan et al., 2019; Drinkard et al., 2000).

Clarithromycin failed to exhibit mutagenic potential in several in vitro tests, including the Salmonella mammalian microsome test, bacterial induced mutation frequency test, rat hepatocyte DNA synthesis assay, mouse lymphoma assay, mouse dominant lethal test and mouse micronucleus test (American Society of Health-System Pharmacists, 2012). However, high doses of clarithromycin (> 100 mg/kg) caused significant increase in the frequency of chromosomal aberrations in bone-marrow and splenocyte cells of mice and a significant increase in the frequency of sister chromatid exchanges in mice bone-marrow (Aziza and El-Sherbeny, 2006). No mutagenic effect was found after an Ames test (Isidori et al., 2005) and no mutagenic potential has been described for different clarithromycin human medicinal products.

No residue depletion study is available for horses or other food-producing animals. These studies are available for other macrolides (e.g. erythromycin). Clarithromycin, as well as other macrolides, accumulate in different lung tissue compartments, but from pharmacokinetic studies available in horses it can be expected that no residues will remain after a six-month withdrawal period. Furthermore, a limited oral bioavailability has been described. Therefore, it can be accepted that the substance 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.

Fusidic acid mainly used in humans as part of a combination therapy against staphylococcal infections or topically for treatment of skin or eye infections. It is authorized in the EU for use in cats and dogs for eye, ear and localized skin infections. As a steroid antibacterial, it is classified under D 'prudence' in the 'Categorisation of antibiotics in the European Union' (EMA/CVMP/CHMP/682198/2017).

Pharmacokinetic data in humans is available. Oral suspensions containing fusidic acid (as sodium fusidate) are absorbed from the gastrointestinal tract with a reported bioavailability of around 70%. Fusidate is widely distributed into tissues and body fluids. It has been found in the foetal circulation and in breast milk. About 95% is bound to plasma proteins. Fusidate has a plasma half-life of about

10-15 hours, and it is excreted in the bile (almost entirely) as metabolites; only about 2% appears unchanged in the faeces (Scriba, 2009).

Thorn and Johansen (1997) measured the biological half-life of fusidic acid in tear fluid samples of humans after instillation of one drop of viscous fusidic acid eye drops (i.e. topical ocular formulation). The biological half-life corresponded to 7.3 hours. Little systemic absorption following topical eye administration has been reported (Enna, 2008). This is also supported by information in human SPCs. Human studies where tear film levels were measured revealed half-lives of 2-6 hours. From these studies it is concluded that there is a sustained presence of fusidic acid on the surface of the human eye for several hours (Doughty and Dutton, 2006).

No pharmacokinetic data is available in the horse. Some studies have been performed in laboratory animals. A single instillation of fusidic acid 1% into rabbit eyes was reported to result in very high concentrations of the drug in the tear film. The concentrations estimated at 4 and 6 h were less than half of those reported at 1 and 2 h, but a sustained presence of fusidic acid at the ocular surface of rabbits was observed. The concentrations in the conjunctiva and corneal tissue stayed at similarly high levels for 1-2 h and then declined quite rapidly (Doughty and Dutton, 2006).

Fusidic acid has a good safety profile. Most common adverse events reported are considered of minor significance (e.g. diarrhoea, abdominal discomfort) (Huttner and Harbath, 2017). Repeated dose toxicity studies have been performed for sodium fusidate in rats and dogs by oral and intravenous administration. Sodium fusidate appears to be well tolerated too. Fertility and reproduction studies in rats revealed no significant differences between treated and control dams. No teratogenic or clastogenic potential has been observed (as reported from human SPCs).

No pharmacokinetic or residues studies are available in horses. However, considering the available pharmacokinetic information in humans and laboratory animals, the proposed indication (to treat eye infections) and the toxicological profile of fusidic acid, it can be accepted that the substance 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.

Moxifloxacin is a fluoroquinolone and broad-spectrum antibiotic which blocks the bacterial DNA replication by inhibiting the activity of two enzymes, i.e. DNA topoisomerase II (DNA gyrase) and topoisomerase IV. It is water-soluble (0.168 mg/mL) and fat-soluble (logP 2.9). It can be administered orally as hydrochloride, as an intravenous solution, or topical into the eyes (Drugbank, 2024). In horses it is used as an ophthalmic medicine. The lipophilicity leads to high corneal penetration but also good aqueous solubility at physiological pH (PubChem, 2024).

In one clinical and pharmacokinetics horse study, the ocular penetration and systemic absorption was studied using 0.5% moxifloxacin with repeated topical administration (seven times 0.2 mL every four hours) in seven healthy horses. Blood was collected at 24, 24.25, 24.5, and 25 hours after the final dose. Concentrations in plasma showed a peak value of 0.015 µg/mL at 24 and 24.25 hours, decreasing to < 0.004 at 25 hours. A plasma half-life of 0.35 ± 0.10 hours and an area under the curve extrapolated to infinity of 0.01 ± 0.01 h·µg/mL was estimated in horses after ophthalmic administration. It was concluded that the minimal plasma concentrations are not expected to be associated with any therapeutic effect (Clode et al., 2010). Further, the pharmacokinetic values of moxifloxacin in six mares after oral administration of 5.8 mg/kg of three doses at 24 h intervals without reaching steady-state were: maximum concentration C_{max} of 3.12 µg/mL (SD 0.86), time of maximum concentration T_{max} of 2.75 h (SD 2.86), apparent volume of distribution (area method) corrected for oral absorption VD(area)/F of 5383.83 mL/kg (SD 2782.28), elimination half-life $t_{1/2}$ of 33.98 h (SD 22.21), i.e. after a rapid absorption, a high C_{max} was reached with persistence of high

intracellular concentrations in alveolar cells and a long elimination half-life. The elimination half-life after oral administration in the horse was prolonged compared with humans (Gardner et al., 2004).

Human pharmacokinetics are described in more detail, however, there is no publicly available information after topical administration into the eyes. After oral administration the bioavailability is high (approximately 90%). Moxifloxacin is widely distributed with higher concentration in tissues than in plasma (volume of distribution 1.7 to 2.7 L/kg). In blood it is approximately 30-50% bound to serum proteins. Moxifloxacin was detected in saliva, nasal and bronchial secretions, mucosa of the sinuses, skin blister fluid, subcutaneous tissue, skeletal muscle, abdominal tissues and fluids after oral or intravenous administration of 400 mg. Moxifloxacin is excreted partly unchanged, and partly via metabolites via urine and faeces. The clearance is described to be 12±2 L/hr (PubChem, 2024). Moxifloxacin undergoes glucuronide and sulphate conjugation without involvement of the cytochrome P450 system (Stass and Kubitzka, 1999; PubChem, 2024). The biological half-life is described in the range 11.5 to 15.6 hours following single oral dose, and the mean elimination half-life from plasma as 12±1.3 hours (PubChem, 2024). Stass and Kubitzka (1999) observed fast absorption and a bioavailability of 86% in a study with healthy volunteers after oral administration; recovery from urine and faeces was 96% or 98% after single oral or intravenous administration, respectively. The elimination half-lives were 15.6±1.15 and 15.4±1.17 hours (geometric mean) after oral and intravenous administration of 400 mg moxifloxacin, respectively.

In the summary of product characteristics of a human ophthalmic medicinal product, the plasma half-life of moxifloxacin is estimated to be 13 hours following topical administration to both eyes, three times daily, for four days in 21 male and female subjects; moxifloxacin was absorbed systemically.

Siefert et al. (1999a) compared pharmacokinetics after oral and intravenous administration of moxifloxacin to human different animal mammalian species (male mice and rats, female dogs, monkeys and minipigs). A high bioavailability and a low plasma protein binding was found in all species. Due to a large volume of distribution, the binding to tissues is high in all species. Body clearance decreases with increasing weight of animals, so that an elimination half-life of 1.3 to 12 hours after oral administration and 0.93 to 13 hours after intravenous administration in the different species was observed (geometric mean). A good correlation between species using allometric scaling could be demonstrated for clearance, volume of distribution, and mean residence time. In a different study with six male goats the estimated plasma elimination was 0.74±0.04, 0.96±0.19, and 0.82±0.11 hours after intravenous, intramuscular, and subcutaneous administration, respectively (Patel et al., 2011).

Absorption from the eye into systemic circulation was observed for rabbits (Zhao et al., 2022). The C_{max} in rabbit plasma after single topical administration of 50 µl moxifloxacin hydrochlorid (25 mg-eye drops) in each eye was 18.2±5.5 µg/L (N=36), the elimination half-life from cornea, aqueous humor and plasma were 2.2, 1.3 and 1.6 hours, respectively.

No publicly available toxicity studies were found. According to the classification provided by companies to ECHA in CLP notifications and according to safety data sheets of companies there were no data available or no chronic toxicities observed. Jeffrey et al. (2000) observed no photochemical mutagenicity for moxifloxacin when compared with other fluoroquinolones. In a mechanistic chronic toxicity and carcinogenicity study in rats over 24 weeks (cancer bioassay ABC) moxifloxacin showed no initiation nor promotion potential of neoplasia in any of the critical organs or tissues examined (Iatropoulos et al., 2001). Moxifloxacin is not examined or classified for carcinogenic hazards by IARC (IARC, 2024). In human medicinal product information it is noted that, as with other quinolones, moxifloxacin was genotoxic in vitro in bacteria and mammalian cells due to the interaction with bacterial gyrase. The mechanism of action (in bacteria) may not be completely selective, so that

gyrase inhibitors gyrase inhibitors may also have cytostatic properties (in mammalian cells). A threshold-based effect was assumed. However, in the end, moxifloxacin was not genotoxic according to in vivo tests. Von Keutz and Schlüter (1999) concluded that the concentrations of the quinolones (including moxifloxacin) that are genotoxic in vitro are far in excess of those that can be achieved in patients at therapeutic dosages and that the negative in-vivo results accurately reflect the in-vivo situation in terms of genotoxicity. Moreover, there is no indication that moxifloxacin is toxic to reproduction.

Side effects described in human medicinal products are gastrointestinal disorders, in particular nausea, diarrhoea, vomiting and indigestion, headaches and drowsiness, superinfections caused by resistant bacteria or fungi, QT prolongation with the risk of dangerous cardiac arrhythmias (in high-risk patients; Khan et al., 2018), especially with known hypokalaemia and elevated liver values. There was rarely a clinically apparent liver injury observed (PubChem, 2024).

No residue depletion data were found for moxifloxacin administered in horses or in closely related species of the *Equidae* family. After single intravenous and intramuscular administration, moxifloxacin passes into the milk of sheep (Goudah et al., 2008). In male rats with periprosthetic infection of the left hind leg (N=36) the distribution into organs compared with plasma was investigated after repeated peritoneal injection (day 7 to 21 after operation). As expected, the tissue concentrations of moxifloxacin were higher in all tissues than in plasma. The relative tissue-to-plasma-concentrations were in muscle (6:1), lung (4:1), bone (3:1), and fat (2:1) after 14 days of treatment. The plasma elimination was rapid. Tissue depletion was not investigated (Beckmann et al., 2007). Two rat strains (Wistar, FB 30) after single intravenous and oral administration showed high tissue affinity of moxifloxacin in several organs by whole-body autoradiography. The tissue-to-plasma-concentration in lung was observed to be 3:1 with a similar concentration time curve as in plasma with rapid elimination within hours, both after intravenous and oral administration (Siefert et al., 1999b). Thus, there were no indications for accumulation in rat.

Considering the pharmacokinetics in horse after ophthalmic use, underpinned by data after ophthalmic use in rabbit and other administration routes in different species, the assumed rather low bioavailability after ophthalmic use, probably leading to not-relevant concentrations of residues in the tissues, which did not show a potential of accumulation at least in the lungs of rats, and the apparent low toxicity despite cytostatic effects may be possible in humans, it can be accepted that moxifloxacin 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.

ii. Antifungals

Amphotericin B is a systematic antifungal for the treatment of (serious) mycotic infections parenterally (intravenous or subcutaneous infusion, generally). Is a fungistatic that can be fungicidal against some organisms depending on the drug concentration. It works by binding to sterols (primarily ergosterol) in the cell membrane and altering the permeability of the membrane allowing intracellular potassium and other cellular constituents to leak out. Mammalian cell membranes do contain sterols (mainly cholesterol) and the toxicity of the drug may be due to a similar mechanism of action, although amphotericin binds less strongly to cholesterol than ergosterol. Amphotericin B is insoluble in water; its salts are more water soluble but have less antifungal activity (Plumb, 2015; Liu et al., 2017).

Amphotericin B is on the EU market as a human medicinal product and four different formulations are available: the so called conventional (formulated with sodium desoxycholate), a cholesteryl sulfate complex, a lipid complex and in liposomal form. The lipid complex formulation is commonly used in

animals. Nephrotoxicity is the main concern, especially with the deoxycholate (conventional) form, i.e. the newer formulations are less nephrotoxic and penetrate better into tissues (Volmer et al., 2010). A study in dogs showed that amphotericin B lipid complex was 8-10 times less nephrotoxic than the conventional form (Plumb, 2015). Differences in distribution and particle size are observed between different forms: amphotericin B deoxycholate (tissue distribution liver > spleen > lung > kidney; size <25 nm); liposomal amphotericin B (tissue distribution spleen > liver > lung = kidney; size <100 nm); amphotericin B lipid complex (tissue distribution spleen > liver =lung = kidney; size <1600-11,000 nm) (Cavasin et al., 2021).

In humans (presumably in animals too) amphotericin B is poorly absorbed from the gastrointestinal tract and is thus administered parenterally to achieve sufficient concentrations to treat systemic fungal infections (Plumb, 2015; Cavasin et al., 2021) The oral bioavailability of amphotericins B is reported to be between 0.2% and 0.9% (Serrano et al., 2017). Recent study showed insignificant degradation of amphotericin the presence of water. Amphotericins have a low aqueous solubility in physiological pH and low stability at high temperatures and acid pH and low permeability through membranes (Cavasin et al., 2021). Lipophilicity of amphotericin B is (logP) 2.296 ± 0.894 (Wang et al., 2021)

One contributing factor to decreased bioavailability of orally administered drugs is their susceptibility to efflux proteins such as P-glycoprotein, which are highly expressed in the small intestine (Azman et al., 2022; Fairuz, 2022) Hydrolysis by enzymes present in the GIT can also break the ester bonds, further reducing the stability of amphotericin B (Pinheiro, 2020).

After intravenous injection, the drug penetrates well into tissues, not well in pancreas, muscle, bone, aqueous humor or pleural, pericardial, synovial and peritoneal fluids. The lipid formulation enhances amphotericin B lung, liver and spleen penetration; a colloidal formulation provided higher amphotericin B concentrations in lung tissue than liposomal formulation (Vogelsinger et al., 2006), but with substantial interpatient variability (Felton et al., 2014). The drug does enter the pleural cavity and joints, when inflamed; CSF (cerebro-spinal fluid) levels are approximately 3% of those found in the serum; approximately 90-95% of amphotericin B in the vascular compartment is bound to serum proteins (Plumb, 2015).

While metabolic pathways of amphotericin B are unknown, it seems to exhibit biphasic elimination. An initial serum half-life of 24-48 hours - conventional amphotericin (half-life of 10-24 hours), lipid amphotericin (half-life of 24 hours), and liposomal amphotericin (half-life of 6-23 hours) (Felton et al., 2014), and a longer terminal half-life of about 15 days have been described (Plumb, 2015). Seven weeks after therapy has stopped, amphotericin can still be detected in the urine. Approximately 2-5% of the drug is recovered in the urine unchanged (biologically active form) (Plumb, 2015).

Risovic et al. (2003) studied the effects of amphotericin deoxycholate and lipid and mixed micelle amphotericin B oral formulations on plasma, tissue concentrations (kidney, liver, spleen, heart and lung) and renal toxicity (in male rats). Rats received a single (1ml) dose by oral gavage containing 1, 5, or 50 mg amphotericin B/kg bw of different formulations. Amphotericin B levels in tissues were analysed by HPLC (high-pressure liquid chromatography). Residues in the kidney were around 9 µg amphotericin B/g tissue at 24 hours for the sodium deoxycholate form; no residues were observed for other forms. The highest levels were found in lung (i.e. 731 ± 602 µg amphotericin B/g tissue) for the sodium deoxycholate form. It should be noted that amphotericin B tissue distribution displays high variability, even within the same species (Vogelsinger et al., 2006).

For antifungal drug the distribution of agents from the bloodstream to various tissue subcompartments is often characterized by considerable variability, beyond that observed in plasma alone. Consequently, target site concentrations often differ markedly from those measured in plasma, especially in sanctuary

sites such as the eye or central nervous system (CNS). Furthermore, there may be discordance in the shape of the concentration-time profiles for plasma and tissues. This phenomenon is called hysteresis. (Felton et al., 2014)

Data in human medicinal products indicates that the safety of amphotericin B during pregnancy has not been established, but there seem be no reports of teratogenicity associated with the drug (Plumb, 2015).

Gangneux et al. (1996) reported levels in plasma, liver, spleen and lung of mice with two forms of amphotericin B deoxycholate and one liposomal form). Amphotericin B levels in plasma, liver, spleen and lung were determined by high-performance liquid chromatography analysis 3, 43 and 103 days after the end of early treatment (day 7 to 17 post infection) at doses of 0.8 mg/kg (deoxycholate forms), 5 mg/kg and 50 mg/kg (liposomal form only) and 2 and 55 days after cessation of delayed treatment (day 60 to 70 post infection) with 0.8 mg/kg (deoxycholate forms), 5 mg/kg and 50 mg/kg (liposomal form only). Amphotericin B formulations were administered intravenously on days 7, 9, 11, 13, 15 and 17 in an early treatment group and on days 60, 62, 64, 66, 68 and 70 in a delayed treatment group. In the early treatment group, in mice treated with the deoxycholate forms (0.8 mg/kg) plasma and tissue levels were consistently low or undetectable at 3, 43 and 103 days after treatment. Mice treated with the liposomal form (0.8 mg/kg) also had low to undetectable levels of amphotericin B in plasma. However, there was a marked accumulation of the drug in the tissues examined, with liver and spleen levels of 33.94 and 23.84 µg/g, respectively, on day 3 and 3.05 and 5.48 µg/g, respectively, on day 43. In addition, amphotericin B remained detectable in the spleen until 14 weeks after the cessation of treatment with the liposomal form. When the liposomal form was administered at 5 or 50 mg/kg, drug concentrations in the liver reached 209.7 and 2,575.4 µg/g, respectively, and those in the spleen reached 98.8 and 929.2 µg/g, respectively. Drug levels in these organs decreased very slowly, with persistent levels of drug detectable 14 weeks after treatment. Amphotericin B was detectable at much lower levels in the lungs of mice given the liposomal form at either dose, and remained at low to undetectable levels in the plasma. In the delayed treatment group, similar results were seen when treatment was given from day 60 to 70. Only undetectable or low levels of amphotericin B were found after treatment with the deoxycholate or the liposomal forms at 0.8 mg/kg. In mice treated with the liposomal form at higher doses, high drug levels were found in tissues 8 weeks after the end of therapy, while low levels were found in plasma at the same time.

Amphotericin B is detected in the liver, spleen, and kidneys for up to 1 year after the end of therapy (Benson and Nahata, 1988). Treatment duration in animals might be long (> a month) and is dependent on clinical response and toxicity. Generally, accumulative doses are not well described in the literature in horses, while some reports suggest some accumulation following a 6.75 mg/kg dose in foals (Stewart et al., 2008; Plumb, 2015).

In light of a possible long-term persistence of residues in tissues, as described in some scientific sources, an estimate of the risk that could arise from the possible presence of residues after the six-month withdrawal period for amphotericin B at an accumulative dose of 6,75 mg in horses has been performed and calculated (see section 7.2.1 for further details). Considering amphotericin B disposition from mice tissue (liver, spleen) (Gangneux et al., 1996) at the level of what is considered a worst-case maximum dose in horses, residues are expected to possibly occur in horse tissues. Considering a 1% oral absorption of amphotericin B in humans (Serrano et al., 2017), after consumption of horse meat containing residues of amphotericin B and comparing the exposure with a toxicological reference value derived from a low human therapeutic dose, a sufficient margin of exposure (MoE) can be established. Thus, no unacceptable risk is identified. It can be accepted that amphotericin B 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.

Voriconazole is a triazole compound used to treat fungal infections. In human medicine, it may be given topically to the eyes, orally or intravenously. In horses it is proposed for ophthalmic fungal keratitis by topical administration only because of the below assessment.

In humans, voriconazole exhibits non-linear pharmacokinetics due to saturable metabolism. It is rapidly and almost completely absorbed from the gastrointestinal tract, with an oral bioavailability over 90%. Plasma protein binding of voriconazole is about 58%. The volume of distribution is 2-4.6 L/kg, suggesting extensive distribution. It is metabolized by hepatic cytochrome P450 isoenzyme CYP2C19 and about 80% is excreted in the urine (Scriba 2009; Theuretzbacher et al., 2006).

Scholz, et al. (2009) compared the pharmacokinetics of voriconazole after oral and intravenous administration. After a single oral dose of 400 mg voriconazole, the absolute bioavailability was high (82.6%). Maximum plasma concentrations of 9.3 ± 3.2 nmol/ml were observed after 1.5 h. After parental application, C_{max} was 12.1 ± 2.3 nmol/ml at the end of the 2-h infusion period. Terminal elimination half-life was not different between oral and intravenous application (8.71 h vs 8.57 h). Of the three metabolites quantified in this study (voriconazole-N-oxide, hydroxy-voriconazole and dihydroxy-voriconazole), voriconazole-N-oxide showed the highest plasma concentration. It was concluded that the liver is the main organ of metabolic elimination. In another study, voriconazole pharmacokinetics was assessed after the first dose and during steady-state treatment, after intake for at least 14 days. Mean plasma concentrations were higher during steady state and there was a statistically significant increase in C_{max}. Clearance was 523 and 1291 ml/min and the terminal elimination half-life was 11.97 and 10.89 h after the first dose and during steady-state treatment, respectively. Pharmacokinetic parameters of the metabolites voriconazole-N-oxide, hydroxy-voriconazole and dihydroxy-voriconazole were also investigated. Terminal elimination half-lives of the three metabolites during steady-state treatment were 21.15, 13.18 and 12.45 h respectively (Geist et al., 2013).

Besides, from the product information of human medicinal products, after administration of a radiolabeled dose, approximately 80-83% is recovered in the urine after multiple dosing and the majority of the total radioactivity (>94%) is excreted in the first 96 hours. It should be noted that because of the non-linear pharmacokinetics, the terminal half-life is not useful in the prediction of the accumulation or elimination of voriconazole.

Pharmacokinetics of ocular, intravenous and oral administrations of voriconazole to horses has also been studied. One study concluded that voriconazole effectively penetrated healthy equine corneas and achieved detectable concentrations in the aqueous humor after topical administration and that the drug enters noninflamed equine ocular tissues after oral administration. In this study, drug was detected at low concentrations in the plasma of horses after 7 topically administered doses at 4-hour intervals; the concentrations persisted for <1 hour after the final administration. The minimal absorption observed suggested that there would be no detectable adverse systemic effects, even with chronic use (Clode et al., 2006). After intravenous administration of 1 mg/kg, mean value for clearance was low (1.89 ± 0.46 ml/kg/min), the volume of distribution was 1.35 L/kg and the terminal half-life was 8.89 ± 2.31 hours. After an oral administration of 4 mg/kg, voriconazole was adequately absorbed, with a systemic bioavailability of 135.75%. The C_{max} was 2.43 ± 0.4 µg/ml at 2.92 ± 1.2 hours. The elimination half-life was 13.11 ± 2.85 h (Davis et al., 2006). In another study, Colitz et al. (2007) assessed the pharmacokinetics of voriconazole following intravenous and oral administration to horses. Half-lives of 11-15 hours were observed after a single intravenous administration of 10 mg/kg. The apparent volume of distribution at steady state was 1.6 ± 0.4 L/kg and the mean clearance was

87.2 ± 30.7 ml/h/kg. After oral administration, C_{max} of 4.7 to 8.5 µg/ml were detected at 1 to 4 hours; these values declined exponentially with a half-life of 7.8 to 12.9 hours. The oral bioavailability was 95 ± 19%. Subsequently, voriconazole was administered PO (3 mg/kg) twice daily for 10 days and voriconazole was found in CSF, synovial fluid, urine and preocular tear film.

Regarding toxicity of voriconazole, repeated-dose toxicity studies indicated the liver to be the target organ. In humans, transient elevations in serum aminotransferase levels occur in 11 to 19% of patients. These elevations are usually asymptomatic and self-limited but 1% of patients require discontinuation because of ALT elevations (Pubchem, 2024). Toxicity notably in the liver and possibly in the kidney may be expected in humans at therapeutic doses. Teratogenicity, notably cleft palate and visceral anomalies in the renal area has been observed in reproduction studies in rats. Voriconazole was not teratogenic in rabbits but became embryotoxic at 100 mg/kg. A standard battery of genotoxicity tests was carried out and it was concluded that voriconazole does not pose a genotoxic hazard. No risk for carcinogenicity in humans has been described either (EMEA, 2005). Voriconazole is currently not listed by the IARC.

No residues depletion study and no information about tissue accumulation are available. In spite of the non-linear pharmacokinetics and the wide distribution of voriconazole in tissues, it is assumed that there is a large margin of safety when comparing the terminal half-lives described in the different studies with different doses and the 6-month withdrawal period. Furthermore, the topical administration showed low concentrations of voriconazole in plasma, so no systemic toxic effects are expected. Therefore, it can be accepted that the substance will not pose an unacceptable risk for consumers when used topically in food-producing animals of the equine species and a six-month withdrawal period is respected. In the case of systemic voriconazole, there are several uncertainties from consumer safety perspective precluding a recommendation for use. Uncertainties relate to its high oral bioavailability, non-linear PK, potential accumulation, wide distribution and long treatments required, together with the lack of residue depletion data studies and the teratogenicity study results in rats.

iii. Antiprotozoals

Not warranted since none of the above is considered essential.

iv. Antivirals

Ganciclovir is a guanosine derivative that, upon phosphorylation, inhibits DNA replication by herpes simplex viruses (HSV). Ganciclovir is transformed by viral and cellular thymidine kinases (TK) to ganciclovir triphosphate, which works as an antiviral agent by inhibiting the synthesis of viral DNA in 2 ways: competitive inhibition of viral DNA-polymerase and direct incorporation into viral primer strand DNA, resulting in DNA chain termination and prevention of replication. Ganciclovir is on the EU market as a human medicinal product with different pharmaceutical forms (intravenous, oral, eye gel). It is used (use outside of the terms of the marketing authorisation) for treatment of keratitis in animals at doses of 1 drop/eye every 4-6 hours for 21 days (Plumb, 2015).

In humans, oral ganciclovir has recently been approved for use in long-term maintenance therapy in the treatment of cytomegalovirus (CMV) retinitis in immunocompromised patients. Information from summary of product characteristics of human medicinal products indicates convenience and practicality of oral maintenance therapy (3000 mg/day) makes it desirable, though it is moderately less effective than intravenous administration (5 mg/kg as a 1-hour infusion every 24 hours). In humans, two dosing regimens (1000 mg three times daily; 500 mg every six times daily) have shown to be efficacious (Anderson et al.,1995). The bioavailability of these was 8.84% and 8.53%, respectively.

The metabolism in (human) liver is minimal; the substance is renally excreted unchanged (94-99%) with short elimination half-lives of 1.7-5.8 hours (Ford et al., 2001).

Carmichael et al. (2013) investigated the pharmacokinetics of ganciclovir (IV 2.5 mg/kg) and its oral prodrug, valganciclovir (orally 1800 mg per horse), in six adult horses using a randomized cross-over design. Intravenously ganciclovir was best described by a three-compartment model with a prolonged terminal half-life of 72 + 9 h. Plasma ganciclovir concentrations remained above the assay's limit of quantification (LOQ:0.005 µg/ml) throughout the sampling interval of seven days. Pharmacokinetic modelling suggested that intravenous administration of ganciclovir at 2.5 mg/kg every 8 h for 24 h followed by maintenance dosing of 2.5 mg/kg every 12 h would maintain effective ganciclovir serum concentrations in most horses throughout the dosing interval. No adverse effects were noted in any horse. It is possible that drug accumulation would occur with multiple doses of ganciclovir in horses, due to the prolonged elimination half-life of ganciclovir in this species. However, such accumulation was predicted to impact the peak concentrations of ganciclovir by a relatively small amount (>10%) due to the pronounced rapid distribution phase of ganciclovir disposition.

Adverse effects of ganciclovir administration reported in humans include bone marrow suppression, seizures, nephrotoxicity, and decreased spermatogenesis (Jacobsen and Sifontis, 2010). Myelosuppression is the main dose-limiting toxicity effect of ganciclovir, with neutropenia occurring in 40% of patients, thrombocytopenia in 20%, and anaemia in 2%; neutropenia occurs typically during the second week of treatment and is usually reversible within 1 week of drug cessation (Jacobsen and Sifontis, 2010). Myelosuppression and nephrotoxicity may occur in cats after systemic treatment (Plumb, 2015). Information from human medicinal products indicates that CNS side effects have also been reported in approximately 5% of patients, and may include confusion, seizure, coma, psychosis, hallucinations, mental status changes, anxiety, and ataxia. A distinctive side effect can occur in patients with cytomegalovirus CMV retinitis, namely, ganciclovir-induced retinal detachment. This complication has been reported in up to 30% of ganciclovir-treated patients with CMV retinitis but is seen rarely in patients without CMV retinitis. Overdoses of ganciclovir have not been reported in the literature.

Ganciclovir is not listed in the IARC database.

During the course of an Article 30 referral (under Directive 2001/83/EC) the European Medicines Agency completed a review of a ganciclovir-containing product. It was concluded that in animal studies ganciclovir impaired fertility in male and female mice. Based on the occurrence of aspermatogenesis at exposures below therapeutic levels in animal studies, it is considered likely that ganciclovir may cause temporary or permanent inhibition of human spermatogenesis (Seidel et al., 2017; McLeroth et al., 2020). The safety of ganciclovir for use in pregnant women has not been established. However, it is known that ganciclovir readily diffuses across the human placenta (Tomi et al., 2011). According to the product information of this ganciclovir-containing product, it should not be used in pregnant women unless the clinical need for treatment of the woman outweighs the potential teratogenic risk to the foetus. Ganciclovir was found to be mutagenic and carcinogenic in humans (de Kanter et al., 2021) and teratogenic in animal reproduction studies (Pescovitz, 1999) . It is unknown if ganciclovir is excreted in human breast milk, but the possibility of ganciclovir being excreted in breast milk and causing serious adverse reactions in the breastfed infant cannot be excluded. Therefore, it is advised that breastfeeding be discontinued during treatment (Alcorn et al., 2002). From registration data in ECHA's C&L inventory it was noted that for valganciclovir (an oral prodrug that is rapidly converted to ganciclovir) is also classified as mutagenic, carcinogenic and associated with reproductive toxicity.

The elimination half-life in humans is short and bioavailability is relatively low, but information in human medicinal products (based on data from animal studies) indicates that the substance is

mutagenic, teratogenic, carcinogenic and affects fertility. It cannot be excluded that accumulation would occur with multiple doses of ganciclovir in horses. There is not residue distribution nor depletion data in horses. Therefore, based on the properties of the substance, only local treatment of keratitis, where the drug absorption is limited, is recommended. In this case, it can be accepted that ganciclovir 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.

Valacyclovir is an antiviral commonly used in humans for treatment of herpes virus infections. It is used in horses for the same indication. Valacyclovir is the prodrug of active acyclovir, obtained by addition of an L-valine amino acid. Valacyclovir is on the EU market as a human medicinal product.

Its antiviral activity is linked to its affinity to the enzyme thymidine kinase (TK). After valacyclovir is converted to acyclovir, it is phosphorylated by TK to the monophosphate form. Acyclovir monophosphate accumulates in cells infected with herpes virus and is converted by guanylate cyclase to acyclovir diphosphate and subsequently to the triphosphate form, which is an inhibitor of viral DNA polymerase. According to Papich (2021) resistance is possible because of changes in TK or in the DNA polymerase.

The information in human medicinal products indicates that the bioavailability of valacyclovir is 3.3 to 5.5-fold greater than that reported for oral acyclovir. The bioavailability is 54% and it is not affected by feeding status (fasted vs fed). Valacyclovir is eliminated in the urine, principally as acyclovir, which makes up more than 80% of the recovered dose; the metabolite CMMG represents about 14% of the recovered dose; the metabolite 8-OH-ACV is detected only in small amounts in urine (< 2% of the recovered dose); less than 1% of the administered dose of valacyclovir is recovered in the urine as unchanged drug. In patients with normal renal function the plasma elimination half-life is approximately 3 hours (following either single or multiple valacyclovir administrations). In patients with end-stage renal disease, the average elimination half-life increases to approximately 14 hours.

After oral valacyclovir administration to horses, the oral absorptions reported range from 26% to 48%, depending on the study, with peak concentrations in the range 4.2 to 5.26 mcg/mL (i.e. 20 to 26.6 mg/kg) (Papich, 2021). Garré et al. (2007, 2009) studied the oral bioavailability and pharmacokinetics of acyclovir and valacyclovir in vitro and following oral administration. In the clinical study three doses were administered to healthy adult horses: (i) 10 mg acyclovir/kg bw as intravenous infusion over 1 hour; (ii) 20 mg acyclovir/kg bw (tablets) via nasogastric intubation; 20 mg valacyclovir/kg bw (tablets) via nasogastric intubation. Peak concentration following intravenous acyclovir was 10 mg/ml and the half-life ranged between 5.05 and 11.9 hours for total and unbound acyclovir, respectively; very poor bioavailability was noted following oral acyclovir; oral valacyclovir resulted in 10-times higher C_{max} and 8-times higher bioavailability than that noted for oral acyclovir. Intravenous administration of 10 mg acyclovir/kg and oral administration of 20 mg valacyclovir/kg achieved therapeutic concentrations within the sensitivity range of equine herpesvirus type 1 (EHV-1) (Garré et al., 2007, 2009). The higher bioavailability of valacyclovir makes it an attractive candidate for the prophylactic and/or therapeutic treatment of horses. Results from PK/PD modelling showed that 40 mg valacyclovir/kg three times daily would reach therapeutic plasma concentrations. Oral administration in horses does not seem affected by feeding status (Papich, 2021).

Information in human medicinal products reveal no hazard for humans based on conventional safety pharmacology studies, repeated dose toxicity, genotoxicity, and carcinogenic studies (non-clinical data); valacyclovir did not affect fertility in male and female rats dosed orally and was not teratogenic in rats or rabbits. Valacyclovir is almost completely metabolised to acyclovir in humans (based on information in human medicinal products). Acyclovir is considered as not classifiable as to its carcinogenicity to humans by the IARC (Group 3). Subcutaneous administration of acyclovir in

internationally accepted tests did not produce teratogenic effects in rats or rabbits. In rats, foetal abnormalities and maternal toxicity were observed at subcutaneous doses of 100 micrograms/mL. A limited amount of data on the use of valacyclovir is available from pregnancy registries. Data following oral or intravenous acyclovir and post-marketing experience show no signs of malformations or toxicity. Acyclovir, the main metabolite of valacyclovir, is excreted in breast milk.

At therapeutic doses of valacyclovir no effects on the breastfed newborns/infants are anticipated according to information in human medicinal products (the anticipated dose ingested by the child is less than 2% of the therapeutic dose of intravenous acyclovir). No human fertility studies were performed with valacyclovir; no changes in sperm count, motility or morphology were reported in 20 patients after 6 months of daily treatment with 400 mg to 1000 mg acyclovir. At high parenteral doses of acyclovir, testicular atrophy and aspermatogenesis have been observed in rats and dogs.

No residue data are available from horses.

Valacyclovir is the prodrug of active acyclovir (proposed for inclusion in the list). A 10-times-higher C_{max} and 8-times-higher bioavailability were noted following oral administration of valacyclovir in horses compared to oral acyclovir. Considering also some of the pharmacokinetic and toxicological properties reported for acyclovir (e.g. its short elimination half-life), it can be accepted that valacyclovir 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.

4.3.4. Conclusion

Based on the above assessment and justifications, the following recommendations are proposed:

1. The following active substances, currently in Regulation (EU) 1950/2006 as amended by Regulation (EU) 122/2013, are proposed to be retained in the list, either without modification or with an amendment of the current entry as shown below:

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
i. Antibiotics			
Amikacin	for the treatment of septicemia in horses and foals	gentamicin, ceftiofur	better safety profile in the target animal
Azithromicin	to treat *Rhodococcus equi* infections susceptible to azithromicin	clarithromycin, erythromycin, gamithromycin, tulathromycin, doxycycline	added clinical benefit in cases of *R. equi* infections in foals that can be resolved as monotherapy or in combination with doxycycline only
Ofloxacin	treatment of external eye infections caused by gram-positive and gram-negative micro-organisms susceptible to ofloxacin	moxifloxacin	clinical experience; penetrates the entire cornea up to the anterior chamber of the eye
Polymyxin B	for the treatment of bacterial keratitis, topical use	ofloxacin, moxifloxacin	effective alternative to systemic treatments; different mechanism of action to other topical alternatives
ii. Antifungals			
Miconazole	for the treatment of fungal infection of the eye	natamycin, nystatin, voriconazole	broad spectrum of activity; less irritant compared to other topical antifungals
Nystatin	for the treatment of fungal and yeast	miconazole	treatment of choice for yeast infections

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
	infections of the eye and genital tract		
iii. Antivirals			
Acyclovir	to treat cases of equine herpes virus infection associated with complications, topical use only	ganciclovir	treatment of choice for ocular ulcers when the implication of a viral pathogen is suspected

2. The following active substances, currently in Regulation (EU) 1950/2006 as amended by Regulation (EU) 122/2013, are proposed to be removed from the list: griseofulvin, idoxuridine, isometamidium, ketoconazole, ponazuril, pyrimethamine, rifampicin, ticarcillin.

3. The following active substances, suggested for addition to the list in the survey to stakeholders, are proposed to be added to the list with an entry as shown below:

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
i. Antibiotics			
Clarithromycin	to treat *Rhodococcus equi* infections susceptible to clarithromycin	azithromycin, erythromycin, gamithromycin, tulathromycin, doxycycline	more active against *R. equi* in vitro than erythromycin or azithromycin; achieves greater concentrations in pulmonary epithelial lining fluid and alveolar macrophages than either erythromycin or azithromycin, though the half-life is shorter
Fusidic acid	topical treatment of eye infections caused by gram-positive bacteria susceptible to fusidic acid	ofloxacin, moxifloxacin	broad spectrum for treatment of gram-positive infections; primary choice in superficial, uncomplicated corneal ulcers and acute conjunctivitis in horses
Moxifloxacin	topical treatment of external eye infections caused by gram-positive cocci, gram-negative, atypical and anaerobic bacteria such as *P. aeruginosa* species susceptible to moxifloxacin	ofloxacin	advantageous pharmacokinetic profile; spectrum of activity includes gram-positive cocci and anaerobic bacteria that may be resistant to other quinolones
ii. Antifungals			
Amphotericin B	treatment of fungal pneumonia, systemic use	none identified	treatment of choice
Voriconazole	treatment of fungal keratitis, topical use	miconazole	broad spectrum of activity; available as oral and systemic formulations
iii. Antivirals			
Ganciclovir	to treat cases of equine herpes virus infection associated with complications, topical use	acyclovir, valacyclovir	wealth of evidence for the treatment of different virus-types causing herpetic infections
Valacyclovir	to treat cases of equine herpes virus infections, oral use	acyclovir	better pharmacokinetic profile and a different route of administration

4. The following active substances, suggested for addition to the list in the survey to stakeholders, are not proposed for inclusion: buparvaquone, ciprofloxacin, fluconazole, rifamycin sodium, tenoic acid (or thenoic acid), tobramycin.

4.4. Substances for respiratory disorders

4.4.1. Overview

	Active substance (ATCvet code)
Substances in Regulation (EU) 1950/2006 **proposed to be retained**, with or without amendment of the current entry	Ambroxol (QR05CB06); Fluticasone (QR03BA05); Ipratropium bromide (QR03BB01); Oxymetazoline (QR01AA05); Phenylephrine[33] (QS01FB01)
Substances in Regulation (EU) 1950/2006 **proposed to be removed**	Budesonide (QR03BA02)
Substances from stakeholders' survey **proposed for inclusion**	None
Substances from stakeholders' survey **not proposed for inclusion**	None

4.4.2. Review of the existing entries in Regulation (EU) 1950/2006, as amended by Regulation (EU) 122/2013, considering the survey results

A. Considerations on the essentiality of the substance(s)

Ambroxol is a secretolytic agent used in the treatment of respiratory diseases associated with viscous or excessive mucus. It displays mucokinetic properties, mucociliary activity, stimulation of surfactant production, anti-inflammatory and antioxidative actions (Allegra et al., 1985; Disse and Ziegler, 1987; Robertson, 1985). Local anaesthetic effects have also been described for this substance (Weiser, 2006). Due to all these properties, ambroxol restores the physiological clearance mechanisms of the respiratory tract and stimulates synthesis and release of surfactant by type II pneumocytes (Malerba and Ragnoli, 2008).

Ambroxol is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), as a stimulant of surfactant in the premature foal. No alternatives are identified, which is also stated as its specific advantage.

The entry in the current list is for a narrow indication. Alternatives such as bromhexine or dembrexine, which have MRL entries and are included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with a 'No MRL required' status and, in the case of dembrexine, a specific entry for *Equidae*, could constitute satisfactory alternative treatments for a wider indication (secretolytic agent, anti-tussive); there are veterinary medicinal products authorised for food-producing animals of the equine species containing dembrexine. The surfactant-stimulating properties of ambroxol are well described in the human medicine literature (Zavattini, 1987; Lusuardi et al., 1992; Hohlfeld et al., 1997). In a recent review, ambroxol was shown to exert additional activities (i.e. secretolytic, anti-inflammatory and local anaesthetic effects through sodium channel blocking at the level of the cell membrane), thus promoting mucus clearance, facilitating expectoration, and easing productive cough; these effects were considered relevant in the reduction of chronic obstructive pulmonary disease exacerbations in humans (Malerba and Ragnoli, 2008). While published scientific evidence in horses is scarce, expertise within the expert group preparing this scientific advice confirmed ambroxol is used as a surfactant-stimulant for premature foals, being the preferred clinical choice.

[33] This substance is discussed in detail in section 4.11 (substances for ophthalmology).

Alternative treatments for surfactant issues in premature foals are the use of steroids or harvesting surfactant from a healthy living donor (via a broncho-alveolar lavage) and transferring to the premature foal (via an endoscopic pulmonary lavage). For the expectorant properties dembrexine or acetylcysteine are satisfactory alternatives.

Respiratory disease associated with viscous or excessive mucous, if untreated, is potentially life-threatening and causes unacceptable suffering of the animal.

Ambroxol was mentioned (five times) in the survey to stakeholders proposing to remove it from the list since existing alternative treatments (i.e. bromhexine and acetylcysteine) yield equally satisfactory results. However, while alternative expectorants (e.g. dembrexine, bromhexine and acetylcysteine) are available, no published reports could be found supporting the view that these represent better clinical alternative to ambroxol for the stimulation of surfactant in foals. It is therefore accepted that ambroxol brings added clinical benefit for the stimulation of surfactant in foals.

A search in the veterinary medicines database does not retrieve any ambroxol-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance ambroxol is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: stimulation of surfactant in the premature foal. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Budesonide is a synthetic glucocorticoid used to treat lower respiratory conditions by reducing inflammation by decreased leukocyte migration to the site of inflammation and bronchodilator effects (Malerba and Ragnoli, 2008). At high doses, glucocorticoids result in immunosuppressive effects (Yasir et al., 2023).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), as an inhalation corticosteroid for control of allergic pulmonary disease. Beclomethasone is identified as its alternative and several reasons are given as specific advantages: inhalation corticosteroid therapy causes less adreno-cortical suppression, with more rapid return to normal function after therapy ends, and fewer systemic side effects than systemic corticosteroid therapy because of limited systemic absorption; inhalation allows reduced doses and local delivery of high concentrations of active substance and hence greater efficacy; budesonide is especially useful for control of mild-moderate disease and long-term maintenance therapy; additional substances with greater potency and different durations of effect than beclomethasone are required to titrate the dose based on clinical response and provide optimum disease control; budesonide has intermediate steroid potency between beclomethasone and fluticasone.

Allergic pulmonary disease, if untreated, is potentially life-threatening and causes unacceptable suffering of the animal.

Budesonide was mentioned (two times) in the survey to stakeholders. Contradictory proposals are noted: one responder suggested to 'add' it to the list (though this substance is already listed in Regulation (EU) 1950/2006, as amended by Regulation (EU) 122/2013) while a second responder proposed that the substance be removed from the list. Arguments provided in support of the view (that applies also to fluticasone) that budesonide (and fluticasone) cannot be considered essential were that, in the opinion of the responder, satisfactory alternative treatments for the proposed indication are authorised for food-producing animals of the equine species and neither of these two substances bring

added clinical benefit compared to other treatment options. Ciclesonide[34] is an inhalation corticosteroid available as a registered VMP, with an established maximum residue limit for the active substance and indicated for the alleviation of clinical signs of severe equine asthma (formerly known as recurrent airway obstruction (RAO) and summer pasture-associated recurrent airway obstruction (SPA-RAO)). The responder states that this substance offers an additional advantage with regards to minimising unwanted systemic effects when compared to beclomethasone (the treatment alternative currently captured in Regulation (EU) 1950/2006, as amended by Regulation (EU) 122/2013), budesonide and fluticasone; it is also stated that all the above-mentioned indications (i.e. control of allergic pulmonary disease, clinical signs of severe equine asthma and summer pasture-associated recurrent airway obstructions), which are not always distinguished or differentiated, can be treated with different inhalation corticosteroids. To justify this proposal, the responder refers to published data (provided for the marketing authorisation of the centrally authorised veterinary medicinal product containing ciclesonide) (Lavoie, 2015).

The arguments provided are noted. In functional in vitro assays of anti-inflammatory activity, the relative potency of inhalation corticosteroids was assessed. The active moieties of ciclesonide and budesonide were roughly equipotent at repressing the activity of the pro-inflammatory transcription factor nuclear factor-κB in A549 lung epithelial cells, whereas fluticasone was approximately 10-fold more potent (Biggadike et al., 2004). It is acknowledged that in vitro potency alone does not predict in vivo activity because the pharmacokinetic profile and drug delivery devices influence both pulmonary efficacy and therapeutic ratio. Therefore, small differences in potency may not be clinically meaningful. Moreover, ciclesonide use is at present for the time course of 10 days only. Some studies support that potency differences observed might not translate into clinical relevance with chronic therapy (Leung et al., 2003, 2005; Nave et al., 2004).

The centrally authorised ciclesonide-containing VMP is indicated for severe cases of equine asthma, not for mild to moderate cases of equine asthma, including subtypes. The older scientific literature refers to different types of equine non-infectious lower airway diseases as distinctly separate conditions (e.g. RAO, inflammatory airway disease-IAD, eosinophilic pneumonia, etc.). These have been amalgamated under equine asthma (mild, moderate, severe). However, there are likely differences in pathogenesis since different cell-types dominate. For example, severe equine asthma is dominated by neutrophils and allergic reactions, whereas mild-moderate equine asthma is more dominated by mast cells, or eosinophils or mixed cell populations and not always linked with allergies. Since fluticasone was shown to be more potent than budesonide, which compared to the authorised ciclesonide treatment alternative, and is indicated for mild to moderate equine asthma, including subtypes, it is proposed to keep fluticasone on the list, but not budesonide.

A search in the veterinary medicines database does not retrieve any budesonide-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance budesonide is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Fluticasone is a synthetic glucocorticoid with similar properties to budesonide. It is more potent than beclomethasone dipropionate, budesonide, triamcinolone acetonide, and mometasone furoate for inhibiting human T-cell migration and proliferation, inhibiting CD4+ T-cell cytokine and basophil

[34] Ciclesonide (*Equidae*), European public MRL assessment report (https://www.ema.europa.eu/en/documents/mrl-report/ciclesonide-equidae-european-public-maximum-residue-limit-assessment-report-epmar-cvmp_en.pdf)

histamine release, attenuating adhesion molecule expression, stimulating inflammatory cell apoptosis, and inducing cellular antiprotease release (Johnson, 1998). Systemically, in vitro experiments show that fluticasone activates glucocorticoid receptors, inhibits nuclear factor-κB, and inhibits lung eosinophilia in rats (Spadijer et al., 2017). It is a very potent glucocorticoid with high topic anti-inflammatory glucocorticoid activity and low systemic bioavailability (Hogger and Rohdewald, 1994; Sastre, 1997). It is used as an inhalation agent for horses suffering from chronic obstructive pulmonary disease, chronic obstructive bronchitis, recurrent airway obstruction (terms now all amalgamated under equine asthma) (Giguere et al., 2002).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), as an inhalation corticosteroid for control of allergic pulmonary disease. Beclomethasone is identified as its alternative and several reasons are given as specific advantages: inhalation corticosteroid therapy causes less adreno-cortical suppression, with quick rebound after therapy ends and fewer systemic side effects than systemic corticosteroid therapy because of limited systemic absorption; inhalation allows local delivery of high concentrations of active substance and hence greater efficacy; fluticasone is especially useful for control of mild-moderate disease and long-term maintenance therapy; additional substances with greater potency and different durations of effect than beclomethasone are required to titrate the dose based on clinical response and provide optimum disease control; fluticasone is 50% more potent than beclomethasone and has longer half-life (6 hours versus 2.8 hours), providing added benefit for more severely affected or refractory cases.

As previously indicated, fluticasone has been shown to be more potent than budesonide in functional in vitro assays of anti-inflammatory activity while the active moieties of ciclesonide and budesonide were roughly equipotent (Biggadike et al., 2004). The centrally authorised ciclesonide-containing VMP, which constitutes an alternative treatment, is indicated for severe cases of equine asthma and not for mild to moderate cases of equine asthma, including subtypes; fluticasone could thus qualify as bringing added clinical benefit for all types of asthma, including cases refractory to ciclesonide therapy.

Allergic pulmonary disease (including mild to moderate cases of equine asthma and subtypes), if untreated, is potentially life-threatening and causes unacceptable suffering of the animal.

Fluticasone was mentioned (two times) in the survey to stakeholders. As for budesonide, contradictory proposals are noted: one responder suggested to 'add' it to the list (though this substance is already listed in Regulation (EU) 1950/2006, as amended by Regulation (EU) 122/2013) while a second responder proposed that the substance be removed from the list. For arguments provided in support of the latter please refer to budesonide (see above).

A search in the veterinary medicines database does not retrieve any fluticasone-containing veterinary medicinal product authorised for use in equine species (neither in other animal species). As indicated previously, there is an alternative treatment (a ciclesonide-containing VMP) authorised for the treatment of severe cases of equine asthma, not for mild to moderate cases of equine asthma, including subtypes. There are likely differences in pathogenesis since different cell-types dominate. For example, severe equine asthma is dominated by neutrophils and allergic reactions, whereas mild-moderate equine asthma is more dominated by mast cells, or eosinophils or mixed cell populations and not always linked with allergies.

The substance fluticasone is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: for control of allergic pulmonary disease including mild to moderate cases of equine asthma and subtypes via inhalation. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Ipratropium bromide is a quaternary ammonium derivative of atropine belonging to the muscarinic antagonists' class and acting as anticholinergic agents. It is indicated for the treatment of lung diseases due to its bronchodilator efficacy. It is a short-acting active substance (3-6 hours).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), for bronchodilatation. No alternatives are identified in the current list and the specific advantage captured is its anticholinergic action, which makes it a necessary therapeutic choice as, in some cases, it is more efficacious than β-agonists.

The anticholinergic activity of ipratropium bromide in the pathophysiology of human chronic obstructive pulmonary disease (COPD) and it's use as an alternative to beta-agonists is well established (Massey and Gotz, 1985). Similarly, the effectiveness for horses is described in the literature (Duvivier et al., 1997, 1999) and data from clinical trials suggest that ipratropium bromide is an effective bronchodilator for equine asthma, at rest, but has little effect during the recovery period from strenuous exercise (Duvivier et al., 1999). In a randomized controlled trial, revatropate (a selective M(3) and M(1) muscarinic antagonist) was evaluated against ipratropium (a non-specific antimuscarinic agent) aiming to show its potential advantages. Inhaled revatropate and ipratropium had similar effects on equine airway function, with no significant difference between their efficacies; however, it was noted that only revatropate significantly improved equine clinical scores for breathing effort (dyspnoea) (McGorum et al., 2013). An alternative for bronchodilation included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 is clenbuterol, a beta-2 adrenergic receptor stimulant for which there are veterinary medicinal products authorised for use in food-producing animals of the equine species.

Bronchoconstriction due to equine asthma, if untreated, is potentially life-threatening and causes unacceptable suffering of the animal.

Ipratropium bromide was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any ipratropium-bromide-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance ipratropium bromide is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: as a bronchodilator in horses with asthma. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal. Available alternatives can be given orally or intravenously; ipratropium bromide is given via the inhalation route for immediate effects.

Oxymetazoline is an imidazoline derivative found in topical ophthalmic and nasal decongestants as well as in topical creams. Imidazolines are sympathomimetic agents with primary effects on α1-adrenergic receptors and little effect on β-adrenergic receptors, if at all (Khan, 2022). In humans, effectiveness of imidazoline decongestants is well established and are generally used as topical vasoconstrictors in nose and eyes for temporary relief of nasal congestion due to colds, hay fever, upper respiratory allergies, or sinusitis (Liu et al., 2023).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), for treatment of nasal oedema. Phenylephrine is identified as its alternative and the specific advantage captured is that substance being an α-adrenoceptor agonist with strong vasoconstrictive properties which is used in preference to phenylephrine due to the fact that it is longer-acting.

While published scientific evidence in horses is scarce, expertise within the expert group preparing this scientific advice confirmed oxymetazoline is used for the treatment of nasal oedema in *Equidae*, being

the preferred clinical choice; it is a safe and effective treatment option (Yokota et al., 2013). Phenylephrine is an alternative treatment proposed for addition to the list.

Nasal oedema, if untreated, is potentially life-threatening and causes unacceptable suffering of the animal.

Oxymetazoline was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any oxymetazoline-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance oxymetazoline is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: treatment of nasal oedema. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Phenylephrine is discussed in detail in section 4.11 (substances for ophthalmology) where it is proposed for treatment of glaucoma and epiphora (please refer to section 4.11 for the detailed assessment of the substance). Phenylephrine is also proposed under this section for the treatment of nasal oedema. This indication for phenylephrine is currently included in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013).

Nasal oedema can develop in anesthetized horses. Nasal congestion can result in significant upper airway obstruction after extubating. While oedema quickly disappears in most horses after extubating and does not require treatment, occasionally it may be severe enough to cause a functional obstruction requiring treatment, i.e. reintubation. Phenylephrine treatment reduces the need for insertion of nasal tubes during recovery (Bednarski, 2009). Oxymetazoline is retained in the list as alternative.

Nasal oedema, if untreated, may cause unacceptable suffering of the animal and could possibly be life-threatening.

The substance phenylephrine is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: treatment of nasal oedema. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Knowledge regarding respiratory medicines in horses for this assessment was derived from textbooks, review articles, retrospective studies and clinical trials.

B. Considerations regarding consumer safety

Ambroxol is structurally related to bromhexine and dembrexine and is present in (human) medicinal products as ambroxol hydrochloride. All three substances are very lipophilic.

Both dembrexine and bromhexine are listed in Table 1 of the Annex to Commission Regulation (EU) No 37/2010, but only dembrexine has an MRL entry for *Equidae* (with a 'No MRL required' status). Their respective European Public MRL Assessment Reports can be consulted on the Agency's corporate website[35].

Ambroxol is rapidly and completely absorbed after oral administration and its systemic bioavailability is high (approximately 80%) whereas dembrexine and bromhexine have a strong first-pass-effect

[35] Maximum residue limit assessment reports | European Medicines Agency (europa.eu)

(EMEA/MRL/080/96-FINAL; EMEA/MRL/503/98-FINAL; Malerba and Ragnoli, 2008; Löscher and Richter, 2016). In humans, the ambroxol maximum blood concentration is reached after approximately two hours (T_{max}); 90% of ambroxol is bound to plasma protein, is rapidly distributed and has high affinity to lung tissue (Malerba and Ragnoli, 2008; Yang et al., 2015). Ambroxol in horses and humans is not completely metabolised. Half of the unchanged drug is hydroxylated, and a small amount oxidised (Uboh et al., 1991; Hug, 2008). Excretion of ambroxol in horses (Uboh et al., 1991), rabbits and humans, takes place almost exclusively via the kidneys; in dogs, approximately equal parts can be found in faeces and urine; rat excretes approximately 30% by the renal route and 70% via faeces (Hammer et al., 1978). Comparable half-life values for ambroxol were found after oral administration in rats (25h), dogs (20h) and humans (22h) (Hammer et al., 1978). However, after intravenous administration, an elimination half-life of about 10 hours was reported for humans (Malerba and Ragnoli, 2008). Also, a half-life in humans of 7 to 12 hours is indicated (Drugbank, 2023a). Rabbits clearly differ from the other species with a half-life of approximately two hours (Hammer et al., 1978). In comparison, the half-life for dembrexine (an alternative to ambroxol) in horses is about one to three hours (Löscher and Richter, 2016). No drug accumulation and no gender differences were observed in humans (Seki et al., 1977; Yang et al., 2015).

According to the chemical classification proposals notified by companies to the European Chemicals Agency (ECHA), ambroxol is of low acute toxicity (i.e. harmful if swallowed), and is seriously to mildly locally toxic to eye, skin and respiratory tract (ECHA, 2023a). No toxicological information is available in the ECHA registration dossier. Malerba and Ragnoli (2008) cite several toxicity studies with ambroxol, including repeated-dose and long-term studies from the period 1973-1986. However, these are not available in the English language (Malerba and Ragnoli, 2008). According to Malerba and Ragnoli (2008), subacute and chronic oral toxicity studies in rats, rabbits and dogs have not shown "any serious functional adverse effects or distinct target organ toxicity up to 2500 mg/kg (rat) and 250 mg/kg (rabbit and dog)". The lowest NOAEL observed was 10 mg/kg bw/day in dogs (from a 52-week study), i.e. dogs are slightly less sensitive to ambroxol than to dembrexine (NOAEL 2 mg/kg bw/d, 90 day–study; EMEA/MRL/080/96-FINAL). Other relevant outcomes noted are: "ambroxol was not embryotoxic and teratogenic at oral doses up to 3000 mg/kg in rats and 200 mg/kg in rabbits, (…) did not impair fertility and postnatal development, (…) was not mutagenic in the Ames and mouse bone marrow micronucleus test". For long-term studies there was no carcinogenic potential observed in mice (max. 800 mg/kg) and rats (max. 1000 mg/kg) over 105 and 116 weeks (treated diet) (Malerba and Ragnoli, 2008).

No publicly available residue studies are available for ambroxol. Similarly, there are no studies about the transfer of the active substance into milk. However, ambroxol as bromhexine and dembrexine is expected to get into milk (i.e. very lipophilic substances). Dembrexine is rapidly eliminated from treated horses. It is expected that exposure to residues of dembrexine from ingestion of animal commodities is well below the ADI within 24 hours of the last treatment (EMEA/MRL/080/96-FINAL); this is similarly expected for bromhexine in cattle, pigs and poultry, which is extensively metabolised and eliminated (EMEA/MRL/503/98-FINAL). Noting the increased half-life of ambroxol compared to dembrexine, it can nevertheless be concluded that residues in food commodities would lead to consumer exposure well below the ADI within a matter of days of treatment.

In humans, a referral revealed that there is a small risk of allergy and skin reactions with ambroxol and bromhexine used as expectorants in human medicinal products (EMA/168579/2015).

Therefore, considering the available data for horses, noting that pharmacokinetic data for species other than horses indicates rapid and almost complete absorption of ambroxol, rapid elimination and no potential for accumulation, and considering the apparent low toxicity of the substance, it can be

accepted that ambroxol 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.

Fluticasone, either in the form of propionate (i.e. fluticasone-17-propionate, hereinafter FP) or furoate (i.e. fluticasone furoate, hereinafter FF) is a tri-fluorinated glucocorticoid used in human medicinal products. The furoate salt is a relatively newly developed active substance with deviating properties and enhanced effects compared to the propionate salt (Biggadike, 2011). Both are presented as solids and/or powders commonly administered by inhalation (either as a suspension or as dry inhalation). They are very lipophilic and insoluble in water.

Inhaled FF has a longer lung retention time in comparison to FP. Time for 90% absorption from the lung in human males was 20-30 hours for FF and 8 hours for FP (Allen et al., 2013). The mean bioavailability values after single inhalation administration in humans were, depending on the inhaler system, 11.9% (95% CI: 9.0-15.7%) and 16.6% (95% CI: 13.6-20.3%) (Mackie et al., 2000), or 9.0% (90% CI: 6.9, 11.7) (Allen et al., 2013) for FP, expressed as geometric means; 6.3% (90% CI: 5.2, 7.6), 13.3% (90% CI: 11.0, 16.0) or 18.4% (90% CI: 15.2, 22.1) for FF, expressed as geometric means (Allen et al., 2013). Bioavailability following oral administration was found to be negligible compared to that of the inhalation route due to an almost complete hepatic first pass inactivation (Dempsey et al., 1999; Allen et al., 2013).

The product information of human medicinal products suggests that both FP and FF are rapidly and widely distributed in the body and bound to tissue due to their lipophilic nature. With systemic exposure, both substances are subject to rapid inactivation from the systemic circulation (i.e. high clearance), predominantly by metabolism via the cytochrome P450 system in the liver to inactive carboxylic acid derivatives. The two substances do not have common metabolites as the ester bindings are stable and the common steroidal backbone fluticasone (M 444.51 g/mol) appears not to be a breakdown product (Biggadike, 2011). Elimination of FF and FP takes place almost completely via the faeces.

The current text in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), states "fluticasone is 50% more potent than beclomethasone and has longer half-life (6 hours versus 2.8 hours)" and this is considered to relate to FP only. In male humans, Minto et al. (2000) found the plasma elimination half-life for inhaled FP to be 2.7 hours (1.4±5.2), absorption included, whereas Allen et al. (2013) noted 11 hours; for inhaled FF, the plasma elimination half-life was observed to be in the range of 17-24 hours (also in male humans) (Allen et al., 2013). The product information of human medicinal products suggests plasma half-lives of 7.8 hours for FP and 15 hours for FF, after intravenous administration.

For both FP and FF, proposals for chemical classifications according to the CLP Regulation[36] were notified by companies to ECHA (ECHA, 2023b; ECHA, 2023c). Some data submitters indicated the classification as toxic to reproduction for both, i.e. suspected of damaging the fertility or the unborn child. A chemical registration dossier is not available.

The product information of human medicinal products containing FP and/or FF as active substances do not suggest genotoxicity or carcinogenicity; suspected developmental toxicity for FP is reported. Commonly found contraindications like hypersensitivity or local irritation following administration are noted.

[36] 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

Therefore, considering the available pharmacokinetic data that indicates both fluticasone propionate and fluticasone furoate are extensively metabolised and rapidly eliminated despite assumed wide distribution in the tissues, it can be accepted that fluticasone (i.e. either salt, propionate or furoate) 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.

Ipratropium bromide, due to its bronchodilator effect, is used in horses for treatment of lung diseases. It is a short-acting substance (half-life 3-6 hours), with limited gastric absorption, which is clinically important (Almadhoun and Sharma, 2023). It is commonly administered via inhalation, producing a local effect without presenting significant systemic absorption.

In humans, the bioavailability of the drug by inhalation ranges 0.03–6.9%. Brittain (2003) found bioavailability of 6.9% following inhalation of 2 mg ipratropium bromide; almost half (48%) of the drug is excreted through the faeces, with around 2.8% being excreted through kidneys; the elimination half-life is 2-3.8 hours. According to Almadhoun and Sharma (2023), about 80% to 100% is excreted in the urine (i.e. less than 20% is excreted in faeces). As stated above, it is a short-acting bronchodilator with a half-life of 3 to 6 hours (Almadhoun and Sharma, 2023).

Human data also suggests ipratropium bromide is poorly absorbed from the gastrointestinal tract, with an apparent systemic availability of approximately 2%; it is known to be highly distributed to tissues. Its protein binding is very low (0-9% of the administered dose) and the concentration of circulating parent drug is also low (Drugbank, 2023b). It has been reported to be partly metabolised and excreted in urine and faeces, either as parent drug or metabolites (eight have been identified, one of them being N-isopropyl-methyl-nortropium bromide); none of these have significant anticholinergic activity. Insignificant amounts of ipratropium are expected to be excreted into breast milk, since ipratropium is a lipid-insoluble quaternary base and is poorly absorbed following inhalation (Brittain, 2003).

Data from laboratory animals (i.e. dog and rat) is also available. When orally treated with radioactive marked (14C) ipratropium, blood concentrations reached plateau over a period of 2 to 8 hours after administration; subsequent elimination occurs with a half-life of 7 hours in rat and 10 hours in dog. Following intravenous administration, half-life of 1.9 hours in rat and 3.4 hours in dog are noted. In the rat, biliary excretion accounts for 3.2% and renal excretion is 5.5% following oral administration; following intravenous administration, biliary excretion accounts for 17.7% and renal excretion is 58%. In dogs, renal excretion averaged 28% and 55% following oral and intravenous administration, respectively (Förster et al., 1976).

Förster et al. (1976) compared blood concentrations as area-under-the-curve (AUC) and renal excretion following oral and intravenous administration to calculate 12% absorption in the rat and 38% in the dog, not including absorption from gastrointestinal tract; for rat, after 1-3 hours, absorption was calculated at 17-35% including from the gastrointestinal tract. Four metabolites and the parent compound could be detected in rat's urine after 8 hours; for dogs, the percentage of parent compound fell from 81% (maximum 1 hour after administration) to 20% after 47 hours.

Ipratropium bromide was not shown to have carcinogenic, teratogenic, or mutagenic potential and it did not present effects on fertility (Drugbank, 2023b). While it is noted that the substance has no MRL assessment, it is structurally related to its derivative, atropine. Significant clinical differences between the two drugs are noted, and the poor gastric absorption of ipratropium bromide has been demonstrated. For atropine, no residue depletion studies were considered necessary as pharmacokinetic data indicated rapid absorption and elimination (EMEA/MRL/517/98-FINAL).

Therefore, considering the available pharmacokinetic data that indicates that ipratropium bromide is poorly absorbed from the gastrointestinal tract and rapidly eliminated despite being widely distributed

in tissues, and considering its apparent low toxicity, it can be accepted that it 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.

Oxymetazoline, as previously indicated, is a sympathomimetic agent with primary effects on α1-adrenergic receptors (Khan, 2022). As other imidazole derivatives, it is quickly absorbed through mucosal membranes (McEvoy, 2006). In humans, imidazoline decongestants are generally used as topical vasoconstrictors in the nose for temporary relief of nasal congestion due to colds, hay fever, upper respiratory allergies, or sinusitis. When sprayed intranasally, single dose oxymetazoline relieved nasal congestion and improved nasal airflow in patients with acute coryzal rhinitis, for up to 12 hours (Druce et al., 2018).

In humans, effects on α-receptors from systemically absorbed oxymetazoline hydrochloride may persist for up to 7 hours, after a single dose. Following intranasal application, local vasoconstriction usually occurs within 5-10 minutes and persists for 5-6 hours, with a gradual decline over the next 6 hours. Following topical application, local vasoconstriction usually occurs within minutes and may persist for up to 6 hours. Occasionally, enough oxymetazoline may be absorbed to produce systemic effects (McEvoy, 2006).

The product information of human medicinal products suggests that oxymetazoline has a rather short terminal half-life, is moderately bound to plasma proteins and is minimally metabolised hepatically (Mahajan, 2011). While the excretion of oxymetazoline following nasal, topical, or ophthalmic administration has not been fully characterized in humans, it is considered that renal excretion is predominant.

With regards to its acute and local toxicity, according to the notified classification proposals provided by companies to ECHA, the pure chemical, oxymetazoline, may present a hazard if swallowed or inhaled or if it comes in contact with the eye (ECHA C&L, 2023). However, no toxicological information is available in the ECHA registration dossier. The product information of human medicinal products containing oxymetazoline do not suggest that the substance is mutagenic or clastogenic. Similarly, available product information did not indicate any potential for developmental toxicity, nor is the substance reported to be associated with an increased incidence of neoplastic or proliferative changes.

Considering the available pharmacokinetic data indicating that oxymetazoline is rapidly eliminated even though no specific pharmacokinetic data in horses could be found, it can be accepted that it 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.

4.4.3. Assessment of new substances proposed to be added to the list in the stakeholders survey

None proposed.

4.4.4. Conclusion

Based on the above assessment and justifications, the following recommendations are proposed:

1. The following active substances, currently in Regulation (EU) 1950/2006 as amended by Regulation (EU) 122/2013, are proposed to be retained in the list, either without modification or with an amendment of the current entry as shown below:

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Ambroxol	stimulation of surfactant in premature foals	steroids, bromhexine, dembrexine, surfactant transfer from healthy donor	preferred clinical choice for premature foal
Fluticasone	for control of allergic pulmonary disease including mild to moderate cases of equine asthma and subtypes via inhalation	beclomethasone	inhalation leads to less adreno-cortical suppression, quicker rebound after therapy ends and fewer systemic side effects than systemic corticosteroid therapy because of its limited systemic absorption; especially indicated for control of mild-moderate and refractory severe asthma as well as long-term maintenance therapy
Ipratropium bromide	as a bronchodilator in horses with mild-moderate asthma	clenbuterol	anticholinergic action, as an alternative to beta-agonists
Oxymetazoline	treatment of nasal oedema	phenylephrine	α-adrenoceptor agonist with strong vasoconstrictive properties and longer acting effect
Phenylephrine[37]	treatment of nasal oedema	oxymetazoline	reduces the need for insertion of nasal tubes during recovery

2. The following active substance, currently in Regulation (EU) 1950/2006 as amended by Regulation (EU) 122/2013, is proposed to be removed from the list: budesonide.

4.5. Substances for cardiology

4.5.1. Overview

	Active substance (ATCvet code)
Substances in Regulation (EU) 1950/2006 **proposed to be retained**, with or without amendment of the current entry	Amiodarone (C01BD01); Quinidine sulfate/gluconate (QC01BA01)
Substances in Regulation (EU) 1950/2006 **proposed to be removed**	Digoxin (QC01AA05); Procainamide (QC01BA02); Propranolol (QC07AA05)
Substances from stakeholders' survey **proposed for inclusion**	Propafenone (QC01BC03); Quinapril (QC09AA06); Sotalol (QC07AA07); Verapamil (QC08DA01)
Substances from stakeholders' survey **not proposed for inclusion**	Flecainide (QC01BC04)

[37] This substance is discussed in detail in section 4.11 (substances for ophthalmology).

4.5.2. Review of the existing entries in Regulation (EU) 1950/2006, as amended by Regulation (EU) 122/2013, considering the survey results

A. Considerations on the essentiality of the substance(s)

Amiodarone belongs to the class III anti-arrhythmic drugs. It blocks potassium currents that cause repolarization of the heart muscle during the third phase of the cardiac action potential, thus, increasing the duration of the action potential as well as the effective refractory period for cardiac cells. The cardiac muscle cell excitability is reduced, preventing and treating abnormal heart rhythms. Unlike other class III agents, amiodarone also interferes with the functioning of beta-adrenergic receptors, sodium channels and calcium channels (Florek et al., 2023).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) as an anti-dysrhythmic for systemic and oral treatment of atrial fibrillation, supraventricular and ventricular tachycardias. Alternatives identified in the current list include quinidine sulphate, procainamide and propranolol. The specific advantages captured in the current list are its different mode of action from the alternatives (class III anti-dysrhythmic); new evidence that amiodarone is effective in atrial fibrillation and better than alternative quinidine sulphate; effective for different types of arrhythmias including ventricular arrhythmias.

Quinidine sulphate/gluconate has been and remains the first-choice medication for the treatment of atrial fibrillation in horses (McGurrin, 2015). However, it is still considered necessary to have an available alternative to quinidine sulphate/gluconate. Amiodarone does add clinical benefit for systemic and oral treatment of atrial fibrillation, supraventricular and ventricular tachycardias; amiodarone has been shown to be effective in treatment of drug-refractory ventricular tachycardia (De Clercq et al., 2007) and supraventricular tachyarrhythmias other than atrial fibrillation in the horse (Whelchel et al., 2017). While two of the currently listed alternatives, procainamide and propranolol are proposed to be removed from the list as these are considered not to bring added benefit compared to other treatment options, from the survey propafenone, sotalol and verapamil are proposed to be added since an added benefit is recognized for each of these three (see below).

Atrial fibrillation, supraventricular and ventricular tachycardias, if untreated, are life-threatening and cause unacceptable suffering of the animal.

Amiodarone was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any amiodarone-containing veterinary medicinal products authorised for use in equine species (neither in other animal species).

The substance amiodarone is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: for systemic and oral treatment of atrial fibrillation, supraventricular and ventricular tachycardias. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Digoxin is a cardiac glucoside with positive inotropic and negative chronotropic action, i.e. it increases the force of the heartbeat and decreases the heart rate. Two principal mechanisms of action are responsible for these effects: reversible inhibition of Na + K+ ATPase enzyme, which results in increased intracellular sodium and calcium levels in the myocardial cells causing an increased contractile force of the heart, and stimulation of the parasympathetic nervous system via the vagus nerve, resulting in a decrease in heart rate.

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for treatment of heart failure. No alternatives are identified in the current list and the specific advantage captured is that digoxin is also the only treatment for the side effects of quinidine treatment.

Digoxin is indeed indicated for the treatment of heart failure, ventricular rate control in supraventricular tachycardia or atrial fibrillation in horses. There are no alternatives for treatments authorised for this indication in horses and digoxin remains the drug-of-choice. For supraventricular tachycardia or atrial fibrillation, other substances are retained in the list and would be a preferred clinical option. Thus, it is proposed that the current indication remain unchanged.

Heart failure, if untreated, is life-threatening and causes unacceptable suffering of the animal.

Digoxin was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any digoxin-containing veterinary medicinal products authorised for use in equine species. There are veterinary medicinal products authorised for use in species other than the equine (i.e. cat and dog).

The substance digoxin is proposed to be qualified as essential because no satisfactory alternative treatments are authorised for food-producing animals of the equine species for the following indication: treatment of heart failure.

Procainamide is a class IA antiarrhythmic drug and binds to fast sodium channels inhibiting recovery after repolarization. It also prolongs the action potential and reduces the speed of impulse conduction (Thompson, 2023). It has similar electrophysiological actions as quinidine (Barrel, 2015). In equine medicine, it is used to treat a variety of supraventricular and ventricular arrhythmias (Muir and McGuirk, 1985), and is also used for the treatment of atrial fibrillation and subsequently persistent atrial tachycardia (Barrell, 2015).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for treatment of cardiac arrhythmias. The identified alternatives in the current list are quinidine sulphate and quinidine gluconate, and propranolol and the specific advantages are as follows: anti-dysrhythmic agent; use is rare but important therapeutic choice, different mode of action necessary for different types of arrhythmias.

For the treatment of cardiac arrhythmias quinidine, as quinidine sulphate and quinidine gluconate, is considered the drug-of-choice and is retained in the list of essential substances. Procainamide has not demonstrated antiarrhythmic activity comparable to that of quinidine (Muir and McGuirk, 1985). Other alternatives are retained, which have shown added clinical benefit compared to the drug-of-choice and it is considered that procainamide does not add clinical benefit compared to any of these: amiodarone has added benefit for treatment of atrial fibrillation, supraventricular and ventricular tachycardias; propafenone has added benefit by means of its mechanism of action, i.e. a sodium channel antagonist that slows down the influx of sodium ions into the myocardium decreasing the excitability of the heart; sotalol is more suitable in horses requiring long-term anti-arrhythmic therapy; verapamil, being a calcium-channel blocker, is also proposed to be added to the list. It is considered that procainamide does not bring added clinical benefit compared to any of these.

Cardiac arrhythmias, if untreated, are life-threatening and cause unacceptable suffering of the animal.

Procainamide was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any procainamide-containing veterinary medicinal products authorised for use in equine species (neither in other animal species).

The substance procainamide is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indication in animals of the equine species; it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Propranolol is a non-selective beta-adrenergic antagonist, also classified as a class II antiarrhythmic. It exerts its response by competitively blocking beta-1 and beta-2 adrenergic stimulation in the heart, typically induced by epinephrine and norepinephrine. In humans, it is used to treat hypertension, angina, supraventricular and ventricular arrhythmias and myocardial infarction (Grandi and Ripplinger, 2019).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for treatment of cardiac arrhythmias in horses. The identified alternatives in the current list are quinidine sulphate and quinidine gluconate, and procainamide, and the specific advantages are as follows: anti-hypertensive, which is used because it also exerts some anti-arrhythmic activity; use is rare but important therapeutic choice; due to the different pathophysiology of arrhythmias it is essential to have a variety of different acting medicines in order to be able to treat the specific condition; use of these medicines consists usually of a single treatment to convert back to normal rhythm, which may have to be repeated on only rare occasions.

Propranolol is rarely used in horses, primarily for rate control in supraventricular tachycardia and atrial fibrillation, for catecholamine-induced arrhythmias, and for unresponsive supraventricular and ventricular arrhythmias (Hinchcliff et al., 1991). Quinidine sulphate and quinidine gluconate are retained in the list of essential substances, and it is considered that propranolol does not bring added clinical benefit compared to quinidine, despite its mode of action. Sotalol, which is proposed to be added to the list of essential substances for an equivalent indication, is considered superior to propranolol in its combined class II and III antiarrhythmic actions. That is supported by data in humans from a study comparing the antiarrhythmic efficacy of sotalol and propranolol in 181 patients with organic heart disease and frequent repetitive ventricular premature complexes. Sotalol showed better efficacy results than propranolol in suppressing ventricular arrhythmias (Deedwania, 1997). It is considered that propranolol does not bring added clinical benefit compared to quinidine nor sotalol in treatment of cardiac arrhythmias in horses.

Cardiac arrhythmias, if untreated, are life-threatening and cause unacceptable suffering of the animal.

Propranolol was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any propranolol-containing veterinary medicinal products authorised for use in equine species (neither in other animal species).

The substance propranolol is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indication in animals of the equine species; it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Quinidine sulphate and gluconate are class 1A antiarrhythmic drugs causing increased action potential duration and prolongation of QT interval. Quinidine is a D-isomer of the antimalarial agent quinine. It has a narrow therapeutic window; complications of quinidine toxicity may include hypotension, cardiovascular collapse, depression, ataxia, weakness, colic, nasal mucosal congestion, convulsions, acute laminitis, dyspnea, and sudden death (Muir and McGuirk, 1985; Reef et al., 1995).

Quinidine sulphate and gluconate are listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for treatment of cardiac arrhythmias. The identified alternatives in the current list are procainamide and propranolol and the specific advantages are as follows: anti-dysrhythmic agent; use is rare but important therapeutic choice, different mode of action necessary for different types of arrhythmias; treatment of choice for atrial fibrillation.

Quinidine sulphate/gluconate has been and remains the first-choice medication for the treatment of atrial fibrillation in horses (McGurrin, 2015). Oral administration of quinidine sulphate is the most common treatment option for treatment of atrial fibrillation in horses, with reported success rates 70 to 80%, despite the various side effects (Burns et al., 2022). Intravenous administration of quinidine gluconate is also safe and effective for treatment of atrial fibrillation in some horses (Muir 3rd et al., 1990). As indicated previously, it is still considered necessary to have available alternatives to quinidine sulphate/gluconate and a number of substances are proposed for inclusion in the list based on their added clinical benefit.

Cardiac arrhythmias, if untreated, are life-threatening and cause unacceptable suffering of the animal.

Quinidine sulphate and quinidine gluconate were not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any quinidine-containing veterinary medicinal products authorised for use in equine species (neither in other animal species).

The substances quinidine sulphate and quinidine gluconate are proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: treatment of cardiac arrhythmias. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering for the animal.

Knowledge regarding substances for cardiology in horses for this assessment was derived from textbooks, review articles, and retrospective studies.

B. Considerations regarding consumer safety

Amiodarone is a class III antiarrhythmic drug. It can be administered intravenously and orally in animals (i.e. horses and dogs). Amiodarone is authorised in the EU as human medicinal products.

It is a lipophilic powder with a pKa of approximately 6.6, is widely distributed throughout the body and can accumulate and persist in adipose tissue. Levels in myocardial cells are significantly higher than in plasma. Amiodarone is metabolised by the liver into the active metabolite desethylamiodarone. Amiodarone contains high levels of iodine and interferes with normal thyroid function in humans (Narayana et al., 2011). Clinical experience with amiodarone in veterinary patients is limited. After oral administration of a single dose in normal dogs, amiodarone's plasma half-life averaged 7.5 hours, but repeated dosing increased its half-life from 11 hours to 3.2 days (Papich, 2021).

In horses amiodarone has a low oral bioavailability, ranging from 6-34% and peak levels of amiodarone and desethylamiodarone occur about 7-8 hours after oral administration (Plumb, 2015); oral absorption is inconsistent and oral administration is therefore not clinically recommended (Papich, 2021). After intravenous administration of 5 mg/kg/h for 1 hour followed by 0.83 mg/kg/h for 23 hours, amiodarone is rapidly distributed with a high apparent volume of distribution of 31 l/kg. It is relatively highly bound to plasma proteins (96%). Clearance was 0.35 l/kg/h and median elimination half-lives for amiodarone and desethylamiodarone were approximately 51 and 75 hours, respectively

(De Clercq et al., 2006; Plumb, 2015). Horses treated with intravenous amiodarone for 36 hours or longer developed short-term hind limb weakness and diarrhoea (Plumb, 2015).

In humans, oral absorption is slow and variable, with bioavailability ranging from 22-86%. Elimination half-lives for amiodarone and desethylamiodarone range from 2.5-10 days after single dose, up to 53 days and 60 days with repeated dosing (Plumb, 2015). In horses the terminal half-life is 38-84 hours (Papich, 2021).

In laboratory animals, amiodarone has been shown to be embryotoxic and congenital thyroid abnormalities have been detected in offspring. In humans, FDA categorizes this drug as category D for use during pregnancy i.e. there is evidence of human foetal risk, but potential benefits from the use of the drug in pregnant woman may be acceptable despite its potential risks (Plumb, 2015). Amiodarone is not listed by the IARC.

Residue depletion data in horses are not available.

Considering the proposed use for the substance, data available from both humans and horses, its relatively low oral bioavailability, that following intravenous administration it has very high bioavailability with rapid distribution and a short half-life, it can be accepted that amiodarone 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.

Digoxin is a cardiac glucoside used to regulate cardiac rate and rhythm and in horses is intended for oral and intravenous administration. In humans, digoxin is used orally on a long-term basis, while intravenous use is only short-term (IARC, 2013). It is a crystal powder, practically insoluble in water and slightly soluble in diluted alcohol (Papich, 2021; Plumb, 2015). Digoxin is authorised in the EU as human medicinal products.

Data in human medicinal products report differences in bioavailability depending (primarily) on the pharmaceutical form and route of administration. Absorption following oral administration occurs in the small intestine and is variable dependent upon the oral dosage form used. Food may delay, but not alter, the extent of absorption in most species studied. Peak serum levels generally occur within 45-60 minutes after oral elixir and about 90 minutes after oral tablet administration. In patients receiving an initial oral dose of digoxin, peak effects may occur in 6-8 hours after the dose (Plumb, 2015). One study in dogs yielded similar values for oral tablets and elixir, but in horses only about 20% of an intragastric dose was bioavailable (Plumb, 2015).

The drug Is distributed widely throughout the body with the highest levels found in kidneys, heart, intestine, stomach, liver and skeletal muscle. Lowest concentrations are found in the brain and plasma. Digoxin does not significantly enter ascitic fluid, so dosage adjustments may be required in animals with ascites. At therapeutic levels, approximately 20-30% of the drug is bound to plasma proteins (Plumb, 2015)

Digoxin is slightly metabolized, and the primary elimination route is renal excretion both by glomerular filtration and tubular secretion; dosage adjustments are required in patients with impaired renal function. Values reported for digoxin's half-life elimination in dogs and cats are variable: 14.4-56 hours for dogs and 30-173 hours for cats. Approximate elimination half-lives are reported for other species which include sheep (7 hours), horses (17-29 hours) and cattle (8 hours) (Plumb, 2015).

The product information of human medicinal products states that the use of digoxin in pregnancy is not contraindicated. Despite extensive antenatal exposure to digitalis preparations, no significant adverse effects have been observed in the foetus or neonate when maternal serum digoxin concentrations are maintained within the normal range. Although it has been speculated that a direct effect of digoxin on

the myometrium may result in relative prematurity and low birthweight, a contributing role of the underlying cardiac disease cannot be excluded. Maternally administered digoxin has been successfully used to treat foetal tachycardia and congestive heart failure. There is no information available on the effect of digoxin on human fertility. No data is available on whether digoxin has teratogenic effects (Plumb, 2015). The FDA categorizes this drug as category C for use during pregnancy i.e. animal studies have shown an adverse effect on the fetus, but there are no adequate studies in humans; or there are no animal reproduction studies and no adequate studies in humans.

Although digoxin is excreted in breast milk, the quantities are minimal and breast feeding is not contraindicated. Studies have shown that digoxin concentrations in mother's serum and milk are similar; however, it is unlikely to have any pharmacological effect in nursing offspring (Plumb, 2015).

Residue depletion data for horses is not available.

Digoxin is considered a possible carcinogen to humans (IARC group 2B). In four case-control studies (including two studies in humans) significant increases in the incidence of breast cancer were observed for digoxin and digitoxin (including an excess risk for male breast cancer). No clear effects of exposure duration or dose were observed, and the tumour incidence decreased after cessation of exposure. IARC concluded that this was consistent with a tumour promoting effect. An association for uterine cancer was found in current users of digoxin (IARC, 2013).

Carcinogenicity data in laboratory animals were neither available to IARC nor for the current assessment.

For the current assessment two studies on genotoxicity of digoxin could be retrieved. A non-GLP, non-guideline compliant in vitro study investigated chromosomal aberrations at several concentrations of digoxin in a single experiment (24 h treatment only). The authors concluded that concentrations causing chromosomal aberrations were in the cytotoxic range of digoxin (Sedigh-Ardekani et al., 2013). In another study (non-GLP, no guideline followed), a bacterial gene mutation test (*S. typhimurium* TA98 and TA100 tested) was negative with and without metabolic activation, whereas an in vitro comet assay resulted in slightly increased DNA damage (only tested without S9). The results of in vitro micronucleus assays reported in the same publication are not entirely plausible. Two independent tests in CHO cells, one with a concentration in the single-digit nanomolar range, the other in the two-digit micromolar range resulted a similar frequency of binucleated cells with micronuclei. In HeLa cells, a slight increase in frequency of binucleated cells with micronuclei was observed. In the same study an 'antimutagenic' activity of digoxin under simultaneous treatment and pre-treatment with the mutagen mitomycin was presented (de Oliveira et al., 2017).

The epidemiologic evidence linking an increased risk of breast cancer with digoxin use may have supported classification in Group 2A. Considering the tumour sites, the fact that the tumour incidence decreases after cessation of treatment and the estrogen-like side-effects of digoxin an endocrine-mediated mechanism could be assumed suggesting a threshold mechanism of action for carcinogenicity of digoxin. However, the mechanistic evidence was considered weak by IARC (2013). Other factors that argued for classification in group 2B were that not all potential confounders were excluded in the epidemiologic studies, that no data were available from studies in experimental animals, and that the molecular mechanism was unclear (IARC, 2013). But based on the additionally available genotoxicity data, an involvement of a genotoxic mechanism of action cannot be excluded.

The case-control studies were performed using data of human patients with a therapeutic dose. Accordingly, no NOAEL can be derived from the data.

Considering that digoxin is possibly carcinogenic to humans (IARC group 2B), i.e. an increased risk of cancer was observed in humans but the evidence for a threshold-based carcinogenicity is weak and a genotoxic mechanism cannot be excluded, it can be concluded that digoxin will pose an unacceptable risk for consumers when used in food-producing animals of the equine species and a six-month withdrawal period is respected.

Quinidine (sulphate and gluconate) is used in equine medicine for the treatment of ventricular arrhythmias (ventricular premature complexes, ventricular tachycardia), refractory supraventricular tachycardias, and supraventricular arrythmias associated with anomalous conduction in Wolff-Parkinson-White (WPW) syndrome. In horses, it can be administered orally or intravenously (Plumb, 2015). Quinidine is authorised in the EU as human medicinal products.

In humans, absolute systemic availability is 70% or greater. It is 70-95% bound to plasma protein, primarily albumin, and binding is reduced in patients with cirrhosis but is not influenced by renal insufficiency (Ochs et al., 1980). After oral administration, quinidine salts are nearly completely absorbed from the gastrointestinal tract; however, the actual amount that reaches the systematic circulation is reduced due to the hepatic first-pass effect. The extended-release formulations of quinidine sulphate and gluconate, as well as the polygalacturonate tablets, are more slowly absorbed than the conventional tablets or capsules (Plumb, 2015).

A study in 9 horses comparing two quinidine sulphate formulations (i.e. oral solution and oral suspension paste) showed that the relative bioavailability of the oral solution was 75±10.2% for the loading dose (20 mg/kg) and 97.18±31.66% after the fourth dose; bioavailability was not reported for the paste formulation as steady-state levels were not reached. There was a large variation in plasma quinidine levels when the paste formulation was administered (Bouckaert et al., 1994). The systemic availability of quinidine sulphate in horses after oral administration of 10 mg/kg dose was 48.5±20.4% (McGruik et al., 1981).

Quinidine is distributed rapidly to all body tissues except the brain. Protein binding varies from 82-92%. The reported volumes of distribution in various species are 15.1 l/kg in horses, 3.8 l/kg in cattle, 2.9 l/kg in dogs, and 2.2 l/kg in cats (Plumb, 2015). In horses receiving intravenous quinidine gluconate and oral quinidine sulphate the apparent volume of distribution was 3.10±0.79 l/kg, total body clearance was 5.49±2.40 ml/min/kg, and plasma half-life was 6.65±3.00 hours. Oral administration of quinidine sulphate in doses of 10 mg/kg and 10 g produced peak plasma concentrations of 0.79 µg/ml at 146 minutes and 1.47 µg/ml at 131 minutes, respectively (McGuirk et al., 1981). Quinidine can reach milk and crosses the placenta (Plumb, 2015).

In humans, the elimination of quinidine combines renal excretion of intact drug (15-40% of total clearance) and hepatic biotransformation to a variety of metabolites (60-85% of total clearance). In the liver quinidine is metabolised primarily by hydroxylation (by cytochrome P450IIIAA). The major metabolite is 3-hydroxy-quinidine, which has a volume of distribution larger than that of quinidine and an elimination half-life of about 12 hours. Other kinetic parameters are reported for humans: volume of distribution 2.0-3.5 l/kg; elimination half-life 5-12 hours; clearance 2.5-5.0 ml/min/kg (Ochs et al., 1980). Acidic urine (pH<6) can increase renal excretion of quinidine and decrease its serum half-life. Serum half-live reported in horses is 8.1 hours (2.3 hours in cattle, 5.6 in dogs and 1.9 in cats) (Plumb, 2015).

Regarding toxicity, a three-month study in rats (including embryo-toxicity and ototoxicity investigations) showed no teratogenic effects or interference with auditory function in doses up to 200 mg/kg (Colley et al., 1989). Regarding genotoxicity, no point mutations were observed in the Ames test. In three cytogenetic tests on small rodents, Chinese hamsters showed no genotoxic

activity, while inbred mice exhibited a dose-dependent increase in sister chromatid exchanges (SCEs), micronuclei incidence, and chromatid breaks (Münzner and Renner, 1983). Quinidine is not listed by the IARC.

Residue depletion data for horses is not available.

Overall data showed that while almost complete absorption from gastrointestinal tract could be expected after oral administration, the actual amount reaching systematic circulation will be reduced due to the hepatic first-pass effect. Considering that the substance is extensively metabolised and is rapidly eliminated in horses (plasma half-life is around 8 hours), it can be accepted that quinidine (sulfate and gluconate) 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.

4.5.3. Assessment of new substances proposed to be added to the list in the stakeholders survey

A. Considerations on the essentiality of the substance(s)

Flecainide was mentioned (once) in the survey to stakeholders and it was suggested for addition to the list. No specific indication was mentioned, and no scientific references were provided.

It is a class IC antiarrhythmic drug and rarely used in the treatment of cardiac arrhythmias in horses (Redpath and Bowen, 2019). It is used in equine medicine for the treatment of acute atrial fibrillation, supraventricular and ventricular arrhythmias resistant to other treatments. Flecainide is a sodium channel blocker. This prevents the influx of sodium ions, preventing the generation of additional action potentials and dampening any associated heart muscle activity. The heart rate slows down overall.

For the treatment of cardiac arrhythmias quinidine, as quinidine sulphate and quinidine gluconate, is considered the drug-of-choice and is retained in the list of essential substances. Other alternatives are retained, which have shown added clinical benefit compared to the drug-of-choice and it is considered that flecainide does not add clinical benefit compared to any of these: amiodarone has added benefit for treatment of atrial fibrillation, supraventricular and ventricular tachycardias; propafenone has added benefit by means of its mechanism of action, i.e. a sodium channel antagonist that slows down the influx of sodium ions into the myocardium decreasing the excitability of the heart; sotalol is more suitable in horses requiring long-term anti-arrhythmic therapy; verapamil, being a calcium-channel blocker, is also proposed to be added to the list. It is considered that procainamide does not bring added clinical benefit compared to any of these. Furthermore, flecainide has been associated with potentially dangerous side effects. Loon et al. (2004) found that intravenous flecainide was associated with potentially dangerous dysrhythmias in horses with naturally occurring atrial fibrillation. In another study of 9 standardbred mares, flecainide caused abnormal QRS complexes in 4 out of 6 horses with atrial fibrillation and 1 out of the three controls (Carstensen et al., 2018). Robinson and Feary (2008) reported two cases of sudden death following oral flecainide administration for chronic atrial fibrillation. Similarly, Dembek et al. (2014) described a case of an 8-year-old horse that developed polymorphic ventricular tachycardia (torsades de pointes) and ventricular fibrillation, leading to sudden death after receiving flecainide for chronic supraventricular tachycardia.

Cardiac arrhythmias, if untreated, are life-threatening and cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any flecainide-containing veterinary medicinal products authorised for use in equine species (neither in other animal species).

The substance flecainide is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indication in animals of the equine species; it does not bring added

clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering for the animal.

Propafenone was mentioned (once) in the survey to stakeholders and it was suggested for addition to the list as an antiarrhythmic drug used in therapy of cardiac arrhythmias in horses due to its mechanism of action (DeClercq, 2008, 2009).

Propafenone is a sodium channel antagonist and slows down the influx of sodium ions into the myocardium, so that the excitability of the heart decreases. Thus, it has a negative bathmotropic effect. In addition, it blocks beta receptors resulting in negative chronotropy. While DeClercq (2008, 2009) showed that propafenone is not effective in chronic atrial fibrillation, its added benefit is for the treatment of ventricular tachycardia and tachyarrhythmia. Quinidine (sulphate and gluconate) is the drug-of-choice for the treatment of cardiac arrhythmias and is retained in the list. Other alternatives are retained (see above). However, propafenone's mechanism of action as sodium channel antagonist brings added clinical benefit.

Cardiac arrhythmias, if untreated, are life-threatening and cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any propafenone-containing veterinary medicinal products authorized for use in equine species (neither in other animal species).

The substance propafenone is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: for the treatment of ventricular tachycardia and tachyarrhythmia. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Quinapril was mentioned (once) in the survey to stakeholders and it was suggested for addition to the list. No specific indication was mentioned, and no scientific references were provided.

It is an antihypertensive agent from the group of angiotensin-converting enzyme (ACE) inhibitors used in the treatment of arterial hypertension in horses (Davis et al., 2014). Through the modulation of the renin-angiotensin-aldosterone system (RAAS), quinapril influences the regulation of blood pressure and the water balance in the human body. It acts via inhibition of angiotensin-converting enzyme and thereby leads to a reduced formation of angiotensin II from angiotensin I. This leads consecutively to reduced vascular tone and, via the falling angiotensin II level, to a lower release of aldosterone from the adrenal cortex. Quinapril is used in equine medicine for the treatment of heart failure and cardiovascular protection in horses with atrial fibrillation (AF) or mitral regurgitation (MR). There are available studies which have explored the effects of quinapril on healthy horses and horses with cardiovascular issues. Intravenously and orally administered quinapril significantly inhibited ACE activity in 6 healthy horses; despite low plasma concentrations, quinapril had sufficient oral absorption to produce the effect (Davis et al., 2014). Gehlen et al. (2003) investigated the impact of quinapril on 20 horses with mitral valve insufficiency noting a moderate improvement in the severity of mitral valve insufficiencies in 5 horses, from moderate to mild, after therapy. There are no veterinary medicinal products authorised for food-producing animals of the equine species from the group of ACE inhibitors and none of the substances retained or added in the list of essential substances belong to this group. Digoxin, however, is used for the treatment of heart failure. Quinapril's different mechanism of action is though considered to bring added clinical benefit in the treatment of heart failure and cardiovascular protection in horses with atrial fibrillation (AF) or mitral regurgitation (MR). Digoxin is finally not proposed for inclusion in the list (see section 4.5.2.B).

Heart failure, atrial fibrillation and mitral regurgitation, if untreated, are life-threatening and cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any quinapril-containing veterinary medicinal products authorised for use in equine species (neither in other animal species).

The substance quinapril is proposed to be qualified as essential because no satisfactory alternative treatments are authorised for food-producing animals of the equine species for the following indication: treatment of heart failure; cardiovascular protection in horses with atrial fibrillation (AF) or mitral regurgitation (MR).

Sotalol was mentioned (once) in the survey to stakeholders and it was suggested for addition to the list.

Sotalol belongs to the group of beta blockers and is used to treat various cardiac arrhythmias in horses. Sotalol closes potassium channels and is therefore a class III antiarrhythmic drug. Both the action potential as well as the refractory period are prolonged. Through an additional blockade of beta-1 receptors in the heart, the heart rate (chronotropy), the conduction velocity (dromotrope), the contractility of the heart muscle (inotropy) and the excitability of the heart (bathmotropic) are reduced. In addition, the sympathetic-system and the renin secretion and the associated renin-angiotensin-aldosterone system is inhibited. In the longer term, this leads to a lowering of the blood pressure.

While quinidine, drug-of choice, and amiodarone, a class III antiarrhythmic agent, are retained in the list, it is considered that sotalol treatment is more suitable in horses requiring long-term anti-arrhythmic therapy (Broux et al., 2016; 2018); it also has less adverse events than amiodarone. Therefore, sotalol is classified as bringing added clinical benefit in the long-term treatment of cardiac arrhythmias in horses.

Cardiac arrhythmias, if untreated, are life-threatening and cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any sotalol-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance sotalol is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: for the long-term treatment of cardiac arrhythmias. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering for the animal.

Verapamil was mentioned (once) in the survey to stakeholders and it was suggested for addition to the list. No specific indication was mentioned, and no scientific references were provided.

It is used as an antiarrhythmic agent (class IV) and belongs to the group of calcium channel blockers. In equine medicine it is used for the treatment of supraventricular arrhythmias, specifically ventricular rate control in atrial fibrillation and interruption of SA/AV nodal-dependent supraventricular tachycardia. There are no veterinary medicinal products authorised for food-producing animals of the equine species from the group of calcium-channel blockers and none of the substances retained or added in the list of essential substances belong to this group. Amiodarone, quinidine sulphate and quinidine gluconate, and sotalol are alternatives retained or added to the list. However, verapamil's different mechanism of action is considered to bring added clinical benefit compared to these anti-arrhythmic agents in the treatment of supraventricular arrhythmias.

Cardiac arrhythmias, if untreated, are life-threatening and cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any verapamil-containing veterinary medicinal products authorised for use in equine species (neither in other animal species)

The substance verapamil is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: treatment of supraventricular arrhythmias. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Knowledge regarding substances for cardiology in horses for this assessment was derived from textbooks, review articles, and retrospective studies.

B. Considerations regarding consumer safety

Propafenone is a class 1C antiarrhythmic agent that can be administered both intravenously and orally. Propafenone is authorised in the EU as human medicinal products.

In humans, pharmacokinetic properties of propafenone differ between individuals (i.e. between the so-called extensive and poor metabolisers) (Bachmakov et al., 2005). Propafenone is well absorbed from the gastrointestinal tract. Maximal plasma concentrations are reached between 2-3 hours after administration and bioavailability is between 4.8-12%. With repeated doses, plasma concentration and bioavailability rise disproportionately due to saturation of the first pass metabolism in the liver (CYP2D6) (Harron and Brodgen, 1987; Papich, 2021).

Propafenone distributes rapidly in the body. In the therapeutic concentration range, more than 95% of propafenone is bound to plasma proteins. The protein binding of propafenone in vitro was assessed in plasma of mouse, rat, rabbit, dog, sheep, man, cow, and horse at two concentration levels. In all species and at both concentrations propafenone was found highly bound (86-99%) to plasma proteins (Puigdemont et al., 1989).

In humans, mean plasma elimination half-life following intravenous administration is 2.8 hours in healthy volunteers, 5 hours in 'non-healthy' cases, and 16.8 hours in healthy 'poor metabolisers'. During long-term oral administration, half-life was 6.2 hours. Less than 1% propafenone is excreted unchanged in the urine, i.e. propafenone is almost exclusively metabolised in the liver. The major metabolites are conjugates of 5-hydroxy-propafenone and N-depropylpropafenone (Harron and Brodgen, 1987).

Propafenone kinetics after intravenous administration have been studied in the horse by a comparative analysis of compartmental and noncompartmental models. Propafenone showed a large distribution with the volume of distribution at steady state (Vdss) of 1021±211 l and a high clearance of 7019±1746 ml/min. The plasma concentrations were very low, below 1 µg/ml in most cases (Puigdemont et al., 1990). In horses receiving a bolus of propafenone (2 mg/kg over 15 minutes), plasma propafenone concentrations (569-1268 ng/ml) reached the human therapeutic range (64-1044 ng/ml) (DeClercq et al., 2009).

The product information of human medicinal products mentions that propafenone is known to cross the placental barrier in humans and the concentration of propafenone in the umbilical cord has been reported to be about 30% of that in the maternal blood. Propafenone was found to be embryotoxic in a study in Wistar rats receiving 46.25 mg/kg bw propafenone daily by gavage from gestation day 5 to 19. Decreased gravid uterine weight, number of implants/litter, viable foetuses, and foetal body weight, and increased placental weight and placental index were noted. Morphological abnormalities,

severe cranial ossification deficiencies, and histopathological changes in liver, kidney, and brain tissues were also observed. Propafenone induced DNA damage in foetal skull osteocytes and hepatic cells in a comet assay (Abd-Allah et al., 2022). Propafenone is not listed by the IARC nor data reported to ECHA in the C&L inventory informs of such properties of the substance.

Considering that the substance shows a large distribution volume and high clearance in horses and is almost exclusively metabolised in the liver, it can be accepted that propafenone 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.

Quinapril is an angiotensin-converting enzyme (ACE) inhibitor that is enzymatically hydrolysed to a pharmacologically active diacid form, quinaprilat, which acts potently and specifically to block conversion of angiotensin I to angiotensin II in both plasma and tissue. It is authorised in the EU as human medicinal products.

In humans, peak plasma concentrations occur within 2.5 hours. About 97% of circulating quinapril and quinaprilat is bound to plasma proteins (Kieback et al, 2009). Plasma radiolabel concentration-time profiles exhibit a polyexponential decay with a prolonged terminal phase at low concentrations in all species. Metabolism to compounds other than quinaprilat is not extensive. After oral quinapril doses of 2.5-80 mg, quinapril and quinaprilat were eliminated from plasma with apparent half-lives of 0.8 and 1.9 hours and apparent plasma clearances were 1.850 and 220 ml/min, respectively (Olson et al., 1989).

In rats, dogs and monkeys, quinapril is rapidly absorbed after oral administration, with absorption rates reported between 53 and 68%. Quinapril is distributed to all tissues except brain tissue and is mainly metabolised to the active diacid metabolite quinaprilat, which is excreted in urine and bile (Olson et al., 1989).

After intravenous administration of quinapril to horses (at 120 mg single dose), mean terminal half-life was 0.694 hours and 1.734 hours for quinapril and quinaprilat, respectively. The mean volume of distribution and clearance for quinapril were 0.242 l/kg bw and 11.93 ml/kg bw/min, respectively. Maximum concentration for quinaprilat was 145 ng/ml at 0.167 hours. Bioavailability of quinapril following oral administration was less than 5%. Quinaprilat was detected in all horses following oral administration of quinapril; however, it was below the limit of quantification of the assay (2.5 ng/ml) for most horses in the lowest dosing group (120 mg vs 240 mg). These results suggest that, despite low plasma concentrations, quinapril has sufficient oral absorption to produce inhibition of ACE in healthy horses (Davis et al., 2014).

Quinapril is well tolerated in a variety of pharmacologic safety screens and its toxicity profile is similar to that of other ACE inhibitors. Acute, subacute, and chronic toxicity studies have been performed with quinapril in rodents and dogs. The preclinical profile of quinapril is similar to that of other ACE inhibitors. Quinapril did not elicit any unexpected toxicity. The acute toxicity of quinapril and quinaprilat in rodents is low. Upon repeated administration of quinapril and quinaprilat at doses far in excess of the maximum recommended human doses, effects common to this class were noted. The principal target organs were the gastrointestinal tract, liver, kidneys, and lungs. Species sensitivity to quinapril is rabbits > mice > rats = dog. Acute quinapril treatment at 3 to 300 mg/kg intraperitoneally in mice caused no signs of central nervous system (CNS) excitation, anaesthesia, convulsions, tremors, twitching, or Straub's tail. There was no depression of grasping, pinnal, corneal, or righting reflexes. Quinapril did not cause aggression or passivity, ptosis, analgesia (tail clip method), or catalepsy and did not prevent electroshock convulsions. There were no changes in body tone, body temperature, or pupillary diameter, nor were there signs of salivation, diarrhoea, piloerection, blanching or flushing,

cyanosis, or dyspnea (Kaplan et al., 1989). Carcinogenicity bioassays in mice and rats, and reproductive and genetic toxicology studies were also conducted. Quinapril does not adversely affect reproduction and is not teratogenic, carcinogenic, or mutagenic (Kaplan et al., 1989; Kieback et al., 2009). It is not listed by the IARC.

Considering that the substance is rapidly eliminated form plasma, has low bioavailability (around 5%) in horses and has apparent low toxicity, it can be concluded that quinapril 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.

Sotalol is a non-selective beta-blocker and Class III antiarrhythmic agent authorised in the EU as human medicinal products.

Sotalol is hydrophilic, well absorbed in the small intestine and does not have any considerable first-pass effect after oral administration. Peak serum concentration in humans occurs 2.5-3 hours after single oral dosage and 2 hours after single intravenous dosage infused over 2.5 hours. Plasma concentrations follow linear kinetics. Sotalol does not undergo any metabolic transformation in the body, so no metabolites are produced (Plumb, 2015; Papich, 2021). In humans, if given fasted bioavailability is 90-100%; food may reduce the bioavailability by approximately 20%. Peak plasma levels occur between 2-4 hours after a dose. There is limited penetration into the central nervous system. Sotalol is distributed into heart, liver, and kidney tissues. The volume of distribution is 1.2-2.4 l/kg. Sotalol is eliminated primarily via the kidney as unchanged drug, with approximately 10% to 20% being excreted unchanged in the faeces. The elimination half-life in humans has been reported to be 12 hours; caution should be paid in patients with renal dysfunction (Antonaccio and Gomoll, 1990; Kpaeyeh and Wharton, 2016). In dogs, the elimination half-life is 5 hours (Plumb, 2015).

Six healthy, unfasted Warmblood horses received 0, 2, 3, or 4 mg/kg sotalol orally twice daily for 9 days in a randomized cross-over design. Mean steady-state plasma concentrations were 287 ng/ml (range 234–339), 409 ng/ml (359–458), and 543 ng/ml (439–646) for the 2, 3, and 4 mg/kg doses, respectively. Sotalol at these doses increased the QT interval and the effective refractory period (ERP) (Broux et al., 2018). Previously to this study, Broux et al. (2016) had already evaluated the pharmacokinetics of intravenously (IV) and orally (PO) administered sotalol in six healthy horses at the dose of 1 mg/kg bw either IV or PO. Mean peak plasma concentrations after IV and PO administration were 1624 ng/ml and 317 ng/ml, respectively. The oral bioavailability was intermediate (48%) with maximal absorption after 0.94 hours, a moderate distribution and a mean elimination half-life of 15.24 hours.

Sotalol did not cause any fetotoxicity or teratogenicity when given to pregnant laboratory animals at high dosages, but clear safety in pregnancy has not been established. Sotalol enters maternal milk in concentrations up to five times found in the serum. Sotalol is excreted in milk and it is not recommended for use in nursing humans (Plumb, 2015).

Sotalol caused developmental toxicity in pregnant rabbits. Doses of 300, 225, and 150 mg/kg given on gestational days 13-16 resulted in total litter loses. Single doses of 100 and 150 mg/kg administered on days 8-17 led to increased embryonic death, especially between days 12-16, with marked embryonic mortality (55-90%), reduced live foetuses per litter, and higher mean foetal weights. Single doses ranging from 50 to 100 mg/kg on day 14 increased embryonic death, with total litter loss at higher doses and around 30% mortality at 50 mg/kg. According to the authors the observed developmental toxicity in the rabbit is most likely secondary to embryonic arrhythmia, similar to other rapidly activating potassium current (Ikr) blockers (Sköld and Danielsson, 2001).

Sotalol is not listed by the IARC. No information was found on of residues in horses.

Considering the available data from horses and that the substance is eliminated relatively quick from the horse, it can be accepted that sotalol 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.

Verapamil is a non-dihydropyridine calcium channel blocker. It exerts its activity by blocking the transmembrane influx of extracellular calcium ions across membranes of vascular smooth muscle cells and myocardial cells. This results in inhibition of the contractile mechanisms of vascular and cardiac smooth muscle (Fahie and Cassagnol, 2023). Verapamil is authorised in the EU as human medicinal products for oral and intravenous administration. It is administered as a racemic mixture containing equal amounts of the S- and R-enantiomer (Busse et al., 2001). It is used in dogs and cats for supraventricular tachycardias. According to the American Association of Equine Practitioners (AAEP) verapamil may be used in horses with tachycardia at a dose of 2.2 mg/kg bw subcutaneously three times a day.

The product information of human medicinal products states that after oral administration, 90% verapamil (or greater) is rapidly absorbed from the small intestine. Mean systemic availability of the unchanged compound after a single dose of immediate release (IR) verapamil is 22% and that of slow release (SR) verapamil approximately 33%, owing to an extensive hepatic first-pass metabolism. Bioavailability is about two times higher with repeated administration. In humans, the half-life of the drug is 2-8 hours after a single intravenous dose, but it can increase after 1-2 days of oral therapy, presumably due to a saturable process of the hepatic enzymes. Verapamil is metabolised in the liver to at least 12 separate metabolites, with norverapamil being the most predominant that carries approximately 20% of the cardiovascular activity of its parent drug. After intravenous infusion, verapamil is eliminated biexponentially, with a rapid early distribution phase (half-life about 4 minutes) and a slower terminal elimination phase (half-life 2-5 hours). Following oral administration, the elimination half-life is 3-7 hours. Approximately 50% of an administered dose is eliminated renally within 24 hours and 70% within 5 days. Up to 16% of a dose is excreted in the faeces. About 3% to 4% of renally excreted drug is unchanged. The total clearance of verapamil is nearly as high as the hepatic blood flow, approximately 1 l/h/kg, with a range of 0.7-1.3 l/h/kg. Food decreases the rate and extent of absorption of orally administered sustained-release tablets, but less so with the conventional tablets. Verapamil's volume of distribution is between 4.5-7 l/kg; approximately 90% of the drug in the serum is bound to human plasma proteins. Verapamil crosses the placenta and milk levels may approach those in the plasma.

In dogs, 90% of an oral dose is absorbed but due to a high first-pass effect bioavailability is only 10-23%. Verapamil's volume of distribution has been reported to be approximately 4.5 l/kg in dogs. Serum half-lives of 1.8 hours and 2.5 hours have been reported in the dog, which appears to be significantly lower than in humans. Elimination occurs primarily via the bile/faeces in dogs (Papich, 2021).

Verapamil administered orally in rats at doses 1.5-6 times the human dose was embryocidal and caused retarded foetal growth and development, probably due to reduced weight gains in dams. In humans, the FDA categorizes this drug as category C for use during pregnancy i.e. animal studies have shown an adverse effect on the foetus, but there are no adequate studies in humans; or there are no animal reproduction studies and no adequate studies in humans (Plumb, 2015). In humans, the product information of some medicinal products indicates verapamil may cross the placental barrier and be detected in umbilical vein blood at delivery. Verapamil hydrochloride and its metabolites are excreted in human breast milk. Limited human data suggests the possibility for a potential low infant relative dose, so verapamil is recommended during lactation only if it is deemed essential for the mother's welfare (Plumb, 2015). Regarding teratogenic effects in human, the product information of

human medicinal products states that there are no adequate and well-controlled study data. However, available animal studies do not suggest direct or indirect harmful effects with respect to reproductive toxicity.

No information was found on pharmacokinetics and depletion of residues in horses. Verapamil is not listed by the IARC. Data reported to ECHA's C&L inventory only notes is an acute toxicant.

Considering the similarity of the pharmacokinetic data in humans and dogs, its first-pass metabolism and the relatively short elimination half-life, it can be accepted that quinapril 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.

4.5.4. Conclusion

Based on the above assessment and justifications, the following recommendations are proposed:

1. The following active substances, currently in Regulation (EU) 1950/2006 as amended by Regulation (EU) 122/2013, are proposed to be retained in the list, either without modification or with an amendment of the current entry as shown below:

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Amiodarone	systemic and oral treatment of atrial fibrillation, supraventricular and ventricular tachycardias	quinidine sulphate/gluconate, sotalol, verapamil	different mode of action: class III anti-dysrhythmic
Quinidine sulphate/gluconate	treatment of cardiac arrhythmias	amiodarone, sotalol, verapamil	treatment of choice for atrial fibrillation

2. The following active substances, currently in Regulation (EU) 1950/2006 as amended by Regulation (EU) 122/2013, are proposed to be removed from the list: digoxin, procainamide, propranolol.

3. The following active substances, suggested for addition to the list in the survey to stakeholders, are proposed to be added to the list with an entry as shown below:

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Propafenone	treatment of ventricular tachycardia and tachyarrhythmia	quinidine sulphate/gluconate	different mode of action: sodium channel antagonist that decreases heart excitability
Quinapril	treatment of heart failure; cardiovascular protection in horses with atrial fibrillation (AF) or mitral regurgitation (MR)	none identified	mode of action: ACE inhibitor
Sotalol	long-term treatment of cardiac arrhythmias	amiodarone, quinidine sulphate/gluconate	more suitable in horses requiring long-term anti-arrhythmic therapy; less adverse events than amiodarone
Verapamil	treatment of supraventricular arrhythmias	amiodarone, quinidine sulphate/gluconate, sotalol	different mode of action: calcium channel blocker

4. The following active substance, suggested for addition to the list in the survey to stakeholders, is not proposed for inclusion: flecainide.

4.6. Substances for diagnostic procedures

4.6.1. Overview

	Active substance (ATCvet code)
Substances in Regulation (EU) 1950/2006 **proposed to be retained**, with or without amendment of the current entry	Barium sulfate (QV08BA01, QV08BA02); Fluorescein (QS01JA01); Iohexol (QV08AB02); Phenylephrine[38] (QS01FB01); Rose bengal (no ATCvet code identified); Thyrotropin releasing hormone (QH01A)
Substances in Regulation (EU) 1950/2006 **proposed to be removed**	Iopamidol (QV08AB04); Radiopharmaceutical Tc99m (QV09BA)
Substances from stakeholders' survey **proposed for inclusion**	None
Substances from stakeholders' survey **not proposed for inclusion**	Disodium oxidronate (no ATCvet code identified); Methylene diphosphonate (no ATCvet code identified); Tetrasodium dihydrogenbutedronate, diphosphono-1,2-propanedicarboxylic acid (no ATCvet code identified)

4.6.2. Review of the existing entries in Regulation (EU) 1950/2006, as amended by Regulation (EU) 122/2013, considering the survey results

A. Considerations on the essentiality of the substance(s)

Barium sulfate is an inorganic compound with the chemical formula BaSO4. This drug is used as a contrast agent in diagnostic radiographic procedures. Therapeutic advantages of barium sulfate in diagnostic procedures include its low water solubility and high level of clearance from the body. Barium sulfate is administered orally allowing for enhanced gastrointestinal tract visualization (NCBI, 2023).

Barium sulfate is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) as a radiographic contrast agent used for oesophageal and gastrointestinal contrast examinations. No alternatives are identified in the current list and the specific advantage captured is the fact that no alternatives are available.

Indeed, barium sulfate is used in equine practice for enhanced gastrointestinal tract visualization and no alternatives are available.

The substance is not intended for the treatment of a specific condition. However, failure to inadequately carry out such gastrointestinal diagnostics due to lack of an appropriate contrast agent may cause unacceptable suffering of the animal and could possibly be life-threatening of the animal.

Barium sulfate was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any barium-sulfate-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance barium sulfate is proposed to be qualified as essential because no satisfactory alternatives are authorised for food-producing animals of the equine species for the following use: enhanced gastrointestinal tract visualization during radiographic examinations.

[38] This substance is discussed in detail in section 4.11 (substances for ophthalmology).

Fluorescein is a fluorescent dye from the group of xanthene dyes and triphenylmethane dyes. By contact with damaged epithelium, it rapidly penetrates so corneal lesions can thus be diagnosed (Williams and Pinard, 2013). It has no known pharmacological effect on its own; however, recent reports have suggested that fluorescein derivatives could have antibacterial activity via membrane depolarization (Nazarov et al., 2020).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), as a diagnostic tool for corneal ulceration and topical use. Rose Bengal is identified as its alternative, and the specific advantage captured is fluorescein has no significant effect on virus replication. Therefore, fluorescein is the diagnostic tool of choice when a viral culture is planned (i.e. the diagnostic use of Rose Bengal prior to viral culture may preclude a positive result).

The use of fluorescein as a diagnostic tool of the eye is well established (Murube, 2013); it is the diagnostic dye of choice for diagnosing eye keratitis/ulcers when a viral culture is needed, since it has no antiviral effect (in contrast with Rose Bengal).

The substance is not used for the treatment of a condition. However, failure to adequately carry out such ophthalmic diagnostics due to the lack of an appropriate contrast agent may cause unacceptable suffering of the animal.

Fluorescein was mentioned (once) in the survey to stakeholders, proposing to add it to the list, though this substance is already included in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013).

A search in the veterinary medicines database does not retrieve any fluorescein-containing veterinary medicinal product authorised for use in equine species. There are veterinary medicinal products authorised for use in species other than the equine (i.e. cat and dog).

The substance fluorescein is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following use: diagnostic tool for corneal keratitis or ulceration, topical use. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Iohexol and **iopamidol** are both effective non-ionic, water-soluble contrast agent for intrathecal administration used in myelography, contrast enhancement for computerized tomography, myelography, arthrography, nephroangiography, arteriography, and other radiographic procedures (Kawada and McLeod, 1985).

Both substances are listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) as radiographic contrast agents used for lower urinary tract studies, arthrography, myelography, sino- or fistulography and dacryocystography. Both are identified as alternatives to each other. Similarly, the specific advantage cited is that these are non-ionic low osmolar contrast agents, stating both are equally acceptable.

While both substances are known contrast agents (Kawada and McLeod, 1985), iohexol has been described as having a better target animal safety profile, with the incidence and severity of adverse side effects being less for iohexol than iopamidol (Lamb, 1985) and causing fewer neurotoxic events than ionic media and some nonionic agents (Haria and Brogden, 1997).

These substances are not intended for the treatment of a specific condition. However, failure to adequately carry out radiographic examinations due to the lack of an appropriate contrast agent may cause unacceptable suffering of the animal.

Neither iohexol nor iopamidol were mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any iohexol- or iopamidol-containing veterinary medicinal products authorised for use in equine species (neither in other animal species).

The substance iohexol is proposed to be qualified as essential because no satisfactory alternative treatments are authorised for food-producing animals of the equine species for the following indication: as a radiographic contrast agent used for lower urinary tract studies, arthrography, myelography, sino- or fistulography and dacryocystography. In contrast, iopamidol is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; it does not bring added clinical benefit compared to iohexol and it is considered that the alternative does yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Phenylephrine is discussed in detail in section 4.11 (substances for ophthalmology) where it is proposed for treatment of glaucoma and epiphora (please refer to section 4.11 for the detailed assessment of the substance). Phenylephrine is also proposed under this section as a diagnostic test for grass sickness.

Equine grass sickness is a polyneuropathy affecting both the central and the peripheral nervous systems of horses. It results in the development of a characteristic array of clinical signs, most of which can be attributed to neuronal degeneration in the autonomic and enteric nervous systems. Despite extensive research efforts, the precise aetiology remains elusive (Pirie et al., 2014). Cases are generally diagnosed on clinical grounds. Certain ancillary diagnostic approaches have also been advocated to improve diagnostic accuracy in a clinical setting, most notably, the topical application of phenylephrine eye drops to temporarily reverse ptosis (Hahn and Mayhew, 2000). However, the histopathological identification of degenerate autonomic and/or enteric neurones remains the accepted means of diagnostic confirmation (Pirie et al., 2014).

The substance is not intended for the treatment of a specific condition. However, failure to inadequately diagnose grass sickness may cause unacceptable suffering of the animal and could possibly be life-threatening of the animal.

The substance phenylephrine is proposed to be qualified as essential because no satisfactory alternatives are authorised for food-producing animals of the equine species for the following use: grass sickness diagnosis.

Radiopharmaceutical Tc99m is a metastable nuclear isomer of technetium-99 (i.e. a radioisotope) that is used in medical diagnostic procedures in human and animal medicine. In equine medicine, it is used for skeletal scintigraphy, which is very sensitive, but relatively nonspecific for determining a definitive aetiology (Winter et al., 2010). While nuclear scintigraphy is effective in localizing pathological changes, magnetic resonance imaging (MRI) provides superior anatomic detail (Daniel et al., 2012).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for scintigraphy. No alternatives are identified in the current list and the specific advantages captured in the current list are: it is the most sensitive diagnostic imaging modality for identification of early bone pathology and fractures, more sensitive than radiography; it allows quantitation and enables imaging of regions not amenable to radiography; essential imaging technique safeguarding welfare of performance horses through early injury detection and prevention of catastrophic fractures; short half-life (6,01 hours) of Tc99m results in rapid clearance of detectable radioactivity (< 72 hours) from the horse.

Radiopharmaceutical Tc99m was not mentioned in the survey to stakeholders.

The expert group preparing the scientific advice under Article 115(5) of Regulation (EU) 2019/6 noted that Article (2)(7)(b) thereof states that this Regulation shall not apply to veterinary medicinal products based on radio-active isotopes. Pursuant to this Article, substances being radio-active isotopes are outside of the scope of this activity and do not need to be assessed according to criteria given in the Commission's request.

Thus, the substance, Radiopharmaceutical Tc99m, cannot be considered for inclusion in the list.

Rose bengal is a bright rose-red xanthene compound belonging to the group of xanthene and triphenylmethane dyes; it was first synthesized in the 19th century as a wool dye, and subsequently used as a food dye in Japan (Mizutani, 2009). The use of Rose Bengal for the visual diagnosis of human ocular surface damage (via ocular instillation) was first described in 1914 by Feenstra et al. (1992) (Feenstra and Tseng, 1992).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), as a diagnostic tool for early corneal damage and topical use. Fluorescein is identified as its alternative, and the specific advantage captured is being the tool of choice to ascertain very early corneal damage.

The use of rose bengal as a diagnostic tool of the eye is well established (Brooks, 2000); it is the diagnostic dye of choice for diagnosing eye keratitis/ulcers.

The substance is not used for the treatment of a condition. However, failure to adequately carry out such ophthalmic diagnostics due to lack of an appropriate contrast agent may cause unacceptable suffering of the animal.

Rose bengal was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any Rose-bengal-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance rose bengal is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following use: diagnostic tool for corneal keratitis or ulceration, topical use. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Thyrotropin releasing hormone (TRH) is a hypothalamic neuropeptide with a wide range of biological responses. Besides its central role in regulating the pituitary-thyroid axis by stimulating the release of thyrotropin, TRH has considerable influence on the activity of a number of neurobiological systems. TRH is known to elicit its biological response through two G-protein coupled receptors for TRH (namely, TRH-R1 and TRH-R2) (Monga et al., 2008).

Thyrotropin releasing hormone is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) as a diagnostic substance for the confirmation of thyroid and pituitary disorders. No alternatives are identified in the current list and the specific advantage captured is the lack of alternatives.

Expertise within the expert group preparing this scientific advice confirmed that thyrotropin releasing hormone would be widely used as a diagnostic substance for the diagnosis of pars pituitary intermedia dysfunction, with no alternatives being available.

The substance is not intended for the treatment of a specific condition. However, failure to diagnose a disorder of an animal may cause unacceptable suffering of the animal and it may be life-threatening.

Thyrotropin releasing hormone was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any TRH-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance, thyrotropin releasing hormone (TRH), is proposed to be qualified as essential because no satisfactory alternatives are authorised for food-producing animals of the equine species for the following use: diagnostic substance for the confirmation of thyroid and pituitary disorders.

Knowledge regarding diagnostic substances in horses for this assessment was derived from textbooks, and review articles.

B. Considerations regarding consumer safety

Barium Sulfate ($BaSO_4$) is commonly used in medical applications as a contrast medium due to its lack of systemic toxicity attributed to its insolubility (SCHER, 2012). The intact sulfate salt of barium has a very low solubility in gastric fluid (< 1%) (ECHA, 2023b). The insoluble compounds of barium, such as barium sulfate, do not generate free Ba(2+) ions in the gastrointestinal tract and therefore are generally nontoxic (EMA/CHMP/ICH/353369/2013). It is thus grouped in the family of poorly soluble particles (PSP) or poorly soluble low toxicity (PSLT) particles (Molina et al., 2019). It is typically minimally absorbed following oral or rectal administration in individuals with a healthy gastrointestinal tract (Oskarsson, 2021; Bhoelan et al., 2014).

However, in cases of potentially damaged gastrointestinal tract (GIT) and influenced by dosage and particle size, publicly available pharmacokinetic studies have reported partial (very low) absorption of barium from barium sulfate in the GIT. Considering such pharmacokinetic study results after administration of barium sulfate, it is to be noted that particle size as well as route of exposure impact elimination kinetics and clearance of BaSO4 particles (Johnston et al., 2013; Konduru et al., 2014; Landsiedel et al., 2014). While micron-scale BaSO4 is commonly used in radio-contrast agents for medical diagnostic purposes (Landsiedel et al., 2014), pharmacology, as described below, has also been studied with nanosized particles potentially leading to overestimation of BaSO4 in body fluids.

After ingesting high doses of 58 to 400 g of BaSO4 as a contrast agent, statistically significant increases in barium concentrations of blood and urine were reported in humans (Mauras et al., 1983; Clavel et al., 1987;). Barium blood levels rose from a baseline of 0.76 µg/ml (mean of 10 values) to 2.53 and 1.31 µg/ml at 4 and 8 hours post oral intake of 350 g of barium sulfate (Mauras et al., 1983). In rodents, measured barium levels in blood were fairly similar when comparing single oral administration of either barium sulfate or barium chloride doses equivalent to 50 mg Ba/kg bw (McCauley and Washington, 1983).

Konduru et al. (2014) compared the pharmacokinetics of radiolabelled, nanosized barium sulfate for different application routes in rats. Barium tissue residues one week after oral gavage of 5.0 mg BaSO4/kg bw were very low to not detectable, with only 0.15% of the total dose still present. Specifically, all tissues, except for stomach, bone and bone marrow, exhibited levels below the limit of detection (LOD) of 0.01 ng/ml constituting less than <0.01% of the total dose. The stomach, bone, and bone marrow showed levels of <0.1% of the total dose. This indicates that generally, only very low or no significant absorption of barium ions from low dosed nanosized BaSO4 in rats took place across the intestinal barrier. Following intravenous injection in rats, the ratio of mean muscle-to-bone concentration was 1:456 after one week (Konduru et al., 2014). Also, Thomas et al. (1973) described that in rats, over time, barium translocated primarily to the skeleton, with a biological half-life of 460 days for this tissue. Thus, barium is practically retained in the bone. A predominantly skeletal barium accumulation has also been shown for humans where approximately 91% of total dietary barium is

stored in bones (Tipton and Cook, 1963; UNEP, 1990). Soft tissues therefore generally have low concentrations of barium, with the exception of the pigmented areas of the eye as was shown in calves, cattle and rabbits (Sowden and Pirie, 1958).

Konduru et al. (2014) documented a half-life of 9.6 days for the clearance of BaSO4 from the lungs in rats. Thomas et al. (1973) described a half-life loss from injection site in the hind leg of 26 days in rats. In human studies it was noted that excretion of barium primarily takes place via faeces and urine (Tipton et al., 1969; Schroeder et al., 1972). In rats, excretion post gavage took place almost exclusively via faeces (Konduru et al., 2014).

According to Loza et al. (2016), BaSo4 does not dissolve at the lower pH present in lysosomes following cellular uptake. Molina et al. (2019) were able to show the contrary in their study on the fate of nanosized BaSO4 following (i) long-term inhalation and (ii) one-off intratracheal application in rats, proving that in fact the barium salt is lysed in phagocytes (here, of the lung) and ionic barium is then distributed to extrapulmonary organs. Four weeks after intratracheal instillation, barium was mainly incorporated in hard bone tissue and deposited in lymph nodes, while concentrations in bone marrow and liver were considerably lower. Intact BaSO4 particles were only seen in lung-associated lymph nodes, and the authors concluded that whole particles – even at nanosize – are very unlikely to cross epithelial barriers.

Regarding toxicity, BaSO4 is not classified according to CLP regulation provided by companies to ECHA (ECHA, 2023a; ECHA, 2023d). Any chronic toxicity results from the availability of Ba2+ cations. A repeated dose study, like other studies, has been performed with the soluble barium chloride dihydrate (BaCl2*2H2O). However, caution is required when reading across, as absorption of barium may be different from BaSO4 and BaCl2*2H2O, so that NOAEL derivation for BaSO4 would be critical here. In an oral sub-chronic study in rats performed according to the relevant guidance, depressed body weight gains, elevated phosphorus levels, neurobehavioral effects and chemically related lesions in the kidney and lymphoid tissue at the highest dose level of 4,000 ppm BaCl2*2H2O was reported. It was concluded that BaSO4 is not genotoxic according to negative results observed for BaCl2*2H2O in in vitro gene mutation tests in bacteria and mammalian cells and chromosome aberration tests. No evidence of carcinogenic activity was shown for BaCl2*2H2O, and thus for BaSO4, up to the highest dose of 2,500 ppm in a 2-year drinking water study in rats and mice.

In an extended one-generation reproduction toxicity study in rats with dose groups of 0, 10, 30 or 100 mg BaCl2*2H2O/kg bw no treatment-related developmental toxicity occurred and the NOAEL for embryo-foetal toxicity was considered to be ≥ 100 mg BaCl2*2H2O/kg bw. However, for maternal toxicity, indicated by spontaneous deaths in the highest dose group, a NOAEL of 30 mg BaCl2*2H2O/kg bw was derived (ECHA, 2023b). Other sources indicate that barium toxicity results in cardiac irregularities, tremors, anxiety, dyspnoea, muscle weakness, numbness or paralysis, and possibly death. Barium can also cause gastrointestinal disturbances and kidney damage and is known to lead to a decrease in body weight (ATSDR, 2007). According to SCHER, barium shows cardiac and renal effects but is not carcinogenic on the basis of a US EPA assessment of human studies following oral exposure (IRIS, 2005; SCHER, 2012). Cordelli et al. (2017) showed that sub-chronic whole-body-exposure to a high dose of 50 mg/m3 BaSO4 nanomaterials did not induce genotoxicity on the hematopoietic system in rats, on DNA, gene, or chromosome level. No relevant inflammatory response was elicited in vitro in rat alveolar macrophages, even with high amounts of phagocytized particulate barium sulfate, at nano- and micron-scale (Loza et al., 2016).

A BMDL of 61 mg barium/kg bw/day was calculated (95% lower confidence limit on the BMD; ATSDR, 2007) based on the results of soluble barium compounds from NTP studies (NTP, 1994), where the

lowest NOAEL in a chronic mice study was reported to be 75 mg barium/kg bw in male mice for nephrotoxicity (SCHER, 2012).

The barium ion blocks passive efflux potassium channels without affecting the Na/K-ATPase pump, leading to extracellular hypokalemia and thus a decreased resting membrane potential (Bhoelan et al., 2014; Krishna et al., 2020). Furthermore, barium possesses chemical and physiological properties that lead to competition with and replacement of calcium in biological organisms, in processes naturally mediated by calcium, also relating to release of adrenal catecholamines and neurotransmitters, such as acetylcholine and noradrenaline (Eco SSL, 2005).

Consumer exposure also includes naturally occurring dietary barium which is estimated to be at least at the level of 0.5 mg barium/person/day and is reported to be up to 1.5 mg barium/person/day (EMEA/MRL/580/99-FINAL).

No pharmacokinetic or residue depletion data were found for barium sulfate administered in horses or in closely related species of the *Equidae* family.

In summary, absorption of barium from BaSO4 in the GIT when applied for diagnostic purposes is commonly assumed to be negligible but several studies in laboratory animals as well as human studies, as reported above, did show very low barium absorption from micron-scale and nanosized BaSO4, as demonstrated by measurable systemic levels of radiolabelled BaSO4. In this regard, it should be considered that barium uptake may be facilitated in the damaged GIT. However, according to the studies found, the main part of the potential, very low, systemic barium levels would be stored in the bones (>90%) from where release of barium is very slow. The proportion of barium ions in all tissues other than bones after six months would be negligible. Considering horse bones as part of the human diet, as worst case the naturally occurring barium exposure in the diet (EMEA/MRL/580/99-FINAL) would be only very slightly increased by consuming horse bones which would not compromise consumer safety. Moreover, there is no indication that barium is genotoxic, toxic to reproduction or carcinogenic.

Considering that barium sulfate (BaSO4) contrast media are routinely administered only once and the gastrointestinal absorption of barium from the insoluble sulfate salt is negligible or as worst case is extremely low, it can be accepted that BaSO4 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.

Fluorescein is a is a fluorescent dye that emits green light when excited with UV or blue light. It is a red solid, almost insoluble in water whereas the sodium salt of fluorescein is water-soluble. It is currently authorized as human medicinal product for ocular use and as an injectable solution; it is authorised in veterinary medicines for ocular use in dogs and cats.

In humans, the oral bioavailability of fluorescein sodium is high (99%) and the maximum concentration is reached in about 2 hours (Barry and Behrendt, 1985); the elimination half-life is around 267 min. Binding to serum protein averaged 63% for fluorescein glucuronide, the main metabolite of fluorescein, and 85% for parent fluorescein (Seto et al., 1986). When administered systemically, fluorescein is distributed widely to body tissues, is rapidly metabolised and is excreted by the kidney (Grotte et al., 1985; Seto et al., 1986).

No kinetic nor residue data following (ocular) administration of fluorescein to horses are found. The systemic absorption of a substance following topical ocular administration also depends on the final formulation, so it is difficult to anticipate the bioavailability in horses following an ocular application without knowing the formulation used. However, it is noted that elimination in human is fast and there was no indication of any accumulation in the literature.

According to the chemical classification reported to ECHA, soluble fluorescein is not classified for any of the health hazards in the ECHA system, due to conclusive data though insufficient for a final classification (ECHA, 2024a; ECHA, 2024b). The acute toxicity of fluorescein sodium is low. No significant general or teratogenic effects on rats or rabbits were found in an oral, non-standardised 14-day test with mated female animals (Burnett and Goldenthal, 1986; ECHA, 2024b). No effects on fertility were observed in a one-dose non-standardised teratogenicity study in rabbits after intravenous administration (McEnerney et al., 1977). Fluorescein sodium is non-mutagenic according to a reliable in vitro gene mutation study in bacteria (Nestmann et al., 1980; ECHA, 2014b). No information on carcinogenicity is available (ECHA, 2024b). The product information of human medicinal products suggests that fluorescein sodium crosses the placenta in rats. According to IARC, fluorescein and its salts were considered but adequate data regarding possible carcinogenicity were not available (IARC, 1982); thus, there is currently no monograph on fluorescein or its salts by IARC (IARC, 2024).

Considering the available data on its pharmacokinetics in species other than the equine and the apparent low toxicity, it can be accepted that fluorescein 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.

Iohexol is a non-ionic and water-soluble contrast medium for myelography with a logarithm of the partition coefficient (logP) of -2.9 (Shaw et al., 1985). In the horse there may be intrathecal administration of e.g. 180 mg iohexol/ml (Widmer et al., 1998).

Iohexol is absorbed from cerebrospinal fluid (CSF) into the bloodstream and is eliminated by renal excretion. No significant metabolism, deiodination, or biotransformation occurs (Drugbank, 2024). Cheng et al. (2018) describe that there is immediate excretion of iohexol. The volume of distribution is reported to be 350-849 ml/kg and the intrathecal half-life is reported to be 3.4 hours. After intravascular administration the half-life was approximately 2 hours in cases of normal renal function (Drugbank, 2024). In a pharmacokinetic study with six male and female patients peak plasma levels of 29-177 µg/ml were found after two to six hours after lumbar puncture of 180 mg iohexol/ml for myelography. The mean terminal elimination half-life was 5.8 hours, with a range of 2.45 to 6.52 hours (Shaw et al., 1985).

Iohexol, along with similar substances, appears to be nephrotoxic especially in patients with preexisting risk factors. This is attributed to its cytotoxicity, especially towards renal cells, which includes mechanisms of apoptosis, cellular energy failure, disruption of calcium homeostasis, disturbance of tubular cell polarity, and oxidative stress (Drugbank, 2024). The result of administration that can follow is called e.g. a "Contrast-induced acute kidney injury" (CI-AKI) or similar (Kirberger et al., 2012; Cheng et al., 2018). Iohexol is also used as intravascular contrast media. In a respective study in isolated perfused kidney, iohexol was filtered by the kidney and also reabsorbed via a saturable mechanism so that accumulation resulted in the tubules. Moreover, intracellular iohexol may affect mitochondrial oxidative metabolism (Masereeuw et al., 1996). Nevertheless, iohexol is also used as a marker for evaluating glomerular filtration rate (GFR) in horses and other species as it is not metabolized by the body or bound to plasma proteins and is supposed to be freely filtered at the glomerulus without absorption (Wilson et al., 2009).

There is no pharmacokinetic study in horses or any residue depletion study in food-producing animals available.

There is toxicological information based on unnamed key studies described in the chemical registration dossier (ECHA, 2023c). In a 28 day-study with three female and three male monkeys administered 0.33, 1 and 3 g iohexol/kg bw/day, a NOAEL of 0.33 g/kg bw/day was determined based on slight

elevation of serum leucine arylamide values, minor vacuolation of the hepatocytes and an increase in kidney weights at 3 g/kg bw/day, and minor vacuolation of the tubular epithelial cells in some animals receiving 1 and 3 g/kg bw/day. The route of administration was not specified. During the test no clinical findings in the animals were observed.

Iohexol was deemed non-genotoxic based on negative findings from two in vitro tests, conducted according to guidelines with restrictions and which included gene mutation in bacteria in four Salmonella strains and mammalian cell gene mutation in mouse lymphoma with/without metabolic activation. A negative finding was also observed in one in vivo test, conducted according to guidelines, which evaluated chromosome aberration and micronucleus formation in CD-1 mice. In a non-guideline one-generation study, no treatment related reprotoxic findings were observed in the F0 generation female rats administrated iohexol intravenously at doses of 0, 1, 2, 4 g/kg bw/day nor in their pups. In a non-guideline developmental study in rats, iohexol was found to be neither embryotoxic nor teratogenic at intravenous doses of 1, 2 and 4 g/kg bw/day (ECHA, 2023c). Other toxicological studies seem not to be available based on literature search findings.

Considering its pharmacokinetics and its apparent low toxicity, it can be accepted that iohexol 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.

Rose bengal is a synthetic fluorescein derivative, used mainly as an ocular diagnostic instrument, although other properties and therapeutic potential have been demonstrated (Demartis et al*.,* 2021). It stains both the nuclei and cell walls of dead or degenerated epithelial cells of the cornea and conjunctiva, and the mucus of the precorneal tear film. However, there are studies that demonstrate cytotoxic effects of rose Bengal in different cell cultures (Tabery, 1998; Lee et al., 1996). This includes cytotoxicity against tumour and microbial cells which is also the basis for some therapeutic uses.

No information on the systemic absorption or metabolism is available. Rose bengal undergoes biliary excretion and can be used as measurement of the hepatic excretory function. Doses between 0.01 and 10 mg/kg bw in rat resulted in a plasma terminal half-life of 100 min, while the biological half-life for excretion is around 30 minutes in rats and rabbits; however, species variation in the biliary excretion of rose bengal was also observed (Klassen, 1976).

According to the classification provided by companies to ECHA this substance causes serious eye irritation, skin irritation and respiratory irritation (ECHA, 2023) due to its photoactivity (Khan-Lim and Berry, 2004). No chemical registration dossier is available from the ECHA website nor were toxicological tests regarding carcinogenicity, mutagenicity, reproductive toxicity properties or chronic toxicity described in the literature. Rose bengal is currently not listed as a carcinogen by IARC or the National Toxicology Program (IARC, 2024).

In spite of its intrinsic toxic effect, rose bengal has been reported to be eliminated rapidly. Thus, it can be accepted that rose bengal 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.

Thyrotropin-releasing hormone (TRH, protirelin, thyroliberin/thyreoliberin), a tripeptide, when used as a diagnostic agent for suspected thyroid or pituitary dysfunction, is commonly administered as a single intravenous injection i.e. TRH-test. For example, in horses, a typical dose of 1 mg per horse is administered for the TRH-test (Beech et al*.,* 2011). There are synthetic active ingredients of medicinal products (e.g. protirelin) that are identical to the hormone which is naturally produced in a part of the diencephalon (hypothalamus). There are other analogues, e.g. montirelin, taltirelin, that seem chemically not identical to protirelin, and are applied in human medicine for conditions related to central nervous system degenerative diseases.

In human diagnostics an adult typically receives approximately 3.3 µg TRH/kg bw (200 µg per person weighing 60 kg), while a child receives 1 µg/kg bw. The maximum level of thyroid-stimulating hormone (TSH) in blood, serving as the diagnostic indicator, is reached approximately 30 minutes after the infusion. In humans, the test should be repeated after 14 days at the earliest. Mild side effects such as headache, sweating, and nausea are common. Very rarely, a seizure or an asthma attack may occur in people with a corresponding predisposition. Additionally, as with any intravenous injection of peptides, a life-threatening hypersensitivity reaction cannot be ruled out.

TRH studies in serum and tissue homogenates of liver, kidney, lung, heart and brain have demonstrated its rapid degradation (Safran et al., 1984). The pharmacokinetics of TRH were studied in rats after intravenous administration over 60 to 90 minutes in two experiments (Safran et al., 1984). Following the treatment, TRH plasma concentrations rapidly decreased and returned to the previous levels within 60 minutes. TRH exhibited wide distribution in the rat body with a volume of distribution ranging from 23% to 34% of body weight. The terminal plasma elimination half-life of TRH lies within minutes, 12.5 and 15.6 minutes. Metabolism studies of TRH in the rat brain, where significant levels were detected following intranasal and intravenous administration, revealed maximum TRH content 30 minutes and 5 minutes post-administration, respectively (Shevchenko et al., 2015). Intranasal administration resulted in a higher brain-to-blood proportion compared to intravenous administration. TRH in the brain undergoes rapid degradation to lower proteins and amino acids due to regulatory processes and proteolytic enzymes (Shevchenko et al., 2015).

In a human pharmacokinetic study, the half-life was determined to be 9.4 minutes for TRH, with an interindividual variation between 4 and 38 minutes. TRH-OH was identified not to be a metabolite of TRH, contrary to previous assumptions regarding rats and humans. No accumulation of degradation products of TRH occurred (Møss and Bundgaard, 1990).

Residue depletion studies in food-producing animals are not available as TRH is rapidly degraded and eliminated.

Regarding toxicity, protirelin as a chemical substance is notified by companies to ECHA to be mildly acutely toxic by inhalation (ECHA, 2023b). The substance is not registered at ECHA, i.e. further toxicological information is not available and there is no indication from available literature studies for chronic toxicity, genotoxicity, toxicity to reproduction or carcinogenicity of TRH/protirelin/thyroliberin.

Given that TRH is an endogenous substance and is likely administered to horses in low doses in diagnostic tests and considering the results of pharmacokinetics studies in vitro and in laboratory animals, it can be concluded that no residues are to be expected in the horse after a six-month withdrawal period. Also considering the apparent low toxicity, it can be accepted that it 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.

4.6.3. Assessment of new substances proposed to be added to the list in the stakeholders survey

A. Considerations on the essentiality of the substance(s)

Three substances, (i) **disodium oxidronate**, (ii) **methylene diphosphonate**, and (iii) **tetrasodium dihydrogenbutedronate, diphosphono-1,2-propanedicarboxylic acid**, were mentioned (once each) in the survey to stakeholders, and they were suggested for addition to the list since, according to the responders, carrier molecules such as the ones proposed are needed in combination with the radiopharmaceutical Tc99m if bone metabolism is to be imaged.

Their proposed addition is thus linked to the presence of the radiopharmaceutical Tc99m in the list. However, since the radiopharmaceutical Tc99m cannot be considered for inclusion in the list pursuant to Article (2)(7)(b) of Regulation (EU) 2019/6, none of these three substances is considered further.

B. Considerations regarding consumer safety

Not warranted since none of the above is considered essential.

4.6.4. Conclusion

Based on the above assessment and justifications, the following recommendations are proposed:

1. The following active substances, currently in Regulation (EU) 1950/2006 as amended by Regulation (EU) 122/2013, are proposed to be retained in the list, either without modification or with an amendment of the current entry as shown below:

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Barium sulfate	enhanced gastrointestinal tract visualization during radiographic examinations	none identified	no satisfactory alternative treatment for enhanced gastrointestinal tract visualisation during radiographic examinations
Fluorescein	diagnostic tool for corneal keratitis or ulceration, topical use	Rose Bengal	diagnostic tool of choice when a viral culture is needed afterwards
Iohexol	radiographic contrast agent for lower urinary tract studies, arthrography, myelography, sino- or fistulography and dacryocystography	none identified	non-ionic, water-soluble contrast agent
Phenylephrine[39]	grass sickness diagnosis	none identified	Ancillary diagnostic approach to equine grass sickness polyneuropathy
Rose Bengal	diagnostic tool for corneal keratitis or ulceration, topical use	fluorescein	diagnostic tool of choice for diagnosing eye keratitis/ulcers
Thyrotropin releasing hormone	diagnosis of pars pituitary intermedia dysfunction	none identified	no satisfactory alternatives for diagnosis of pars pituitary intermedia dysfunction

2. The following active substances, currently in Regulation (EU) 1950/2006 as amended by Regulation (EU) 122/2013, are proposed to be removed from the list: iopamidol, radiopharmaceutical Tc99m.

3. The following active substances, suggested for addition to the list in the survey to stakeholders, are not proposed for inclusion: disodium oxidronate, methylene diphosphonate, tetrasodium dihydrogenbutedronate diphosphono-1,2-propanedicarboxylic acid.

[39] This substance is discussed in detail in section 4.11 (substances for ophthalmology).

4.7. Substances for gastrointestinal disorders

4.7.1. Overview

	Active substance (ATCvet code)
Substances in Regulation (EU) 1950/2006 **proposed to be retained**, with or without amendment of the current entry	Metoclopramide (QA03FA01); Phenylephrine[40] (QS01FB01); Ranitidine (QA02BA02); Sucralfate (QA02BX02)
Substances in Regulation (EU) 1950/2006 **proposed to be removed**	Bethanechol (QN07AB02); Codeine (QR05DA04); Loperamide (QA07DA03); Phenoxybenzamine (QC04AX02); Propantheline bromide (QA03AB05)
Substances from stakeholders' survey **proposed for inclusion**	Misoprostol (QA02BB01)
Substances from stakeholders' survey **not proposed for inclusion**	None

4.7.2. Review of the existing entries in Regulation (EU) 1950/2006, as amended by Regulation (EU) 122/2013, considering the survey results

A. Considerations on the essentiality of the substance(s)

Bethanechol is a synthetic cholinergic ester (similar in structure and pharmacological function to acetylcholine) that causes stimulation of the parasympathetic nervous system (Plumb, 2023). It is a direct muscarinic agonist and stimulates the parasympathetic nervous system by binding to postganglionic muscarinic receptors with only little activity towards nicotinic receptors (Löscher and Bankstahl, 2016; Padda and Derian, 2023). Its parasympathetic activity causes an increased tone and peristalsis throughout the gastrointestinal tract and esophagus and increased secretions from the pancreas and gastrointestinal tract (McEvoy, 1992).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for the treatment of ileus, of gastroduodenal stricture in foals, and of recurrent small colon impactions in adults. Alternatives identified in the current list include metoclopramide and erythromycin. The specific advantages captured are as follows: betanechol is a muscarinic cholinergic agonist that stimulates acetylcholine receptors on gastrointestinal smooth muscles, causing them to contract; it has been shown to increase the rate of gastric and caecal emptying; both betanechol and metoclopramide have been shown to be beneficial in the treatment of post-operative ileus.

The treatment of ileus, gastroduodenal stricture in foals, and recurrent small colon impactions includes, primarily, intravenous fluid substitution and, when necessary, use of painkillers. There are veterinary medicinal products available to meet the need for such fluid substitution. In addition, the substance menbutone, that is included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with a 'No MRL required' status for *Equidae*, could serve as an alternative for which there are veterinary medicinal products authorized for equivalent indications for food-producing animals of the equine species. Moreover, metoclopramide is retained in the list of essential substances.

[40] This substance is discussed in detail in section 4.11 (substances for ophthalmology).

Ileus, gastroduodenal stricture in foals, and recurrent small colon impactions, if untreated, is potentially life-threatening and cause unacceptable suffering of the animal.

Bethanechol was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any bethanechol-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance bethanechol is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Codeine is an opioid analgesic with central analgesic, sedative, hypnotic, antinociceptive, antitussive, and antiperistaltic properties (Bhandari et al., 2011). It displays a high affinity to μ_2 opioid receptors but a 100 times reduced affinity to the μ_1 opioid receptor compared to morphine (Ammer and Potschka, 2010). Codeine is known for its cough suppressant effects (i.e. antitussive) (Bolser et al., 1999). It may reduce intestinal motility through both a local and possibly central mechanism of action, which could lead to constipation (EMA/235820/2015). It has been reported that codeine phosphate increases duration and number of circular muscle contractions of the jejunum and duodenum resulting in a prolonged transit time of the ingesta with little effect on the gastric antrum (Fox et al., 1985). Other studies did not confirm a prolonged transit time but described a prolonged absorption-time of codeine and a reduction of gut motility (Katori et al., 1998).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for the treatment of diarrhoea. The only alternative identified in the current list is bismuth subsalicylate. The specific advantage captured states that codeine has a different mode of action than bismuth subsalicylate; it is an opioid motility modulator acting on μu receptors in the gut that provides effective symptomatic management of non-infectious diarrhoea, especially in foals; frequently, it is used in combination with loperamide; similarity in mode of action to loperamide brings synergistic action.

There are three substances currently listed for the treatment of diarrhoea: codeine, loperamide and phenoxybenzamine. The primary treatment of this condition usually includes intravenous fluid substitution and dietary management, and there are veterinary medicinal products available to meet the need for such fluid therapy. When additional therapy is required, there are satisfactory treatment alternatives available; the substances bismuth subnitrate, bismuth subsalicylate and *piceae turiones recentes extractum* are included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with a 'No MRL required' status for all food producing species, which includes *Equidae*.

Diarrhoea, if untreated, is potentially life-threatening and causes unacceptable suffering of the animal.

Codeine was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any codeine-containing veterinary medicinal product authorised for use in equine species. There are veterinary medicinal products authorised for use in species other than the equine (i.e. dog and cat).

The substance codeine is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do

yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Loperamide is a highly lipophilic synthetic phenylpiperidine opioid agonist. It acts on the μ opioid receptors of the smooth intestinal muscles (Ramsey, 2008). It decreases peristalsis and fluid secretion in the gastrointestinal tract, delays colonic transit time, and increases the absorption of fluids and electrolytes from the gastrointestinal tract (Pannemans and Corsetti, 2018). Loperamide also increases rectal tone, reduces daily faecal volume, and increases the viscosity and bulk density of faeces (Sahi et al., 2023); the release of acetylcholine and prostaglandins are blocked, reducing propulsive peristalsis and prolonging intestinal transit time. It also increases the tone of the anal sphincter and thus reduces excretion (Schaefer et al., 2013). In horses, it has been reported that loperamide, at first, increases intestinal transit time but subsequently reduces it (Alexander, 1978).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for the treatment of diarrhoea in foals. The only alternative identified in the current list is bismuth subsalicylate. The specific advantages captured are as follows: loperamide has a different mode of action than bismuth subsalicylate; it is an opioid motility modulator acting on μ-receptors in the gut providing more effective symptomatic management of non-infectious diarrhoea in foals than other substances; frequently, it is used in combination with codeine; similarity in mode of action to codeine brings synergistic action.

As indicated under 'codeine', the primary treatment of this condition usually includes intravenous fluid substitution and dietary management, and it is considered that there are veterinary medicinal products authorised for an equivalent indication (i.e. for the treatment of diarrhoea and other digestive disorders) for food-producing animals of the equine species; the substances bismuth subnitrate, bismuth subsalicylate and *piceae turiones recentes extractum* are included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with a 'No MRL required' status for all food producing species, which includes *Equidae*.

Diarrhoea, if untreated, is potentially life-threatening and causes unacceptable suffering of the animal.

Loperamide was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any loperamide-containing veterinary medicinal product authorised for use in equine species. There are veterinary medicinal products authorised for use in species other than the equine (i.e. dog).

The substance loperamide is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Metoclopramide is a dopamine D2 antagonist, whose mode-of-action is to shorten the intestinal transit time and accelerate gastric emptying (Plumb, 2023). These prokinetic effects are via inhibitory actions on presynaptic and postsynaptic D2 receptors, agonism of serotonin 5-HT4 receptors, and antagonism of muscarinic receptor inhibition (Lee and Kuo, 2010). In contrast to unspecific, cholinergic stimulation of smooth muscles of the gastrointestinal tract, metoclopramide coordinates gastric, pyloric, and duodenal motor action (McEvoy, 1992). It also increases oesophageal sphincter tone and increases peristalsis of the oesophagus preventing gastric reflux and causing antiemetic action (Forth and Rummel, 1998).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for the treatment of post-operative ileus. Bethanechol and erythromycin are the two alternatives identified in the current list. The specific advantages captured are as follows: it is a substituted benzamide with several mechanisms of action - (1) it is a dopamine receptor antagonist, (2) it augments the release of acetylcholine from intrinsic cholinergic neurons, and (3) it has adrenergic blocking activity; it is effective in restoring gastrointestinal coordination post operatively and it decreases the total volume, rate and duration of gastric reflux; metoclopramide is a prokinetic drug, which acts more in the proximal gastrointestinal tract; both bethanechol and metoclopramide have been shown to be beneficial in the treatment of post-operative ileus.

The treatment of postoperative ileus includes intravenous fluid substitution, primarily, and use of painkillers if needed. There are veterinary medicinal products available to meet the need for such fluid substitution. In addition, there are a number of painkillers, e.g. flunixin, included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with an entry covering *Equidae*, either with 'No MRL required' status or with numerical MRLs proposed, for which there are veterinary medicinal products authorised for food-producing animals of the equine species. Erythromycin is also included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with an entry covering *Equidae.* However, metoclopramide is considered to bring added clinical benefit by being a prokinetic drug. It has been described that better results regarding improvement of gastrointestinal motility were achieved with metoclopramide when compared to other substances, e.g. bethanechol, which also displays a substantial prokinetic capacity (Koenig and Kote, 2006). It is noteworthy that constant rate infusion lidocaine (intravenously) has also been considered in the past as an alternative for post-operative ileus.

Post-operative ileus, if untreated, is potentially life-threatening and causes unacceptable suffering of the animal.

Metoclopramide was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any metoclopramide-containing veterinary medicinal product authorised for use in equine species. There are veterinary medicinal products authorised for use in species other than the equine (i.e. dog and cat).

The substance metoclopramide is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indications: for the treatment of post-operative ileus. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Phenoxybenzamine is a non-selective long-acting alpha-adrenergic antagonist with antihypertensive properties (Plumb, 2023). Its long duration of action is due to irreversible binding to alpha receptors. By non-competitive blocking of alpha receptors, it causes muscle relaxation and widening of the blood vessels. Phenoxybenzamine binds covalently to a component of the alpha-adrenergic receptor, which reduces the sympathetic effects of adrenaline and noradrenaline resulting in an overall decrease in vasoconstriction and a decrease in system vascular resistance (Hjemdahl et al., 1979; Frang et al., 2001). This causes a vasodilative effect since alpha-adrenergic receptors are present throughout the walls of all blood vessels, which leads to reduced blood pressure (Yoham and Casadesus, 2022).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for the treatment of diarrhoea and colitis. The alternatives identified in the current list include bismuth subsalicylate and flunixin, and the specific advantage captured is that it has a different mode of action (alpha-1

antagonist and antisecretory agent) compared to other authorised treatments and codeine; it provides useful symptomatic management of diarrhoea and colitis.

As indicated previously under 'codeine', the primary treatment of diarrhoea usually includes intravenous fluid substitution and dietary management, and it is considered that there are veterinary medicinal products authorised for an equivalent indication (i.e. for the treatment of diarrhoea and other digestive disorders) for food-producing animals of the equine species; the substances bismuth subnitrate, bismuth subsalicylate and *piceae turiones recentes extractum* are included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with a 'No MRL required' status for all food producing species, which includes *Equidae*. With regards to the colitis indication (a term generally referring to inflammation of the cecum (typhlitis), colon (colitis), or both (typhlocolitis), with subsequent rapid onset of diarrhoea in the adult horses), flunixin is included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with MRLs for *Equidae* and veterinary medicinal products are authorised for food-producing animals of the equine species. The mechanism of action of phenoxybenzamine does not make it a drug of choice for the treatment of colitis.

Diarrhoea and colitis, if untreated, are potentially life-threatening and cause unacceptable suffering of the animal.

Phenoxybenzamine was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any phenoxybenzamine-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance phenoxybenzamine is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Phenylephrine is discussed in detail in section 4.11 (substances for ophthalmology) where it is proposed for treatment of glaucoma and epiphora (please refer to section 4.11 for the detailed assessment of the substance). Phenylephrine is also proposed under this section for the treatment of splenic entrapment. This indication for phenylephrine is currently included in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013).

The effect of phenylephrine on splenic dimensions in horses was studied by Hardy et al. (1994) who found that phenylephrine administration caused a dose-dependent splenic contraction that returned to baseline values by 35 minutes after the end of the infusion. Two studies confirmed the clinical value phenylephrine in the resolution of nephrosplenic entrapment of the large colon (NSELC), albeit they concluded differently regarding the clinical significance of the nonsurgical treatments studied (Baker et al., 2011; Fultz et al., 2013). Phenylephrine is also known to have haemodynamic effects (Hinchcliff et al., 1991); in healthy horses, with and without prior atropine administration, it produced a sharp increase in arterial blood pressure in both groups.

Splenic entrapment, if untreated, may be life-threatening and causes unacceptable suffering of the animal.

The substance phenylephrine is proposed to be qualified as essential because no satisfactory alternative treatments are authorised for food-producing animals of the equine species for the following indication: treatment of splenic entrapment.

Propantheline bromide is a synthetic quaternary ammonium anticholinergic belonging to the parasympatholytics group. In general, parasympatholytics block the action of acetylcholine on smooth muscles, myocardium, glandular cells, peripheral ganglia, and the central nervous system (Brown and Taylor, 2001). Propantheline bromide also inhibits the action of acetylcholine at the postganglionic nerve endings of the parasympathetic nervous system. It displays a dose related inhibiting effect on gastric water and acid secretion, inhibits gastrointestinal motility, and prevents spasms (Dajani and Driskill, 1978). It has also been described that propantheline bromide reduces intestinal transit time by 50% in rats (Haruta et al., 1998).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) as an anti-peristaltic. Two alternatives are identified: atropine and lidocaine, the latter given diluted intrarectally as an enema. The specific advantages captured in the current list are as follows: propantheline bromide is a synthetic quaternary ammonium anticholinergic which inhibits gastrointestinal motility and spasm and diminishes gastric acid secretion; it also inhibits the action of acetylcholine at the postganglionic nerve endings of the parasympathetic nervous system; its effects are similar to those of atropine although they last longer (six hours); propantheline bromide is an important choice for decreasing peristalsis to avoid rectal tearing during rectal palpation or to explore and treat a potential rectal tear where it can be difficult to get a lidocaine enema to work effectively.

It is worth noting that the two current alternatives listed, atropine and lidocaine, are included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with a 'No MRL required' status for *Equidae*, thus constituting satisfactory treatment alternatives available for which veterinary medicinal products authorised for food-producing animals of the equine species are available. In addition, as an anti-peristaltic drug, butylscopolaminium bromide is also in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with a 'No MRL required' status, and veterinary medicinal products are authorised for an equivalent indication for food-producing animals of the equine species, it also qualifies as a satisfactory treatment alternative available.

Increased gastrointestinal motility, if untreated, is potentially life-threatening and causes unacceptable suffering of the animal.

Propantheline bromide was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any propantheline-bromide-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance propantheline bromide is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Ranitidine is a competitive histamine H2-receptor antagonist drug. It exerts its activity by blocking the interaction of histamine with the histamine H2-receptors, with a high selectivity and potency for those present within the gastric wall, reducing the production of gastric acid and its concentration in H+. The result is a dose-dependent inhibition of gastric acid secretion.

It is currently listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for gastric ulcer prophylaxis in neonates. The only identified authorised alternative for the same purpose is (oral) omeprazole, which is a proton pump inhibitor belonging to the substituted benzimidazole class of compounds. The current list indicates, as specific advantages, its different mode of action from

omeprazole and the route of administration (intravenous) which brings (according to the current text) added clinical benefit over all other anti-ulcer medications as these require oral administration. In addition, it is stated that intravenous ranitidine preparations are essential for foals that have absent gastrointestinal motility, a group of animals that are at high risk for ulcers.

However, clinical results with ranitidine have been variable. For example, in critically ill neonatal foals the intragastric pH was variable and a limited response to ranitidine administration was noted (Sanchez et al., 2001). This suggests that, in critically ill neonatal foals, the development of gastric ulcers may not only be due to intraluminal gastric acid. Thus, it can only be considered that the different route of administration (intravenous) brings a theoretical added clinical benefit over oral antiulcer medications in e.g. foals that have absent gastrointestinal motility (ileus).

Prophylactic treatment of critically ill neonates for gastric ulcers has been standard for years, due to the evidence of clinically silent "ulcers" that could result in catastrophic rupture. There are reasons why this may not be necessary or the most appropriate approach. For example, the gastric acid environment is protective against bacterial colonization and translocation of bacteria (Barr, 2011). The pathogenesis of gastric ulcers in neonatal foals may not only involve intraluminal gastric acid, but instead may involve hypoxic/ischemic insult to the gastric mucosa. Gastric ulcer disease in equine neonatal intensive care unit patients appears independent of pharmacological prophylaxis (Barr et al., 2000), where one study found no statistically detectable difference between foals given prophylaxis ulcer medications versus no prophylaxis (Barr et al., 2000). Results from a retrospective study evaluating the presence or absence of gastric ulceration in foals dying or euthanised due to critical illness in the neonatal period showed decreased incidence in gastric ulcers despite reduced prophylactic treatment rates (Barr et al., 2001).

Gastric ulcer in critically ill neonates, if untreated, might be life-threatening and causes unacceptable suffering of the animal.

Ranitidine was mentioned (six times) in the survey to stakeholders, and it was proposed to be removed from the list since omeprazole is an authorised alternative with higher clinical value.

A search in the veterinary medicines database does not retrieve any ranitidine-containing veterinary medicinal product authorised for use in equine species. There are veterinary medicinal products authorised for use in species other than the equine (i.e. dog) containing ranitidine (or ranitidine hydrochloride) as active substance, for administration via oral and intravenous routes. A similar search showed there are 105 VMPs containing omeprazole as active substance, for use in a wide range of target species including animals of the equine species, and for administration via the oral route.

Omeprazole thus constitutes an alternative treatment for treatment of gastric ulcers in critically ill neonates and is, generally, the preferred treatment option (i.e. yields equal or even superior clinical profile for the previously referred indication). However, since no intravenous alternative is available, ranitidine is considered to bring added clinical benefit because it is presented as an injectable formulation, which adds a specific advantage.

The substance ranitidine is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: treatment of gastric ulcers in critically ill neonates, intravenous use. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Sucralfate is a hydroxy aluminium salt of sucrose octasulfate that coats damaged gastric tissues by binding to negatively charged particles at the ulcer base (Nagashima, 1981). It is a gastro-duodenal

protective agent used in the treatment of gastric and duodenal ulcers and to prevent duodenal ulcer recurrence. It has little acid neutralizing effect but creates a protective mechanical barrier between the lining of the gastrointestinal tract and damaging substances. Moreover, sucralfate increases levels of growth factors locally and causes an increase in prostaglandins, thus displaying cytoprotective properties and improving healing-time of the mucosa of the gastrointestinal tract (Candelli et al., 2000). After oral administration, sucralfate forms stable complexes with proteins, which adsorb bile acid and pepsin. Sucralfate forms a gel-like protective adhesive layer, through which acid, pepsin, or bile cannot diffuse, thus protecting tissue against further damage (Geyer and Herling, 2016).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for gastric ulcer prophylaxis in neonates. The only alternative identified in the current list is omeprazole, and the specific advantage captured is that sucralfate has a different mode of action than omeprazole and is a valuable adjunctive in gastric ulcer prophylaxis; its unique mode of action (mucosal adherent) provides physical lesion stabilisation.

Use of sucralfate in equine glandular gastric disease is well established in combination with omeprazole for an enhanced clinical performance (Banse and Andrews, 2019; van den Boom, 2022). Its different mode of action than omeprazole (mucosal adherent), which provides physical lesion stabilisation, is considered to bring added clinical benefit. Omeprazole is included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with a 'No MRL required' status for *Equidae*.

Gastric ulcers, if untreated, are potentially life-threatening and cause unacceptable suffering of the animal.

Sucralfate was mentioned (once) in the survey to stakeholders, and it was proposed that the indication be modified to treatment of equine glandular gastric disease. Sucralfate is not only useful for gastric ulcer prophylaxis in neonates; thus, the indication could be modified and amended to 'treatment and prevention of gastric ulcers in horses'.

A search in the veterinary medicines database does not retrieve any sucralfate-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance sucralfate is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indications: treatment and prevention of gastric ulcers in horses. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal, avoiding unnecessary suffering of the animal.

Knowledge regarding gastrointestinal substances in horses for this assessment was derived from textbooks, review articles, and retrospective studies. No clinical trials could be identified in horses.

B. Considerations regarding consumer safety

Metoclopramide is a lipid-soluble antiemetic agent and dopamine D2 antagonist used in both human and veterinary medicine. In veterinary medicine, it can be administered orally and parenterally.

The pharmacokinetics of metoclopramide have been well characterized in humans. Metoclopramide is rapidly and almost completely absorbed from the gastrointestinal tract after oral administration. It undergoes a hepatic first-pass metabolism, hence absolute bioavailability and plasma concentrations are subject to a wide interindividual variation. The bioavailability is about 80% (varies between 30-100%), and peak plasma concentrations occur about 1 to 2 hours after oral dosing. It is weakly bound

to plasma proteins, ranging from 13 to 30%. Metoclopramide is widely distributed in the body and readily crosses the blood-brain barrier into the central nervous system; it also crosses the placenta and is excreted in variable amounts in breast milk. Metoclopramide undergoes metabolism in the liver via oxidation, primarily via cytochrome P450 2D6 enzyme, as well as via glucuronide and sulfate conjugation. Elimination is biphasic, with a terminal elimination half-life of approximately 4 to 6 hours. It is excreted in the urine, with about 85% of a dose being eliminated in 72 hours. In adults with severe renal impairment, there is a reduction in metoclopramide clearance, resulting in a prolongation in the terminal elimination half-life to 7.7 to 17.8 hours. (Scriba, 2009; Ge et al., 2020). The plasma concentration-time profile after administration of metoclopramide intravenously to man is described by a biexponential equation. In another study, after 10 mg intravenous dosing, the volume of distribution was high at 2223.7 ± 183.7 ml/kg, the total body plasma clearance 11.61 ± 1.32 ml/min Kg and the half-life for the β phase was 156.7 min (Bateman et al., 1980). Following intravenous injection of a microdose of [^{11}C]-metoclopramide in healthy volunteers, the majority of administered radioactivity was taken up by the liver followed by urinary excretion (Bauer et al., 2021).

Pharmacokinetic parameters were measured in calves after a single intravenous administration of 0.4 mg/Kg. The volume of distribution was 16.8 ± 7.1 l/kg and the clearance 5.01 ± 2.80 l/h/kg. The mean half-life observed was reported as 9.6 ± 5.7 hours (Takayasu et al., 2015). In goats, a two-compartment model best described the pharmacokinetics of metoclopramide administered intravenously and two-compartment pharmacokinetics with first-order absorption was followed when administered intramuscularly. Metoclopramide distributed very rapidly and widely after intravenous administration of 0.5 mg/kg to goats, the volume of distribution being 3.166 l/kg. The mean half-life for the β phase was 37.038 and 62.603 mins when administered intravenously and intramuscularly, respectively (Huhn et al., 1992).

Extrapyramidal side effects (EPS) have been reported in humans and in the horse as a consequence of metoclopramide treatment (Scriba, 2009; Agass et al., 2016; Ge et al., 2020). In one study performed in children it was observed that when doses of less than 2 mg were administered, the toxicity was minimal; they concluded that the incidence of metoclopramide toxicosis was related to the dose and treatment duration (Allen et al., 1985). Also, other authors report that patients receiving lower doses of metoclopramide, 10-20 mg for adults and 0.1-0.2 mg/kg for children, have a decreased incidence of EPS (Watcha et al., 1992). A systematic review found that extrapyramidal symptoms were the most commonly reported adverse effects (9%) associated with multiple-dose metoclopramide use, while also suggesting that these symptoms are uncommonly reported in children (Lau Moon Lin et al., 2016). Another study that actively evaluated EPS reported that no child (0/14) was observed to develop EPS during treatment with 5-40 mg daily of oral metoclopramide (Nicolson et al., 2005). The use of metoclopramide in elderly patients and for a period of more than 5 days were considered risk factors for developing involuntary movements (Frez et al., 2018). In 2013, the EMA's human medicines committee (CHMP) re-examined metoclopramide and confirmed the risks of neurological effects; it was stated that the risk of acute neurological effects is higher in children and that the risk is increased at high doses or with long-term treatments. A short-term use (up to 5 days) and a restriction of the maximum dose were recommended (EMA/13239/2014 Corr.1).

Metoclopramide at intramuscular doses up to 20 mg/kg/day in male and female rats was found to have no effect on fertility and reproductive performance. The product information of human medicinal products suggests no evidence of impaired fertility or harm to the fetus from teratology studies performed in rats and rabbits at oral metoclopramide doses up to 45 mg/kg/day. Available data on mutagenicity are inconclusive (ECHA, 2023).

Considering the available information on the pharmacokinetics and toxicity of metoclopramide, it can be accepted that the substance 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.

Ranitidine, as previously stated, is a H2 blocker. No other H2 blockers (e.g. cimetidine or famotidine) are listed in the Annex to Commission Regulation (EU) No 37/2010. However, omeprazole (whose pharmacodynamic effect is reached by inhibition of the H+/K+-ATPase gastric proton pump in the secretory membrane of the parietal cells) is listed in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with a "No MRL required" status for *Equidae*, and with the provision "for oral use only".

Ranitidine pharmacokinetics has been well described in humans and there are good correlations in ranitidine ADME characteristics between human and laboratory species. Plasma concentration data after intravenous administration fits a two compartment (biexponential) model (Van Hecken et al., 1982; Roberts, 1984; Madani et al., 2014). The elimination half-life has been described as approximately two hours in humans and approximately four hours in dogs after intravenous administration (Van Hecken et al., 1982; Eddershaw et al., 1996; Madani et al., 2014). In man, ranitidine has been reported to be rapidly excreted in high concentrations through the kidney and metabolized in the liver to inactive metabolites. Accumulation can occur in patients with renal insufficiency. Mean urinary recovery of unchanged ranitidine was 79% after intravenous administration (of 150 mg at 24 hours), while it accounts for only 27% after oral administration. Metabolites in the urine amounted to approximately 9% of excreted drug (after 100 mg intravenous administration). Some unchanged ranitidine and metabolites are also excreted extra renally (Van Hecken et al., 1982). Following oral administration, ranitidine is absorbed rapidly, but undergoes extensive first-pass metabolism. The commonly reported bioavailability value is approximately 50%. Peak plasma concentrations occur at ≈ 2-3 hours after oral dosing. Ranitidine is distributed widely throughout the body and is only 10-19% bound to plasma protein (Plumb, 2015).

In adult horses, the clearance value reported in one study after intravenous administration of 2.2 mg ranitidine/kg bw was 9.8 ml/min/kg; the apparent volume of distribution was 1.1 L/kg; the mean residence time observed was 113 min and the terminal half-life 170.45 min. In foals, the clearance value reported after intravenous administration of 2.2 mg ranitidine/kg bw was 13.3 ml/min/kg, the apparent volume of distribution was 1.5 L/kg, and the mean residence time was 108.9 min. Furthermore, with respect to oral ranitidine, the bioavailability has been described to be ≈ 27% in adults and 38% in foals (Holland et al., 1997a; 1997b). Residue depletion data in horses is not available.

No genotoxic potential has been found for ranitidine nor its main metabolites, using the Ames test. In addition, ranitidine was not genotoxic in a series of in vivo studies. Regarding carcinogenicity, the opinion provided by EMA's human medicines committee (CHMP) regarding ranitidine-containing medicinal products due to the presence of low levels of an impurity called N-nitrosodimethylamine (NDMA) is noted (EMA/599846/2020 Corr. 1). NDMA has been found in several ranitidine medicines above levels considered acceptable; NDMA is classified by the International Agency for Research on Cancer (IARC) as probably carcinogenic to humans. However, this conclusion is not considered to impact on consumer safety of food commodities derived from animals of the equine species treated with ranitidine after a six-month withdrawal period is applied.

Therefore, considering the pharmacokinetic data available and other scientific evidence, it can be accepted that ranitidine 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.

Sucralfate is a hydroxy aluminium salt of sucrose octasulfate that coats damaged gastric tissues by binding to negatively charged particles at the ulcer base. It is used orally in human and veterinary medicine as a gastro-duodenal protective agent.

Sucralfate is only slightly absorbed from the gastrointestinal tract after oral doses. However, there can be some release of aluminium ions and of sucrose sulfate; small quantities of sucrose sulfate may then be absorbed and excreted, primarily in the urine and some absorption of aluminium may also occur (Scriba, 2009). Approximately 3-5 % of an orally administered dose of sucralfate is absorbed, and more than 90% of the dose is excreted unchanged in the faeces (Garnett, 1982).

Sucralfate acts locally with negligible absorption offering a favorable safety profile. The most common side effect is constipation seen in 1 to 10% of patients. Long-term use of sucralfate generally results in minimal aluminium retention, except in cases of renal insufficiency. Uremia increased absorption of aluminium from the gut. Sucralfate should be used with caution in patients with end-stage renal disease or avoided to prevent aluminum intoxication (Kudaravalli and John, 2022). When absorbed, the elimination of aluminium was prolonged in two studies performed in subjects with normal and insufficient renal function. Elevated urinary aluminium excretion persisted for 10 and 14 days, respectively, after 3 weeks of sucralfate therapy (Burgess, 1991).

Risks associated with sucralfate overdosing are minimal as sucralfate has minimal absorption from the gastrointestinal system; many patients that overdosed on sucralfate remained asymptomatic (Kudaravalli and John, 2022).

Considering that sucralfate acts locally with negligible absorption and the absorption of aluminium is related with renal impairment, no residues of sucralfate are expected on the horse. Besides, a withdrawal period of 6 months would cover the urinary aluminium excretion described above. Therefore, it can be accepted that the substance 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.

4.7.3. Assessment of new substances proposed to be added to the list in the stakeholders survey

A. Considerations on the essentiality of the substance(s)

Misoprostol was mentioned (nine times) in the survey to stakeholders and it was suggested for addition to the list as an alternative to omeprazole and sucralfate, when not effective, for the treatment of gastric glandular disease and colitis, and for being superior to the combination omeprazole-sucralfate for the treatment of equine gastric glandular disease (e.g. Varley et al., 2019). Misoprostol is a prostaglandin E1 analogue used to reduce the risk of NSAID-induced gastric ulcers by reducing secretion of gastric acid from parietal cells (e.g. Davis, 2015; Krugh and Maani, 2023). Its cytoprotective effect on gastric mucosal cells is attributed to stimulation of epithelial mucus secretion, bicarbonate secretion, dose-related inhibition of gastric acid and pepsin production and secretion, and regulation of gastric acid and pepsin secretion (e.g. Varley et al., 2019). Recent work has shown that misoprostol inhibits multiple functions of equine leukocytes in vitro, including LPS-stimulated TNFα production (e.g. Martin et al., 2019). Numerous in vitro and ex vivo studies in humans and other species, including horses, have also demonstrated the potential of anti-inflammatory effects of misoprostol through cAMP-mediated pathways (e.g. Martin et al., 2017).

It is considered that misoprostol brings added clinical benefit compared to the existing alternative medicinal product(s) authorised for food producing animals of the equine species due to its specific mode of action, resulting in cytoprotective effects on gastric mucosal cells.

Gastric glandular disease and colitis, if untreated, are potentially life-threatening and cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any misoprostol-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance misoprostol is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: for the treatment of gastric glandular disease and colitis. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal. Misoprostol is superior to combined omeprazole-sucralfate for the treatment of equine gastric glandular disease.

Knowledge regarding gastrointestinal substances in horses for this assessment was derived from review articles.

B. Considerations regarding consumer safety

Misoprostol is used orally in human medicine to treat benign gastric and duodenal ulceration and to prevent NSAID-induced ulcers. It has also uterotonic activity and can induce labor.

In humans, misoprostol (an ester) is rapidly and completely de-esterified to the pharmacologically active carboxylic acid in the stomach after oral administration. Absorption of misoprostolic acid is rapid, with peak plasma concentration after 15-30 minutes. A 200-µg oral dose produced a peak plasma concentration of the acid metabolite of 309 ng/L. The acid has a plasma half-life of 13-40 minutes and is 85% bound to serum albumin plasma protein. Misoprostol fits the criteria of a highly variable drug since it exhibits 43% intersubject variability (Davies et al., 2001). Plasma concentrations are typically detectable for only 2-3 hours after the recommended 20-µg dose. Approximately 80 % of radioactivity is recovered in urine after an oral dose of ^{3}H-misoprostol and a negligible amount of the parent compound appears in urine (Davies et al., 2001). After an oral dose of 400 µg, the bioactive misoprostol acid was rapidly formed and peaked at 19.7 minutes. After approximately 60 minutes it was quickly cleared from the systemic circulation. In this study, two misoprostol tablets were compared, and mean plasma half-lives obtained were 69.7 ± 42.5 and 59.9 ± 33.0 minutes (Wang et al., 2022).

Pharmacokinetics have been evaluated in horses. Misoprostol was rapidly absorbed and eliminated regardless of whether administered orally (PO) or rectally (PR) to horses. The free acid form was measured in plasma in pharmacokinetic analyses of the drug after a single dose of 5 µg/kg. Plasma concentrations were below the LLOQ (100 pg/ml) by 90 minutes PR and by 8 hours during the unfed and fed PO conditions. PR administration resulted in the highest C_{max} and shortest T_{max} and plasma half-life. The C_{max} was 967 ± 492 pg/ml for the PR conditions and 655 ±259 and 352 ±109 pg/ml for the unfed and fed PO conditions, respectively. The T_{max} was 5 minutes for the PR conditions and 21 ± 13 and 30 ± 29 min for the unfed and fed PO conditions. Plasma half-life was 21 ± 29 mins for the PR condition and 170 ± 129 and 119 ± 51 mins for the unfed and fed PO conditions, respectively. A considerable amount of variability among horses in each experimental condition was described (Lopp et al., 2019). Another study performed in horses with a single oral dose of 5 µg/kg, reports mean C_{max} of 0.29 ± 0.07 ng/ml at 23.4 ± 2.4 min following administration and a mean elimination half-life of 40.2 ± 12 min (Martin et al., 2019).

The most common adverse effects of misoprostol are nausea, vomiting, diarrhoea, abdominal pain, chills, shivering and fever. Information relating to human medicinal products indicates that mutagenic and carcinogenic potential has not been observed. There was no evidence of an effect on tumour occurrence or incidence in rats receiving daily doses up to 150 times the human dose for 24 months. Similarly, there was no effect on tumour occurrence or incidence in mice receiving daily doses up to 1000 times the human dose for 21 months. However, exposure to misoprostol in early pregnancy has been associated with multiple congenital defects. The FDA classifies misoprostol as a category X drug (evidence of teratogenesis in animals and humans) in the first and second trimesters of pregnancy. In pregnant rabbits, doses of 300 to 1500 µg/kg on days 7-19 of embryogenesis have been associated with teratogenic effects. In humans, there are several malformations associated with the use of misoprostol in the first trimester of pregnancy (Moebius sequence, arthrogryposis, transverse reduction of extremities and limbs, congenital clubfoot, hydrocephalus, among others). The teratogenic mechanism attributed to these foetal malformations and alterations is a result of vascular disruption caused by intense uterine contractions and vaginal bleeding leading to embryonic hypoperfusion with tissue hypoxia, endothelial cell damage and tissue loss. It is still unclear whether if the risk of teratogenicity is dose-dependent. A relationship between the time of exposure and the range of defects observed has been reported (Pastuszak et al., 1998; Tang et al., 2007; Krugh and Maani, 2023; National Specialized Commission on Maternal Mortality et al., 2023).

No residues depletion studies are available in horses or food-producing animals for misoprostol. However, considering the above-mentioned pharmacokinetic characteristics it can be assumed that misoprostol will be rapidly eliminated from the horse. Although congenital defects have been described with the use of misoprostol, they are associated with the intrinsic uterotonic activity rather than a direct effect on the developing foetus. No other toxicological concerns have been reported. Therefore, it can be accepted that the substance 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.

4.7.4. Conclusion

Based on the above assessment and justifications, the following recommendations are proposed:

1. The following active substances, currently in Regulation (EU) 1950/2006 as amended by Regulation (EU) 122/2013, are proposed to be retained in the list, either without modification or with an amendment of the current entry as shown below:

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Metoclopramide	for the treatment of post-operative ileus	intravenous fluid substitution, painkillers (e.g. flunixin), lidocaine	prokinetic drug
Phenylephrine[41]	treatment of splenic entrapment	none identified	clinical value in the resolution of splenic entrapment; causes a dose-dependent splenic contraction
Ranitidine	treatment of gastric ulcers in critically ill neonates, intravenous use	omeprazole	the intravenous route of administration brings added clinical benefit over other oral antiulcer medications
Sucralfate	treatment and prevention of gastric ulcers in horses	omeprazole	different mode of action than omeprazole (mucosal adherent), which provides physical lesion stabilisation

[41] This substance is discussed in detail in section 4.11 (substances for ophthalmology).

2. The following active substances, currently in Regulation (EU) 1950/2006 as amended by Regulation (EU) 122/2013, are proposed to be removed from the list: bethanechol, codeine, loperamide, phenoxybenzamine, propantheline bromide.

3. The following active substance, suggested for addition to the list in the survey to stakeholders, is proposed to be added to the list with an entry as shown below:

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Misoprostol	for the treatment of gastric glandular disease and colitis	omeprazole, sucralfate	superior to omeprazole for the treatment of equine gastric glandular disease

4.8. Substances for metabolic disorders

4.8.1. Overview

	Active substance (ATCvet code)
Substances in Regulation (EU) 1950/2006 **proposed to be retained**, with or without amendment of the current entry	Insulin (QA10AC03; QA10AC01)
Substances in Regulation (EU) 1950/2006 **proposed to be removed**	None
Substances from stakeholders' survey **proposed for inclusion**	Pergolide[42] (QN04BC02)
Substances from stakeholders' survey **not proposed for inclusion**	Canagliflozin (QA10BK02); Ertugliflozin (QA10BK04); Flutamide (QN01BB09); Goserelin (QN01BB09); Metformin (QN01BB09); Velagliflozin (QA10BK90)

4.8.2. Review of the existing entries in Regulation (EU) 1950/2006, as amended by Regulation (EU) 122/2013, considering the survey results

A. Considerations on the essentiality of the substance(s)

Insulin is a natural hormone secreted by the beta cells of the pancreatic Langerhans islets in various animal species (sometimes with minor structure differences) (Plumb, 2015; Papich, 2021). Insulin interacts with insulin receptors and therewith triggers a complex cell signaling cascade, which results in increased glucose uptake into the cells. The main effects of insulin are the reduction in circulating blood glucose concentrations and the storage of fat. Overall, insulin influences the regulation of the carbohydrate and fat metabolism.

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), for treatment of hyperlipaemia, used in combination with glucose therapy, and for the diagnosis of metabolic disorders. No alternatives are identified in the current list and the specific advantage captured is the absence of available alternatives.

[42] Please refer to the section 'Points for further consideration' within this scientific advice for additional scientific considerations regarding this substance. It is proposed that despite the substance fulfilling the criteria established in the European Commission's request, it is not listed.

Hyperlipaemia is a metabolic disorder, of ponies and donkeys primarily, where a negative energy balance, combined with stress, leads to uncontrolled fatty acid mobilization and an increase of very low-density lipoproteins in circulating blood. This thus results in persistent elevated plasma triglycerides i.e. hyperlipidaemia. If untreated, hyperlipemia might result in multi-organ failure due to fatty infiltration of organs (e.g. lipids deposited in the liver and kidneys).

Early-onset cases can be treated with glucose and other therapies. For refractory or severe cases, where treatments such as fluid therapy (glucose) is not effective, exogenous insulin provides an added clinical advantage to halt or address the metabolic issues associated with hyperlipemia (exogenous insulin can also halt further lipolysis and stimulate gluconeogenesis). However, it should be noted that insulin resistance has been reported to be a factor in cases of hyperlipaemia. For such cases, treatment with insulin may be arguable (Hughes et al., 2004; Durham and Thiemann, 2015).

Regarding diagnostic procedures for identifying metabolic disorders (insulin resistance), exogenous insulin is used for the combined glucose-insulin test (CGIT) or frequently sampled insulin-modified intravenous glucose tolerance test (FSIGTT) (Dunbar et al., 2016). Although less commonly used in practice, the CGIT and FSIGTT remain the most accurate diagnostic tests for insulin resistance in the equine species, because they are function metabolic tests, as opposed to measuring blood endogenous insulin concentrations.

Hyperlipaemia, if untreated, might be life-threatening and causes unacceptable suffering of the animal. Diagnosis of metabolic disorders is critical in enabling suitable treatment and, as a result, avoiding unnecessary suffering of the animal.

Insulin was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any insulin-containing veterinary medicinal product authorised for use in equine species. There are veterinary medicinal products authorised for use in species other than the equine (i.e. dog and cat).

The substance (exogenous) insulin is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: as an aid in the treatment of hyperlipaemia unresponsive to glucose therapy or severe hyperlipemia, used in combination with glucose and other therapies; for the diagnosis of metabolic disorders (e.g. insulin resistance associated with equine metabolic syndrome or pars pituitary intermedia dysfunction). It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal. Low-molecular weight heparin can be used for cases of hyperlipemia, though insulin is the preferred clinical choice.

Knowledge regarding use of insulin in horses for this assessment was derived from textbooks, review articles, and retrospective studies. No clinical trials could be identified for use of insulin in horses.

B. Considerations regarding consumer safety

Insulin is marketed in the EU both as veterinary and human medicinal products, either as intermediate or long-acting formulations.

Insulin is a protein drug. Insulin has a molecular weight of 5800 Daltons and is poorly absorbed through the columnar mucous membrane of the gastrointestinal tract (Wong et al., 2015). The low bioavailability of intact insulin via the oral route can be attributed to its susceptibility to gastrointestinal

enzymes, high molecular weight, and low diffusion rate across the mucin barrier. Oral delivery of insulin is susceptible to proteolysis. Intact protein absorption is typically low (<1%). The digestive enzyme responsible for protein degradation in the stomach is pepsin, while three additional proteolytic enzymes are present within the intestine (trypsin, chymotrypsin, and carboxypeptidase).

Papich (2021) mentions, in connection with the use of insulin in food producing animals, that "No regular information is available for animals intended for food. Because of a low risk of residues, no withdrawal time is suggested".

Considering the nature of the substance and with due consideration of the pharmacokinetic data available, it can be accepted that insulin will not pose an unacceptable risk for consumers when used in food-producing animals of the equine species following a six-month withdrawal period is respected.

4.8.3. Assessment of new substances proposed to be added to the list in the stakeholders survey

A. Considerations on the essentiality of the substance(s)

Canagliflozin (QA10BK02), **ertugliflozin** (QA10BK04), and **velagliflozin** (QA10BK90) (herein after referred to as 'gliflozins') were mentioned once, twice, andonce, respectively, in the survey to stakeholders for the treatment of insulin dysregulation and refractory hyperinsulinemia. In the responders' opinions, no treatment alternatives are available and gliflozins are the only option for horses. Scientific references were provided and are discussed below.

Gliflozins are sodium-glucose co-transporter-2 (SGLT2) inhibitors, which block glucose resorption in the renal proximal tubule. These are a relatively new class of human antidiabetic drugs that lower insulin concentration increasing urinary glucose excretion, thus reducing plasma glucose concentration and reducing insulin secretion from the pancreas. There are currently no registered veterinary medications which specifically target hyperinsulinaemia and hyperinsulinaemia-associated laminitis in horses, this being the reason why some studies have recently been conducted in horses (Meier et al., 2018, 2019; Frank et al., 2023; Sundra et al., 2023a, 2023b).

In humans, sodium-glucose cotransporter-2 (SGLT2), predominantly expressed in the kidney, are responsible for approximately 90% of urinary glucose reabsorption in the proximal convoluted tubule of the kidney (Chao and Henry, 2010). It is well established that inhibition of SGLT2 lowers blood glucose levels by induced glucosuria, providing optimal glycaemic control in human type 2 diabetes with low risk of secondary hypoglycaemia since their action stops when the filtered glucose load falls below approximately 80 g/day (Vallon and Thomson, 2017).

According to the survey respondents, insulin dysregulation in horses manifests, typically, as basal hyperinsulinaemia, excessive or prolonged hyperinsulinaemic response to carbohydrate challenge, or tissue insulin resistance, which is an equine specific endocrine disease that could cause hyperinsulinemia-associated laminitis. In horses, insulin dysregulation might lead to serious or life-threatening conditions. It had been demonstrated that treatment with ertugliflozin and velagliflozin reduced hyperinsulinaemia and improved insulin dysregulation in horses (Meier et al., 2018, 2019; Frank et al., 2023). In the respondents' opinions, gliflozins offer substantial clinical advantage over current practices or available treatments, in particular for animals refractory to husbandry and management changes, as described by Frank et al. (2022). In addition, the responders indicated that gliflozins can be used for the treatment of hyperinsulinaemia in cases of obesity, equine metabolic syndrome (EMS), or pars pituitary intermedia dysfunction (PPID).

With due consideration of the arguments presented, acknowledging that gliflozins could constitute a promising treatment option for reduction of hyperglycaemia in non-insulin-dependent horses, the expert group does not agree that these substances should be considered essential within the meaning and terms provided in the European Commission's request.

Insulin dysregulation (ID) in horses is defined as any combination of basal (resting) hyperinsulinemia, postprandial hyperinsulinemia (response to oral sugar test, OST, or consumed feeds), or tissue insulin resistance (IR, either hepatic and/or peripheral). It is the central endocrine disorder of the equine metabolic syndrome (EMS) and is typically associated with increased generalized or regional adiposity (obese EMS) but can be detected in lean horses (non-obese EMS). It can exist in the absence of EMS in association with conditions such as PPID (pituitary pars intermedia dysfunction, also known as Equine Cushing's disease) and transiently with systemic illness, stress, pregnancy, and starvation (Frank et al., 2022). EMS is highly associated with an increased risk of hyperinsulinemia-associated laminitis (HAL) and potentially other morbidities. Inciting factors associated with EMS development are improper management, exposure to environmental factors, or epigenetic influences on gene expression (Frank et al., 2022).

In contrast to other species, horses rarely show marked hyperglycemia or suffer glucose toxicity, as the equine pancreas can produce large amounts of insulin for prolonged periods without pancreatic failure (McGowan et al., 2004). This can result instead in insulin toxicity (Asplin et al., 2007). Hyperinsulinemia in horses is multi-factorial; however, De Laat et al. (2015) identified the diet as a triggering factor (i.e. a diet high in non-structural carbohydrates in animals with a tendency for enhanced glucose absorption). Gliflozin therapy does not correct hyperinsulinemia.

Therapeutic options for treating hyperinsulinemia are limited. In cases with concurrent PPID, pergolide mesylate is an option (Donaldson et al., 2002). However, modified husbandry practices, including diet, housing modifications to reduce stress and foot care, including restricting access to pasture and diets low in non-structural carbohydrates improves insulin sensitivity, also reducing any risk for developing laminitis (Frank and Tadros, 2014). Exercise also improves insulin sensitivity, with light regular exercise being sufficient to maintain the improvement (Freestone et al., 1992; Menzies-Gow, 2010; Powell et al., 2010). These remain as the first line treatment option.

Meier et al. (2018) studied whether velagliflozin reduced hyperinsulinemia and prevented laminitis in insulin-dysregulated ponies fed a challenge diet high in non-structural carbohydrates (NSC). Lower (45%) maximum insulin concentrations were observed in the treated group. The diet induced Obel grade 1 or 2 laminitis in 14 of the 37 controls (38%), whereas no velagliflozin-treated ponies developed laminitis. Velagliflozin showed promise as a safe and effective compound for treating ID and preventing laminitis by reducing the hyperinsulinemic response to dietary non-structural carbohydrates. However, the effect of the change in diet was not investigated since all animals received a challenge diet high in NSC. Similarly, Meier et al. (2019) further examined the safety and efficacy of velagliflozin over 16 weeks of treatment and over 4 weeks of withdrawal, in 24 ID ponies, concluding velagliflozin appears to be a promising and safe treatment for equine ID, bringing post-prandial insulin concentrations below the laminitis risk threshold, albeit without normalising them. The efficacy of ertugliflozin in the management of hyperinsulinaemia and laminitis in horses has also been studied (Sundra et al., 2023a, 2023b). Similarly to velagliflozin, while results of these two studies indicate ertugliflozin may be effective in reducing insulin concentrations in horses and ponies with EMS, it was also noted that insulin levels did not return to normal in all horses, and increases in triglyceride concentrations were also observed. This result is not surprising, since horses suffering from ID generally display normoglycemia. Thus, glucose reabsorption via SGLT2 transporters may not result in an improvement of the disease, i.e. reduction of insulin levels. More importantly, none of the available

studies suggests that treatment with SGLT2 inhibitors could or should substitute what continues to be the preferred clinical option for treatment of insulin dysregulation and refractory hyperinsulinemia in horses, i.e. husbandry strategies and diet. EMS is generally controlled by dietary strategies and exercise programs that have demonstrated to improve insulin dysregulation (Durham et al. 2019; Frank et al., 2022).

When medical treatment is required for ID horses, levothyroxine continues to be the first line treatment (Frank et al., 2022). Levothyroxine is included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with a 'No MRL required' status for all mammalian food-producing species, which covers *Equidae*. When the animal suffers from severe laminitis, or in cases of HAL, treatment would be necessary, which generally includes pain management (for which there are sufficient alternatives available included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010) and orthopaedic intervention. For PPID treatment please refer to 'pergolide' below. Insufficient evidence is available as to the added clinical benefit of SGLT2 inhibitors compared to current available pharmacological therapy and management options.

Insulin dysregulation, if untreated, is potentially life-threatening and may cause unacceptable suffering.

A search in the veterinary medicines database does not retrieve any gliflozin-containing veterinary medicinal product authorised for use in equine species. There is a (recently authorised) velagliflozin-containing veterinary medicinal product for cats.

The substances canagliflozin, ertugliflozin and velagliflozin are not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; they do not bring added clinical benefit compared to other treatment options like levothyroxine, which continues to be the first line treatment. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Flutamide was mentioned (once) in the survey to stakeholders and it was suggested for addition to the list. No specific indication was mentioned by the responder. No scientific references were provided.

Flutamide is a selective non-steroidal androgen receptor competitive antagonist that is used as an anti-androgenic drug for controlling the sexual behavior of stallions with breeding potential in competitions and trainings (Mendoza et al., 2017). To that effect, the substance deslorelin acetate, which is included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with a 'No MRL required' entry for *Equidae*, meets any therapeutic need for the same indication. Flutamide is not considered to bring added clinical benefit for controlling the sexual behaviour of stallions.

The substance is not intended for the treatment of a specific condition but to control the sexual behavior of stallions with breeding potential in competitions and trainings.

A search in the veterinary medicines database does not retrieve any flutamide-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance flutamide is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indication in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Goserelin (GnRH agonist implant) was mentioned (once) in the survey to stakeholders and it was suggested for addition to the list as a treatment for inducing ovulation in anoestrus mares. Low-dose, slow-release GnRH agonist formulations injected subcutaneously as implants showed potential as a practical method of hastening renewed ovarian cyclicity in anoestrous mares (Allen et al., 1987). The responder stated that deslorelin implants could be used as an alternative for the same indication.

Goserelin is used as GnRH agonist to induce ovulation in seasonally anestrous mares (Fitzgerald et al., 1993) and to determine ovarian cyclicity and follicular activity of mares (Mumford et al., 1994). In small animal medicine it is used for "chemical castration"; it is also possible to use deslorelin for the same purpose. In fact, Schönert et al. (2012) showed, by means of histopathological analysis, that a subcutaneously applied deslorelin acetate implant in a three-year-old stallion reduced the spermatogenesis and decreased the testicular volume after treatment, and while it initially increased the 'male behavior', 15 days after treatment it shifted towards a more 'gelding-like' behavior. Deslorelin acetate is included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with a 'No MRL required' entry for *Equidae* and can be used for inducing ovulation in anoestrus mares. Goserelin has not shown to bring added clinical benefit compared to deslorelin.

Anoestrus, if untreated, is not life-threatening and does not cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any goserelin-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance goserelin is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Metformin was mentioned (two times) in the survey to stakeholders, and it was suggested for addition to the list as an option for treatment of equine metabolic syndrome (EMS) and for managing insulin dysregulation (ID) in horses. It was cited that metformin decreases intestinal absorption of glucose and the corresponding insulin response both in healthy horses and in horses with experimentally induced ID (Rendle et al., 2013; Durham et al., 2019).

It is used as a biguanide antihyperglycemic agent in conjunction with diet and exercise for glycemic control in type 2 diabetes mellitus in humans (Viollet et al., 2012). It is considered an antihyperglycemic drug because it lowers blood glucose concentrations in type II diabetes without causing hypoglycaemia. Metformin administration results in reduced gluconeogenesis and glycogenolysis and reduces resorption of glucose in the gastrointestinal tract (Plumb, 2011). In human medicine, it is commonly described as an 'insulin sensitizer', leading to a decrease in insulin resistance and a clinically significant reduction of plasma fasting insulin levels (Viollet et al., 2012). It also causes modest weight loss, making it an effective choice for obese patients with type II diabetes (Lund et al. 2007). Metformin is indicated as an adjunct to diet and exercise to improve glycaemic control. However, the drug is only effective in a small percentage of horses and may lose efficacy over time (Frank et al. 2022).

Therapeutic interventions for treating equine metabolic syndrome (EMS) and for managing insulin dysregulation (ID) in horses are limited in their efficacy. Thus, management of obese horses with dietary strategies and exercise programs aiming to improve insulin regulation and weight loss is imperative. Moreover, levothyroxine, which is included in Table 1 of the Annex to Commission

Regulation (EU) No 37/2010 with a 'No MRL required' status for all mammalian food-producing species, which covers *Equidae*, remains the first line treatment (Rendle et al., 2018; Durham et al., 2019; Frank et al., 2022).

Equine metabolic syndrome and insulin dysregulation, if untreated, are potentially life-threatening and may cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any metformin-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance metformin is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; it does not bring added clinical benefit compared to other treatment options like levothyroxine, which continues to be the first line treatment. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Pergolide was mentioned (six times) in the survey to stakeholders and it was proposed for addition to the list for the treatment of pituitary pars intermedia dysfunction in horses (PPID, also known as Equine Cushing's disease). In the responders' opinions, no treatment alternatives are available. The scientific references provided to justify its addition to the list are discussed below.

Pituitary pars intermedia dysfunction (PPID) (Equine Cushing's Disease) is a slowly progressive age-related degenerative disease of dopaminergic neurons in the hypothalamus. PPID is the most common endocrine disorder of geriatric horses, affecting 20-25% of horses over the age of 15 years (Durham et al., 2019; Kirkwood et al., 2022). A huge array of clinical abnormalities is associated with PPID, including hypertrichosis, lethargy, muscle atrophy, pendulous abdomen, polydipsia, polyuria, abnormal fat deposition, hyperhidrosis, recurrent infections, infertility, behavioural changes, insulin dysregulation, and laminitis (Kirkwood et al., 2022). PPID is a chronic metabolic disorder of *Equidae* and incurable, but manageable. Appropriate treatment is necessary for the horse's quality of life, and PPID can be managed effectively with pergolide mesylate and husbandry practices (Tatum et al., 2020). In fact, pergolide is the treatment of choice for PPID.

Since PPID was first described in 1932, considerable research has been conducted investigating PPID pathophysiology, prevalence of clinical signs, appropriate diagnostic techniques, and treatment options (Kirkwood et al., 2022). For example, it is now known that the pathogenesis of this disease leads to chronic elevations in adrenocorticotropic hormone (ACTH), and blood cortisol. This further leads to some of the more serious clinical abnormalities, e.g. lethargy, muscle atrophy, polydipsia, polyuria, recurrent infections, insulin dysregulation, and laminitis, while muscle wastage in horses with PPID is due to atrophy of types 2A and 2B muscle fibres (Aleman et al., 2006).

Pergolide is a synthetically produced ergot alkaloid derivative and a potent, long-acting dopamine receptor agonist. Both in vitro and in vivo pharmacological studies have shown that pergolide acts as a selective dopamine agonist and has little or no effect on noradrenergic, adrenergic or serotoninergic pathways at therapeutic doses. Like other dopamine agonists, pergolide also inhibits the release of prolactin. The therapeutic effect of pergolide in horses with dysfunction of the pars intermedia of the pituitary gland (PPID) is mediated by stimulation of dopamine receptors (Kirkwood et al., 2022). In addition, pergolide has been shown to reduce plasma levels of ACTH, MSH and other peptides produced from proopiomelanocortin in horses with PPID (Tatum et al., 2020).

In their review, Tatum et al. (2020) described the state-of-the-art practices in terms of PPID treatment using pergolide. Treatment consists of dose titration to the lowest effective dose per individual based on response to therapy, whether it is effectiveness or signs of intolerance. A starting dose is

recommended, followed by maintenance doses; a lifelong treatment is anticipated for this disease. Clinical improvement with pergolide is expected within 6 to 12 weeks only. Horses may respond clinically at lower or varying doses compared to those generally recommended; thus, following stabilisation of the optimal dose, regular clinical assessment and diagnostic testing should be performed every 6 months to monitor treatment and dose.

Although husbandry management options like controlling the diet and increasing exercise must always be considered and incorporated in the disease management, it is acknowledged that pergolide does bring added clinical benefit compared to these in treating horsed with PPID. Improvement of clinical signs, in particularly hirsutism, impaired shedding, hyperhidrosis, weight loss/muscle wasting, abnormal fat distribution, lethargy, (chronic), polyuria, polydipsia, chronic infections, neurological signs, laminitis, sweating is well reported in the literature.

Pituitary pars intermedia dysfunction (PPID), if untreated, is potentially life-threatening and causes unacceptable suffering of the animal.

A search in the veterinary medicines database retrieves at least six pergolide-containing veterinary medicinal products authorised for use in equine species (non-food producing horses). There are no veterinary medicinal products authorised for use in species other than equine.

The substance pergolide is proposed to be qualified as essential because no satisfactory alternative treatments are authorised for food-producing animals of the equine species for the following indication: symptomatic treatment of pituitary pars intermedia dysfunction in horses (PPID, also known as Equine's Cushing disease). Please refer to the section 'Points for further consideration' within this scientific advice for additional scientific considerations regarding this substance. It is proposed that despite the substance fulfilling the criteria established in the European Commission's request, it should not be listed.

Knowledge regarding metabolic disorders in horses for this assessment was derived from textbooks, review articles, retrospective and prospective studies, and clinical trials in horses.

B. Considerations regarding consumer safety

Pergolide is a synthetically produced ergot alkaloid derivative and a potent, long-acting dopamine receptor agonist. It is used in horses for the treatment of the clinical signs of PPID (Pituitary Pars Intermedia Dysfunction).

In horses, pharmacological parameters following oral application have been reported. After single administration of 10 µg/kg maximum plasma concentrations were 2.11 and 4.05 ng/ml reached within 0.41 and 0.51 hours, respectively. The half-lives were 5.86 and 26.84 hours and volumes of distribution 3.0 and 39.74 l/kg (Gehring et al., 2010; Wright, 2009). In repeated dose studies with a maintenance dose of 4 µg/kg the reported maximum plasma concentrations were 2.11 and 4.05 ng/ml with time to reach these levels not reported and 0.53 hours, respectively. The half-lives were less than 12 hours and 24 hours in each case, and volumes of distribution were not reported and 93 l/kg, respectively (McFarlane et al., 2017; Rendle et al., 2015). Chronic administration in horses did not result in drug accumulation. Following intravenous administration of pergolide mesylate at a dose of 20 µg/kg (equivalent to 15.2 µg of pergolide/kg) to eight healthy horses, clearance and terminal volume of distribution were 959 ± 492 ml/h/kg and 6.87 ± 2.04 l/kg, respectively. A terminal elimination half-life of 5.64 ± 2.36 hours was found after intravenous administration (Rendle et al., 2015).

In humans pergolide is rapidly absorbed with the time to maximum concentration 2 to 3 hours, has a large apparent volume of distribution, was shown to have a mean half-life of 21 hours (Thalamas et al., 2005) and undergoes extensive first-pass metabolism (Blin et al., 2003). It is highly bound to plasma proteins. Elimination takes place predominantly within 48 hours, although low levels of radioactivity have been measured up to approximately 7 days in the urine. After oral administration about 55% is excreted in urine, 40% in faeces, and 5% via breath. HPLC elution profiles of urinary and fecal extracts indicated formation of at least 10 radioactive metabolites of pergolide, each at minute concentrations (Rubin, 1981). Clemens et al. (1993) referenced four identified metabolites of pergolide: the sulfoxide-, sulfone-, despropyl-pergolide, and despropyl pergolide sulfoxides, of which the first two have been shown to exhibit similar dopamine D2-receptor agonist activity as the parent molecule in rats.

Effects of acute tests via oral, intravenous and intraperitoneal routes in rats, mice, rabbits, and dogs, and subchronic and chronic pergolide administration either by gavage or in the diet to rats, mice and dogs were attributed to the pharmacological activity of the compound. Subchronic and chronic studies of pergolide administration by gavage or via diet for up to 1 year investigated daily doses of up to 20 mg/kg for Fischer 344 rats, 45 mg/kg for B6C3F1 mice, and 5 mg/kg for beagle dogs (Francis et al., 1994).

According to CLP notifications provided by companies to ECHA, pergolide is fatal if swallowed, suspected of causing cancer, and suspected of damaging fertility or the unborn child; no study data are provided (ECHA, 2024). No teratogenic effects were seen in mice (Hoyt et al., 1994; Buelke-Sam et al., 1991a). Pergolide has no genotoxic activity in vitro in human lymphoblastoid cell line (Hastwell et al., 2009) or in vivo in mice (Buelke-Sam et al., 1991a, 1991b; Hoyt et al., 1994). Effects in offspring development were attributed to lactation failure.

Pergolide is not listed by the IARC. Hastwell et al. (2009) stated that pergolide mesylate has been identified as a rodent carcinogen, with a low incidence of uterine neoplasms observed in both rats (endometrial adenomas and carcinomas) and mice (endometrial sarcomas). The product information of some human medicinal products state that the development of neoplasms has been linked to the high oestrogen/progesterone ratio that would occur in rodents as a result of the substance's prolactin-inhibiting potency. An ANSES opinion (ANSES, 2016) states that the increased incidence of uterine tumours related to the pharmacological action of pergolide was considered to be non-significant in horses and in humans.

Common side effects of pergolide in humans include nausea and vomiting in the initial phase of the treatment, insomnia and psychotoxic reactions (Jeanty et al., 1984), symptomatic postural hypotension, drowsiness, and dyskinesia (Robin, 1991). Moreover, the use of pergolide has been demonstrated to heighten the risk of fibrotic disorders (mainly in form of cardiac valvular disease, but also as pulmonary, pleural, and/or retroperitoneal fibrosis), pericarditis, pleuritis, and pericardial and/or pleural effusions (Elangbam, 2010; Hofmann et al., 2006). Based on the increased risk of heart valve disease, all manufacturers of pergolide in the USA have decided to voluntarily withdraw the drug (FDA, 2024b). In the EU, as result of a referral under Article 31 of Directive 2001/83/EC as amended, a review of the safety of ergot-derived dopamine agonists was completed by the EMA (EMEA/CHMP/322395/2008). The CHMP concluded that prescribing information on pergolide (and cabergoline)-containing HMPs should be amended to recommend a maximum dose of 3 mg/day, as well as additional information pertaining to specific warnings and contraindications. Pergolide therapy is furthermore associated with transient serum enzyme elevations in a small proportion of patients during treatment and has been implicated in rare cases of acute liver injury. The likelihood score is given as E*, i.e. unproven but suspected cause of clinically apparent liver injury (LiverTox, 2017).

According to DILIrank, a classification derived from analyzing the hepatotoxic descriptions presented in FDA-approved drug labeling documents and assessing causality evidence in literature, the substance was assigned an "ambiguous DILI-concern" in a "severity class 3" (of severity classes 0 to 8), meaning the causality for the risk of developing drug-induced liver injury in humans is undetermined (Chen et al., 2016; FDA, 2024a).

No literature on pergolide metabolites in horses could be found. No depletion data are available for pergolide according to the ANSES opinion (ANSES, 2016), and no new data were found in the current search. Rubin (1981) cited a personal communication, according to which pergolide levels were noted in all tissues except blood in animal experiments. However, the actual tissue distribution was not reported in any of the public literature.

Considering pharmacokinetic data following oral administration of clinically relevant doses of pergolide in horses, which do not result in accumulation judged by measured plasma concentrations, and considering available studies indicating no genotoxic or carcinogenic potential (tumor genesis in rodents considered non-significant for humans), it can be accepted that pergolide 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[43].

4.8.4. Conclusion

Based on the above assessment and justifications, the following recommendations are proposed:

1. The following active substance, currently in Regulation (EU) 1950/2006 as amended by Regulation (EU) 122/2013, is proposed to be retained in the list, either without modification or with an amendment of the current entry as shown below:

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Insulin	as an aid in the treatment of hyperlipaemia unresponsive to glucose therapy or severe hyperlipemia, used in combination with glucose and other therapies; for the diagnosis of metabolic disorders (e.g. insulin resistance associated with equine metabolic syndrome or pars pituitary intermedia dysfunction)	low-molecular weight heparin can be used for cases of hyperlipemia	insulin is the preferred clinical choice

2. The following active substance, suggested for addition to the list in the survey to stakeholders, is proposed to be added to the list and the entry is proposed as shown below:

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Pergolide[44]	symptomatic treatment of pituitary pars intermedia dysfunction in horses (PPID, also known as Equine's Cushing disease)	none identified	no therapeutic alternatives available; improves clinical condition

[43] Please refer to the section 'Points for further consideration' within this scientific advice for additional scientific considerations regarding this substance. It is proposed that despite the substance fulfilling the criteria established in the European Commission's request, it is not listed.
[44] Please refer to the section 'Points for further consideration' within this scientific advice for additional scientific considerations regarding this substance. It is proposed that despite the substance fulfilling the criteria established in the European Commission's request, it is not listed.

3. The following active substances, suggested for addition to the list in the survey to stakeholders, are not proposed for inclusion: canagliflozin, ertugliflozin, flutamide, goserelin, metformin, velagliflozin.

4.9. Substances for musculoskeletal disorders

4.9.1. Overview

	Active substance (ATCvet code)
Substances in Regulation (EU) 1950/2006 **proposed to be retained**, with or without amendment of the current entry	Atracurium (QM03AC04); Dantrolene sodium (QM03CA01); Edrophonium (QV04CX07); Guaifenesin (QM03BX90)
Substances in Regulation (EU) 1950/2006 **proposed to be removed**	Phenytoin[45] (QN03AB02)
Substances from stakeholders' survey **proposed for inclusion**	Cisatracurium (QM03AC11)
Substances from stakeholders' survey **not proposed for inclusion**	Chondroitin sulfate (QM01AX25); Glucosamine (M01AX05); Pentosan polysulphate (QM01AX90); Rocuronium (QM03AC09); Thiocolchicoside (QM03BX05)

4.9.2. Review of the existing entries in Regulation (EU) 1950/2006, as amended by Regulation (EU) 122/2013, considering the survey results

A. Considerations on the essentiality of the substance(s)

Atracurium is a synthetic benzylisoquinolinium compound that is a non-depolarising neuromuscular blocking agent that can be used to produce muscle relaxation under general anaesthesia. It competitively binds to postsynaptic acetylcholine receptors at the neuromuscular junctions to induce paralysis (Martin-Flores, 2013; Scherrer and Hopster, 2021). Neuromuscular blocking agents are indicated for specific types of surgery such as ophthalmic, orthopaedic or abdominal surgeries where relaxation or complete immobilization of skeletal muscle is necessary.

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), for muscle relaxation during anaesthesia. Guaifenesin is the only alternative identified in the current list and the specific advantages captured are as follows: it is a non-depolarising neuromuscular blocking agent; neuromuscular blocking agents are used in particular for eye and deep abdominal surgery; edrophonium is required for reversal; atracurium and edrophonium have the most extensive clinical support data.

In specific circumstances such as ophthalmic surgeries, certain orthopedic repairs and when deep access to the abdominal cavity is needed, increased levels of muscle relaxation are necessary in horses under general anaesthesia. The addition of muscle relaxants to the anaesthetic protocol can be preferable to administration of higher doses of inhalant agents which could lead to cardiac and pulmonary depression. Total intravenous anaesthesia offers a number of advantages compared to inhalational anaesthesia: ease of use, reduced costs as less equipment required, suitability for field anaesthesia, reduced risk to personnel from anaesthetic gases, better cardiovascular function and

[45] This substance is discussed in detail in section 4.10 (substances for nervous system disorders).

higher quality recovery (Lerche, 2013). The use of atracurium in horses is well reported and considered safe (Scherrer and Hopster, 2021). In a recent study looking at the use of low doses of atracurium during anaesthesia for ophthalmic surgeries horses that received atracurium had better recoveries from anaesthesia compared to horses that received lidocaine constant rate infusions (Scherrer and Hopster, 2021). While the horses in that study did not require reversal, it would be considered important to have edrophonium available in cases where repeated or higher doses of atracurium would be used (see also Edrophonium). Lidocaine has an MRL entry in Commission Regulation (EU) No 37/2010 for *Equidae* and there are veterinary medicinal products authorised for food-producing animals of the equine species; cisatracurium and guaifenesin are proposed to be retained in the list.

The substance is not intended for the treatment of a specific condition. However, failure to achieve adequate muscle relaxation under general anaesthesia could be potentially life-threatening and cause unacceptable suffering of the animal.

Atracurium was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any atracurium-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance atracurium is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: to induce muscle paralysis under general anaesthesia. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal, avoiding unnecessary suffering of the animal.

Dantrolene sodium is a skeletal muscle relaxant that acts by suppressing calcium release, subsequently interfering with excitation–contraction coupling in the muscle fiber. Therapeutically it is commonly used before strenuous training to prevent exertional rhabdomyolysis (tying up).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), for the treatment of rhabdomyolysis and the treatment of malignant hyperthermia during anaesthesia. Phenytoin is the only identified alternative in the current list, and the specific advantage captured is that dantrolene exhibits muscle relaxation activity by direct action on muscle as it inhibits the release of calcium from the sarcoplasmic reticulum and thus causes a dissociation of excitation-contraction coupling; both phenytoin and dantrolene sodium have been found to be useful in the treatment of recurrent forms of rhabdomyolysis.

Rhabdomyolysis in horses is a syndrome of muscle fatigue, pain, or cramping associated with either exercise or ingestion of a toxin (e.g. Hypoglycin A from ingestion of seeds of *Acer* spp. – maple trees). Less common exertional myopathies that cause exercise intolerance without muscle necrosis include mitochondrial myopathies, polysaccharide storage myopathy, and myofibrillar myopathy in Warmblood and Arabian horses (Hunt et al., 2008). Most commonly, exertional myopathies produce necrosis of striated skeletal muscle and are termed exertional rhabdomyolysis. Although rhabdomyolysis was previously considered a single disease, it is now known to comprise several myopathies, which, despite similarities in clinical presentation, differ notably in etiopathogenesis. Therefore, different treatment and management approaches are necessary depending on etiopathogenesis. Some genetic-related causes of rhabdomyolysis (e.g. polysaccharide storage myopathy) are incurable and require life-long management (Aleman, 2008).

Malignant hyperthermia is a very rare, genetic disease involving the horse's muscular system. It can be seen in <1% of Quarter Horses and related breeds, like Appaloosas and American Paint Horses. There is no gender or age tied to the condition. Malignant hyperthermia is caused by a genetic

mutation in the RYR1 gene (Aleman et al., 2004, 2009). It is a dominant gene mutation, meaning that the horse needs only one mutated gene, either from their sire or dam to be affected. This gene is responsible for regulating calcium release within the cells of skeletal muscles. The clinical signs that occur during an episode are the result of excessive calcium release causing overactive muscle activity. The most common cause of malignant hyperthermia in horses is the use of inhalant (gas) anaesthetic drugs, but can also be triggered by stress, excitement, apprehension, and exercise.

Dantrolene's effectiveness in racehorses undergoing strenuous exercise is for the most part anecdotal; there are no studies demonstrating the efficacy of dantrolene for either the treatment of exertional rhabdomyolysis or malignant hyperthermia (DiMaio-Knych et al., 2011). As a preventative, McKenzie et al. (2004) administered an oral dose of either 4, 6 or 8 mg/kg bw of dantrolene to horses with recurrent exertional rhabdomyolysis (RER) 90 min prior to exercise and were able to demonstrate significant decreases in creatinine kinase (CK – marker for muscle damage) as well as alleviation of the clinical signs associated with tying up. Similarly, Edwards et al. (2003) were able to demonstrate a significant difference in pre- vs. postrace CK levels, between horses with exertional rhabdomyolysis and those without, following a dose of 800 mg/kg bw of dantrolene orally, 1 hour prior to exercise. Neither of the two papers describing clinical trials is of sufficient power to provide conventional statistical confidence in the absolute risk reduction (Holmes, 2007). Available evidence thus suggests that dantrolene is not a treatment but a preventative for certain types of cases of rhabdomyolysis and malignant hyperthermia.

Alternatives for the treatment for either rhabdomyolysis or malignant hyperthermia depend on the type of rhabdomyolysis, stage of disease and severity of clinical signs, and can include NSAIDs, intravenous fluids, vitamin E/selenium, phenytoin, bicarbonate, vasodilator, diet and exercise management changes (McKenzie and Firshman, 2009); genetic causes for either rhabdomyolysis or malignant hyperthermia are also addressed by breeding programs leading to less animals carrying/expressing genes responsible. Some of these (pharmacological) alternatives listed have an MRL entry in Commission Regulation (EU) No 37/2010 for *Equidae* and there are veterinary medicinal products authorised for food-producing animals of the equine species. However, dantrolene's efficacy does bring added clinical benefit compared to these alternatives for the prevention of rhabdomyolysis or malignant hyperthermia in horses undergoing general anaesthesia.

Rhabdomyolysis and malignant hyperthermia, if untreated, can be life-threatening and cause unacceptable suffering of the animal.

Dantrolene was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any dantrolene-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance dantrolene is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: prevention of rhabdomyolysis; prevention of malignant hyperthermia during anaesthesia. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Edrophonium is a short and rapid acting acetylcholinesterase inhibitor. It works by blocking the action of the acetylcholinesterase enzyme responsible for the hydrolysis of acetylcholine thus increasing the local concentrations of acetylcholine which then competes with any remaining atracurium to bind to receptors at the neuromuscular junctions.

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), for reversal of atracurium muscle relaxation. No alternatives are identified in the current list and the specific advantage captured is that it is a cholinesterase inhibitor, essential for reversal of neuromuscular blockade; edrophonium has the least side effects of the cholinesterase inhibitors in horses.

Edrophonium administration effectively reversed the effects of atracurium-induced muscle relaxation in equids and ponies, leading to increased blood pressure and a smooth recovery to standing after anaesthesia (Hildebrand et al., 1986, 1989). It is considered important to have edrophonium available in cases where repeated or higher doses of atracurium would be used (see also atracurium).

The substance is not intended for the treatment of a specific condition. Failure to revert muscle relaxation under general anaesthesia could be potentially life-threatening and cause unacceptable suffering of the animal.

Edrophonium was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any edrophonium-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance edrophonium is proposed to be qualified as essential because no satisfactory alternatives are authorised for food-producing animals of the equine species for the following indication: to reverse the effects of atracurium muscle paralysis.

Guaifenesin is a centrally acting muscle relaxant. The exact mechanism of action is unclear, but it is postulated that it decreases transmission along spinal pathways and/or internuncial neurons, at the level of the subcortical brain, brainstem and spinal cord, which maintain skeletal muscle tone (McLeay, 2004).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), for muscle relaxation under general anaesthesia. Atracurium is identified as its alternative and the specific advantages captured are as follows: essential alternative to α-2/ketamine regimens in horses where α-2 agents and ketamine are contraindicated such as in horses not responding to these agents or horses having shown adverse effects during a previous administration; invaluable in combination with ketamine and α-2 agents for remarkably safe field anaesthesia for which no effective alternative intravenous techniques have been developed.

Guaifenesin works by binding to specific inhibitory neurotransmitter receptor sites in the brain and spinal cord that are activated by GABA. It is widely used in equine veterinary practice, specifically as part of the most common total intravenous anaesthesia (TIVA) protocol, where it is combined with ketamine and an alpha 2 agonist to produce safe and reliable anaesthesia (Taylor et al., 1998; Lerche, 2012; Valverde, 2013). It is particularly indicated in field (non-hospital) conditions where anaesthesia may be necessary. The reduced cardiopulmonary depressive effects seen with these combinations compared to gaseous inhalant anaesthetic agents facilitates safe anaesthesia without advanced monitoring equipment or mechanical ventilation (Taylor et al., 1998; Valverde 2013). While alternative treatments are authorised for induction of anaesthesia in food-producing animals of the equine species (e.g. isoflurane) or retained in the list (e.g. propofol), guaifenesin may bring additional clinical benefit particularly for induction and maintenance of general anaesthesia in field conditions. Other alternatives that are identified in the current list include atracurium and cisatracurium.

The substance is not intended for the treatment of a specific condition. However, failure to achieve adequate muscle relaxation under general anaesthesia could be potentially life-threatening and cause unacceptable suffering of the animal.

Guaifenesin was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database retrieves at least nine guaifenesin-containing veterinary medicinal products authorised for use in equine species (non-food-producing horses). There are veterinary medicinal products authorised for use in species other than the equine (i.e. dog).

The substance guaifenesin is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: for induction and maintenance of general anaesthesia in field conditions. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Phenytoin is discussed in detail in section 4.10 (substances for nervous system disorders) where it is proposed for the treatment of convulsions in foals (please refer to section 4.10 for the detailed assessment of the substance). Phenytoin is also proposed under this section for the treatment of rhabdomyolysis, myotonia, hyperkalemic paralysis and stringhalt in adult horses. These indications for phenytoin are currently included in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013).

Indeed, use of phenytoin for the treatment of rhabdomyolysis, myotonia, hyperkalemic paralysis and stringhalt in adult horses is well-known. Phenytoin has a different mode of action being a calcium-blocking agent that is considered to bring added clinical benefit for all the currently proposed indications (i.e. for the treatment of rhabdomyolysis, myotonia, hyperkalemic paralysis and stringhalt in adult horses). While dantrolene sodium is retained in the list for treatment of rhabdomyolysis, phenytoin is considered to bring added clinical benefit.

Rhabdomyolysis, myotonia, hyperkalemic paralysis and stringhalt in adult horses, if untreated, can be life-threatening and cause unacceptable suffering of the animal.

The substance phenytoin is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indications: treatment of rhabdomyolysis, myotonia, hyperkalemic paralysis and stringhalt in adult horses. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Knowledge regarding muscle relaxants in horses for this assessment was derived from textbooks, review articles, and retrospective studies.

B. Considerations regarding consumer safety

Atracurium besilate is used in human medicine for endotracheal intubation and to provide muscle relaxation in general anaesthesia for surgical procedures and to aid controlled ventilation.

In humans, atracurium besilate is inactivated via Hoffman elimination, i.e. spontaneous degradation in plasma and tissue at normal pH and temperature and ester hydrolysis, i.e. catalysis by nonspecific esterases to produce laudanosine and other metabolites. The metabolites have no neuromuscular blocking activity. About 80% of atracurium besilate is bound to plasma proteins and the excretion is in urine and bile, mostly as metabolites. The elimination half-life has been reported to be about 20 minutes for atracurium and about 3 to 6 hours for laudanosine (Scriba, 2009). Nondepolarizing neuromuscular blocking agents are poorly absorbed after oral administration and must be administered by injection (Buck and Reed, 1991).

The non-compartmental pharmacokinetics of atracurium have been studied in children dosed with total doses from 4.5 to 73.9 mg/kg for orthotopic liver transplantation. The mean terminal half-life was 18.8 minutes with a range of 12 to 32.3 minutes. The mean steady-state plasma clearance was 13.9 ml/min/kg ranging from 7.9 to 20.3 ml/min/kg and the mean terminal volume of distribution was 390 ml/kg, ranging from 124 to 551 ml/kg (Chow et al., 2000). A one-compartment pharmacokinetic model was used in other study, which described half-lives from 5.8 to 23.4 minutes, volumes of distribution from 13100 to 18000 ml and clearances from 449 to 1560 ml/min, for the different atracurium isomers. In this study, patients were given a bolus of 0.5 mg/kg or an initial infusion of 0.6 mg/kg/h or both (Boyd et al., 1996).

A two-compartment model was used to describe the pharmacokinetics of atracurium in young and elderly adults infused at 16.3 ± 2.8 µg/kg/min for approximately 10.2 minutes. The elimination half-life and the total clearance were similar for both groups, 15.7 and 21.8 minutes and 5.3 and 6.5 ml/kg min, for young and the elderly, respectively, while the volume of distribution at steady state in the elderly was larger than in young adults with 188 and 98 ml/kg, respectively (Kitts et al., 1990). Pharmacokinetic parameters were compared between infants, children and adults using a two-compartment pharmacokinetic model and a dose of 15.8 ± 1.7 µg/kg/min for 6-11 minutes. Volume of distribution at steady state (210, 129 and 100 ml/kg for infants, children and adults, respectively) and total clearance (7.9, 6.8 and 5.3 ml/kg/min for the three groups) decreased with increasing age. Neither elimination half-life (20, 17.2 and 15.7 minutes for infants, children and adults, respectively) nor the steady state plasma concentration that resulted in 50% neuromuscular blockade (363, 444 and 436 ng/ml for the three groups) varied with age (Fisher et al., 1990).

The pharmacokinetics of the main metabolites have also been reported in humans. Mean plasma elimination half-life of 20 minutes for atracurium, 234 minutes for laudanosine and 39 minutes for quaternary alcohol have been described in a study using a two-compartment model in patients with normal renal function and in patients in renal failure after a bolus dose of 0.3-0.4 mg/kg (Ward and Weatherley, 1986). Laudanosine mean terminal half-life of 3.9 hours, with a range of 1.1 to 6.7 hours has also been described by other authors (Chow et al., 2000). Likewise, mean plasma laudanosine levels in patients with normal renal function peaked at 199 ± 31 ng/ml at 2 minutes after 0.5 mg/kg atracurium administration and slowly decreased over the next 4 hours (Fahey et al., 1984). Mean concentrations between 200 and 300 ng/ml have also been described at 2 minutes after intravenous dose of atracurium of 0.5 to 0.6 mg/kg, declining afterwards, up to the eight or tenth minute (Nigrovic and Fox, 1991).

In animals, laudanosine can cause electrophysiological seizure activity; a steady state laudanosine concentration of 17 µg/ml produces seizures in dogs. However, the relationship between plasma laudanosine and seizure activity appears to be species dependent. In cats, no evidence of epileptiform activity occurs at plasma laudanosine concentration of up to 100 µg/ml (Chow et al., 2000). Intravenous administration of laudanosine of 10-20 mg/kg in mice and rats, 14-22 mg/kg in dogs and infusion of 0.5 mg/kg/min in rabbits are reported to cause convulsions (Fodale and Santamaria, 2002). In humans, Chow et al. (2000) reported concentrations of 19 µg/ml in a child without any untoward effect. Furthermore, despite long-term use of high doses of atracurium infusion, only moderate accumulation of laudanosine occurred in a woman with a normal electroencephalogram (EEG) (Grigori et al., 1998).

The common adverse reaction is the effect of nondepolarizing neuromuscular blockers-induced histamine release. The effects of histamine reaction include hemodynamic instability, bronchospasm and urticaria (Clar and Liu, 2023). Information relating to human medicinal products indicate non-mutagenicity in the Ames Salmonella assay at concentrations up to 1000 mcg/ml, and in the rat bone

marrow cytogenicity assay performed at sub-paralyzing doses. Positive responses were observed in the mouse lymphoma assay.

There is no available information on pharmacokinetics in horses. However, studies in humans suggest a rapid degradation/elimination and a poor oral absorption. There is also some information on the main metabolite, laudanosine, that suggest a rapid elimination and that toxic concentrations are unlikely to be accumulated because of atracurium use. Furthermore, the limited use of atracurium in horses should be noted as well.

Therefore, it can be accepted that atracurium 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.

Dantrolene sodium is a lipid soluble hydantoin derivative. It is a muscle relaxant which has been shown to act directly on skeletal muscle; it is currently authorized as human medicinal products for administration orally or intravenously.

In humans, a relatively high bioavailability of oral dantrolene was noted. Approximately 70% of a single oral dose of dantrolene is absorbed, with peak plasma concentrations of about 1 mg/L occurring 4 to 6 hours after administration of a 100 mg dose. Dantrolene is bound to human serum albumin, plasma proteins and to skeletal muscle sarcoplasmic reticulum. Dantrolene is metabolised in the liver primarily to 5-hydroxydantrolene, which has similar pharmacological action to the parent compound and with repeated administration may exceed the serum concentrations of dantrolene. Approximately 15 to 25% of dantrolene is excreted in the urine (primarily as the hydroxy metabolite) with negligible faecal excretion. The elimination half-lives of dantrolene and 5-hydroxydantrolene are about 6 to 9 hours and 15.5 hours, respectively (Ward et al., 1986). Similarly, in ten malignant hyperthermia–susceptible human patients given a total dose of 5 mg/kg bw of dantrolene in 3 or 4 doses, every 6 hours, an elimination half-life of 15.8 ± 6.0 hours was determined (Allen et al., 1988). Dantrolene is rapidly metabolized to 5-hydroxydantrolene both in vivo and in vitro. After a single dose of 500 mg, peak plasma concentrations were 28.9 ± 21.6 ng/mL for oral capsules and occurred at 3.8 hours. Dantrolene and its major metabolite were both below the limit of detection in both plasma and urine by 168 h post administration.

In horses, following oral administration of dantrolene the absorption is limited and the terminal half-life is short. The pharmacokinetics of dantrolene sodium were investigated in four horses following both intravenous and intra-gastric administration (2 and 4 mg/kg bw, respectively). Distribution and elimination of dantrolene was rapid, resulting in an elimination half-life of 129 ± 8 min and a whole-body clearance of 4.16 ± 0.52 ml/min/kg. Following intra-gastric administration, dantrolene rapidly achieved peak concentrations within 1.5 h, but was incompletely absorbed, with a bioavailability of 39 ± 10%; small amounts of intact drug were recovered in urine and bile (Court et al, 1987). Dantrolene is rapidly metabolized to 5-hydroxydantrolene both in vivo (in horses) and in vitro (hepatic microsomes). The maximal concentration was observed 4 hours after the oral administration of 500 mg of dantrolene in 8 horses. The terminal half-life is about 4 hours. (DiMaio-Knych et al., 2011).

Dantrolene sodium is currently not classified as a carcinogenic hazard by IARC. There is no monograph available (IARC, 2024).

Given its use in humans and considering the pharmacokinetics data available in horses, in particular its rapid plasma elimination of 2 hours, it can be accepted that dantrolene 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.

Edrophonium is a reversible inhibitor of cholinesterase activity. It has a rapid onset but short duration of action; because of its brief action the drug is not suitable for the routine treatment of myasthenia gravis. Its effect is manifest within 30 to 60 seconds after injection and lasts an average of 10 minutes. The polar quaternary ammonium structure of cholinesterase activity inhibitors reduces their oral bioavailability and their ability to penetrate the blood-brain barrier, making intravenous administration the route of choice (Kleinz and Spence, 2008).

Some pharmacokinetic data is available in humans. Edrophonium given intravenously shows first order elimination. A three-compartment model was used in a study comparing infants, children and adults. Mean elimination half-lives were 73,99 and 126 minutes for the three groups, respectively. Volumes of distribution at steady state were 1.18, 1.22 and 0.99 l/kg, respectively, while total clearances observed were 17.8, 14.2 and 8.3 ml/kg/min (Fisher et al., 1984). Plasma clearance of edrophonium of 8.2 ± 2.7 ml/kg/min in normal patients and 2.7 ± 1.4 ml/kg/min in patients without renal function have been reported. Thus, approximately 67% of plasma clearance appears to be dependent on renal excretion. Hepatic metabolism and biliary excretion have been demonstrated in animals and represent probable pathways for non-renal clearance (Morris et al., 1981). The exact method of its metabolism is unknown.

As edrophonium increases acetylcholine levels at both nicotinic and muscarinic receptors throughout the body, muscarinic receptor-mediated adverse effects can be elicited such as bradycardia, bronchoconstriction, increased secretions and other parasympathomimetic side effects. Since its ability to permeate the blood brain barrier is low, the anticholinesterase effects it elicits are primarily mediated outside the central nervous system (Pope, 2017).

No information on the pharmacokinetics or residues of edrophonium is available in horses. However, edrophonium is a short-acting anticholinesterase, with inhibition being rapidly reversible, it is rapidly eliminated, and a reduction of the oral bioavailability has been described for polar quaternary ammonium structures. Besides, the limited use of edrophonium in horses should be also noted.

Therefore, it can be accepted that edrophonium 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.

Guaifenesin is a centrally acting muscle relaxant used intravenously in veterinary medicine during anaesthesia, and orally in human medicine as an expectorant for the symptomatic relief from congested chests and coughs.

Guaifenesin is well absorbed in humans after oral administration. From information relating to human medicinal products, it appears to undergo both oxidation and demethylation followed by excretion in the urine. Approximately 40% of a dose is excreted as the metabolite beta-2-methoxyphenoxy-lactic acid in the urine within 3 hours. Following an oral dose of 600 mg guaifenesin to 3 healthy male volunteers, the half-life was approximately 1 hour, and the drug was not detectable in the blood after 8 hours. Thompson et al. (2016) studied the relationships between guaifenesin pharmacokinetic parameters and age after oral dosing. It was observed that the oral clearance and terminal volume of distribution increased significantly with age; however, following allometric adjustment, no age-related change was observed. Oral clearance was 50.07 L/h for children 2-5 years old given 100 mg guaifenesin, while it was 182 L/h for children 6-11 years old and 156.8 L/h for children 12-17 years old, all administered 200 mg guaifenesin. The terminal exponential half-life was 0.6, 0.7 and 1.03 hours, respectively.

Some pharmacokinetic data is available in horses. Guaifenesin was rapidly absorbed following oral administration. The terminal plasma half-life was 2.62 ± 1.24 hours, and no bioaccumulation was observed (Knych et al., 2016). Guaifenesin was also studied in horses and donkeys after intravenous

administration. Recovery time to standing was 36 min for both, donkeys administered with 125 mg/kg and horses administered with 211 mg/kg. The terminal plasma half-life was 1.77 ± 0.32 hours and the clearance was 313 ± 62 ml/h kg in horses while it was 0.84 ± 0.09 h and 546 ± 73 ml/h kg in donkeys, respectively. Volumes of distribution were 794 ± 25 ml/kg and 678 ± 92 ml/kg for horses and donkeys, respectively (Matthews et al., 1997).

Regarding toxicity, gastrointestinal discomfort, nausea and vomiting have occasionally been reported with guaifenesin, particularly after administration of very high doses. Urinary calculi have been reported in human patients consuming large quantities of over-the-counter preparations containing guaifenesin (Scriba, 2009). Few data are available on carcinogenic, mutagenic and teratogenic potential of guaifenesin. Guaifenesin was associated with significant developmental toxicity when administered to pregnant rats at 250, 350, 500 and 600 mg/kg, from gestation day 6 to 17 (Shabbir et al., 2016).

Although little information is available on the mutagenic, teratogenic or carcinogenic potential of guaifenesin, it has been observed that it is rapidly eliminated from the body. Therefore, considering its pharmacokinetic characteristics and its use in veterinary medicine, it can be accepted that guaifenesin 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.

4.9.3. Assessment of new substances proposed to be added to the list in the stakeholders survey

A. Considerations on the essentiality of the substance(s)

Chondroitin sulfate was mentioned (once) in the survey to stakeholders and it was suggested for addition to the list. No specific indication was mentioned by the responder nor references provided.

Chondroitin sulphate is a naturally occurring glycosaminoglycan, a long chain of repeating sugar molecules that is a major component of cartilage, which is the connective tissue found in joints. Chondroitin sulphate is postulated to counteract cartilage degradation by inhibiting enzymes that are degradative to cartilage, and to stimulate synthesis of extracellular matrix components of cartilage by providing the substrates or "building blocks". Most reports of the use of chondroitin sulphate in equines relates to oral nutraceutical supplements, and evidence as to their efficacy is low (Neil et al., 2005; McIlwraith et al., 2015; Yamada et al., 2022; Brito et al. 2023), with some studies suggesting that there is low oral bioavailability. Similar results are found in other species (Kampa et al., 2023).

The treatment of choice for joint disease and/or osteoarthritis continues to be anti-inflammatory medicines, for which there are alternatives included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with an entry for *Equidae* (e.g. flunixin) or glucocorticoids, for which there are veterinary medicinal products authorised for food-producing animals of the equine species. Triamcinolone acetonide is an alternative proposed to be retained in the list of essential substances.

Joint disease or osteoarthritis, if untreated, is not life-threatening but may cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any chondroitin-sulfate-containing veterinary medicinal product authorised for use in equine species. There are products authorised for non-food-producing horses containing glycosaminoglycan polysulphate (neither in other animal species).

The substance chondroitin sulphate is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; satisfactory

alternative treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Cisatracurium was mentioned (once) in the survey to stakeholders and it was suggested for addition to the list. No specific indication was mentioned by the responder. The responder noted its advantage in animals with renal or hepatic disorder as it is mostly metabolised by the Hofman reaction. It has been safely used in horses under general anaesthesia (Tutunaru et al., 2019).

It is a benzylisoquinolinium, an isomer of atracurium. Cisatracurium is a non-depolarising neuromuscular blocking agent that can be used to produce muscle relaxation under general anaesthesia. It has been suggested that it may be more potent and produce less side effects than atracurium in humans. The use of cisatracurium in horses has been reported (Tutunaru et al., 2019; Martin-Flores and Sakai, 2022).

In specific circumstances such as ophthalmic surgeries, certain orthopedic repairs and when deep access to the abdominal cavity is needed, increased levels of muscle relaxation are necessary in horses under general anaesthesia. The addition of muscle relaxants to the anaesthetic protocol can be preferable than administration of higher doses of inhalant agents which could lead to cardiac and pulmonary depression. Total intravenous anaesthesia offers a number of advantages compared to inhalational anaesthesia: ease of use, reduced costs as less equipment required, suitability for field anaesthesia, reduced risk to personnel from anaesthetic gases, better cardiovascular function and higher quality recovery (Lerche, 2013). The successful use of cisatracurium in horses is reported and it is considered safe (Tutunaru et al., 2019). Atracurium is proposed to be retained in the list. It may have, however, some limitations in terms of availability; thus, cisatracurium is proposed for addition to the list noting the reversal agents that work for atracurium would also work for cisatracurium and are already on the list.

The substance is not intended for the treatment of a specific condition. However, failure to achieve adequate muscle relaxation under general anaesthesia could be potentially life-threatening and cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any cisatracurium-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance cisatracurium is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: to induce muscle paralysis under general anaesthesia. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Glucosamine was mentioned (once) in the survey to stakeholders and it was suggested for addition to the list. No specific indication was mentioned by the responder nor references provided.

Glucosamine is a key component of the glycosaminoglycans, which are long chains of sugars that make up the structural framework of cartilage. Glucosamine is thought to work by providing the building blocks needed for the synthesis and repair of cartilage. While it has been postulated to have anti-inflammatory effects and help reduce the activity of enzymes that contribute to cartilage breakdown, evidence to that effect is low (Neil et al., 2005; McIlwraith et al., 2015; Yamada et al., 2022; Kampa et al., 2023). Most reports of the use of glucosamine in equines relate to oral nutraceutical supplements for which there is evidence of their low oral bioavailability.

As indicated previously, the treatment of choice for joint disease and/or osteoarthritis continues to be anti-inflammatory medicines, for which there are alternatives included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with an entry for *Equidae* (e.g. flunixin) or glucocorticoids, for which there are veterinary medicinal products authorised for food-producing animals of the equine species. Triamcinolone acetonide is an alternative proposed to be retained in the list of essential substances.

Joint disease or osteoarthritis, if untreated, is not life-threatening but may cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any glucosamine-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance glucosamine is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Pentosan polysulphate was mentioned once in the survey to stakeholders and it was suggested for addition to the list as an aid in the treatment of non-infectious, inflammatory joint disease in horses. The responder stated that it can be administered intramuscularly 4 times with a 5-7 day interval and the non-requirement of intra-articular injection is beneficial for the treatment and management of such conditions. Pentosan polysulphate is licensed in Australia. No scientific references were provided.

Pentosan polysulphate is a relatively novel glycosaminoglycan-like molecule, with human medical uses for the treatment of systemic and musculoskeletal disorders (Herrero et al., 2015). Polysulphated glycosaminoglycan is listed in Table 1 of the Annex to Commission Regulation (EU) 37/2010 pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin with a 'no MRL required' status for *Equidae*; there are veterinary medicinal products authorised for food-producing animals of the equine species.

Non-infectious inflammatory joint diseases in horses, if untreated, are not life-threatening but cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any pentosan-polysulphate-containing veterinary medicinal product authorised for use in equine species. There are veterinary medicinal products authorised for use in species other than the equine (i.e. dog).

The substance pentosan polysulphate is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Rocuronium bromide was mentioned (once) in the survey to stakeholders and it was suggested for addition to the list as a neuromuscular blocking agent as an alternative to atracurium due to atracurium not being widely available. No scientific references were provided.

It is an aminosteroid non-depolarizing neuromuscular blocker or muscle relaxant. The use of rocuronium in horses has been reported (Martin-Flores and Sakai, 2022) for muscle relaxation or

neuromuscular blockade under general anaesthesia particularly for ophthalmic surgeries (Auer et al., 2007; Auer and Moens, 2011; Martin- Flores and Sakai, 2022).

As indicated under atracurium and cisatracurium, in specific circumstances such as ophthalmic surgeries, certain orthopedic repairs and when deep access to the abdominal cavity is needed, increased levels of muscle relaxation are necessary in horses under general anaesthesia. The addition of muscle relaxants to the anaesthetic protocol can be preferable than administration of higher doses of inhalant agents which could lead to cardiac and pulmonary depression. Total intravenous anaesthesia offers a number of advantages compared to inhalational anaesthesia: ease of use, reduced costs as less equipment required, suitability for field anaesthesia, reduced risk to personnel from anaesthetic gases, better cardiovascular function and higher quality recovery (Lerche, 2013). While the successful use of rocuronium in horses is reported (Auer et al., 2007) it has not been shown to bring clinical added benefit compared to atracurium and cisatracurium. Both substances are proposed to be retained in the list for improved availability, together with guaifenesin.

The substance is not intended for the treatment of a specific condition. However, failure to achieve adequate muscle relaxation under general anaesthesia could be potentially life-threatening and cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any rocuronium-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance rocuronium is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indication in animals of the equine species; it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal and avoiding unnecessary suffering of the animal.

Thiocolchicoside was mentioned (once) in the survey to stakeholders and it was suggested for addition to the list as a muscle relaxant, either with intramuscular or mesotherapy routes of administration. The indication suggested in the survey would seem to be mainly for administration via the mesotherapy route, a commonly used complementary technique in sports medicine that would not be indicated in very painful conditions where welfare is a concern. No scientific references were provided.

It is a muscle relaxant with anti-inflammatory and analgesic effects. Its mechanism of action is unknown, but it is believed to act via antagonism of nicotinic acetylcholine receptors. It may also be a competitive antagonist of GABA-A and glycine receptors. While there are sporadic reports where the use of thiocolchicoside is described as part of a treatment protocol with other medications for the treatment of flexural limb deformity in a foal (de Amorim et al., 2020) or tetanus (Pereira et al., 2020), it does not seem to be widely used in horses. The most common application reported in veterinary medicine appears to be in mesotherapy in working canines (Alves and Santos, 2017; Alves et al., 2018).

There are muscle relaxant alternatives proposed for inclusion in the list (i.e. atracurium and cisatracurium) and sufficient anti-inflammatory and analgesic alternatives, both in the list and in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with a MRL entry for *Equidae* and for which there are veterinary medicinal products authorised for food-producing animals of the equine species. Thiocolchicoside has not been shown to bring added clinical benefit compared to any of these.

The substance is not intended for the treatment of a specific condition. However, failure to achieve adequate muscle relaxation could potentially cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any thiocolchicoside-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance thiocolchicoside is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indication in animals of the equine species; it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal and avoiding unnecessary suffering of the animal.

Knowledge regarding muscle relaxants in horses for this assessment was derived from textbooks, review articles, and retrospective studies. No clinical trials could be identified in horses.

B. Considerations regarding consumer safety

Cisatracurium is used in medicinal products in its salt form, cisatracurium besilate or benzolsulfonate.

It has a high polarity and a large molecular weight (Schmith et al., 1996), therefore a low volume of distribution and a rapid clearance have been observed (Kisor and Schmith, 1999). In humans, metabolism and elimination of cisatracurium besilate is slightly influenced by age, renal or hepatic function (Boyd et al., 1996; Dhonneur et al., 2001; Liu et al., 2012), as the predominant pathway for cisatracurium clearance (77%) is initiated by the Hofmann degradation in tissues and plasma, meaning that cisatracurium is cleared non-enzymatically throughout the whole body (Kisor et al., 1996). In vitro studies in human plasma have shown that this reaction leads to the metabolite laudanosine and a proposed monoquaternary acrylate, which is further subject to ester hydrolysis, forming monoquaternary alcohol (Welch et al., 1995). The metabolites do not possess clinically significant neuromuscular or autonomic effects (Schmith et al., 1996). In healthy human patients, 95% of an administered dose of 0.1 mg/kg [14C]cisatracurium besilate was recovered in the urine, the majority as conjugated desmethyl metabolites of laudanosine and less than 10% as unchanged cisatracurium besilate (Schmith et al., 1996). Low quantities are thought to be biliary excreted in humans (Kisor et al., 1996). In rat plasma, cisatracurium is metabolized to the monoquaternary alcohol and the monoquaternary acid by non-specific carboxylesterases, thus, representing the rate-limiting step in this species (Dear et al., 1995).

Elimination half-lives are generally short in humans with the highest recorded value being approximately 36.3 minutes (Kisor and Schmith, 1999). Investigation of cisatracurium pharmacokinetics in anaesthetised dogs revealed a similar in vivo plasma elimination half-life of 16.4 ± 2.7 minutes, as well as about two-fold this half-life in vitro in plasma, i.e. 32.9 ± 3.7 minutes, with a higher share of organ clearance, i.e. 56 ± 12 % of total clearance than known for humans (Chen et al., 2009).

No pharmacokinetic studies were found for other animal species, including horses. Further reports are limited to the description of the efficacy in horse, pig, and dog (Adams et al., 2001; Tutunaru et al., 2017, 2019).

According to the classification provided by companies to ECHA in CLP notifications, cisatracurium besilate is toxic if swallowed and causes serious eye irritation (ECHA, 2024). Cisatracurium is not listed by the IARC or NTP. No published studies on toxicity were found.

Information on toxicity tests is only available from the product information of human medicinal products. Cisatracurium was not found to be mutagenic in three out of four tests (Ames Salmonella assay, rat bone marrow cytogenetic assay, and in vitro human lymphocyte cytogenetics assay). No

structural or numerical chromosome aberrations were observed in the presence or absence of metabolic activation. The fourth test conducted, on the mutation-inducing ability of cisatracurium at the heterozygous thymidine kinase (tk+/-) locus in the L5178/tk+/- mouse lymphoma assay conducted in presence or absence of exogenous metabolic activation [rat liver S9]), revealed a dose-related mutagenic response at concentrations ≥ 40µg/ml in the absence of exogenous mammalian metabolic activation, as well as minimal evidence of mutagenicity in the presence of metabolic activation at an isolated concentration of 300 µg/ml. A clinical relevance of this test result was not seen.

The product information of human medicinal products cite the outcome of a teratology study with no evidence of embryotoxicity, fetotoxicity, or teratogenicity in anesthetized, mechanically respired rats, given intravenous doses of 0.5 or 1 mg/kg on gestational days 6 to 15. A second rat teratology study was described to have shown maternal toxicity, including some deaths, at the high subcutaneous dose of 10 mg/kg/day, but not at 4 mg/kg/day, and no evidence of embryotoxicity, fetotoxicity, or teratogenicity at any dose.

The literature review of Szakmany and Woodhouse (2015) lists known adverse effects of cisatracurium in humans, such as ICU-acquired muscle weakness/new-onset critical illness neuropathy, barotrauma, tachyphylaxis, and cardiovascular events. In contrast to atracurium, cisatracurium causes considerably less histamine release leading to a reduced risk of anaphylaxy, and less formation of laudanosine (Larsen, 2016). Laudanosine at high doses has been shown to induce seizures in animals (Chapple et al., 1987).

No residue depletion studies were found. The only report found for tissue levels is one in rabbits, where cisatracurium was administered at a dose of 0.4 mg/kg intravenously, leading to concentrations of 0.14 µg/ml in plasma, and 0.11 µg/g in the tibialis anterior muscle and the diaphragm, respectively. The exact time of sampling was different for each individual animal and was chosen depending on the recovery of a certain twitch tension (Sun et al., 2019).

Considering the rapid clearance of cisatracurium, as well as the apparent low toxicity, it can be accepted that cisatracurium 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.

4.9.4. Conclusion

Based on the above assessment and justifications, the following recommendations are proposed:

1. The following active substances, currently in Regulation (EU) 1950/2006 as amended by Regulation (EU) 122/2013, are proposed to be retained in the list, either without modification or with an amendment of the current entry as shown below:

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Atracurium	to induce muscle paralysis under general anaesthesia	cisatracurium, guaifenesin	brings added clinical benefit in horses under general anaesthesia in cases where increased muscle relaxation is necessary such as ophthalmic surgeries, certain orthopaedic repairs and when deep access to the abdominal cavity is needed.
Dantrolene sodium	prevention of rhabdomyolysis;	NSAIDs, intravenous fluids, vitamin E/selenium	efficacious as preventative, inhibiting

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
	prevention of malignant hyperthermia during anaesthesia		the release of calcium from the sarcoplasmic reticulum and thus causing dissociation of excitation-contraction coupling
Edrophonium	to reverse the effects of atracurium muscle paralysis	none identified	cholinesterase inhibitor, essential for reversal of neuromuscular blockade; least side effects of the cholinesterase inhibitors in horses
Guaifenesin	for induction and maintenance of general anaesthesia in field conditions	atracurium, cisatracurium	particularly indicated in field (non-hospital) conditions where anaesthesia may be necessary; the reduced cardiopulmonary depressive effects facilitate safe anaesthesia without advanced monitoring equipment or mechanical ventilation

2. The following active substance, currently in Regulation (EU) 1950/2006 as amended by Regulation (EU) 122/2013, is proposed to be removed from the list: phenytoin[46].

3. The following active substance, suggested for addition to the list in the survey to stakeholders, is proposed to be added to the list with an entry as shown below:

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Cisatracurium	to induce muscle paralysis under general anaesthesia	atracurium, guaifenesin	brings added clinical benefit in horses under general anaesthesia in cases where increased muscle relaxation is necessary such as ophthalmic surgeries, certain orthopaedic repairs and when deep access to the abdominal cavity is needed.

4. The following active substances, suggested for addition to the list in the survey to stakeholders, are not proposed for inclusion: chondroitin sulfate, glucosamine, pentosan polysulphate, rocuronium, thiocolchicoside.

4.10. Substances for nervous system disorders

4.10.1. Overview

	Active substance (ATCvet code)
Substances in Regulation (EU) 1950/2006 **proposed to be retained**, with or without amendment of the current entry	Diazepam[47] (QN05BA01)

[46] This substance is discussed in detail in section 4.10 (substances for nervous system disorders).
[47] This substance is discussed in detail in section 4.12 (substances for sedation and premedication (and antagonism)).

	Active substance (ATCvet code)
Substances in Regulation (EU) 1950/2006 **proposed to be removed**	Carbamazepine (QN03AF01); Phenytoin (QN03AB02); Primidone (QN03AA03)
Substances from stakeholders' survey **proposed for inclusion**	None
Substances from stakeholders' survey **not proposed for inclusion**	Alprazolam (QN05BA12); Fluoxetine (QN06AB03); Phenobarbital (QN03AA02); Trazodone (QN06AX05)

4.10.2. Review of the existing entries in Regulation (EU) 1950/2006, as amended by Regulation (EU) 122/2013, considering the survey results

A. Considerations on the essentiality of the substance(s)

Carbamazepine is an anticonvulsant. Its mechanism of action is not fully understood. One major hypothesis is that carbamazepine inhibits sodium channel firing treating seizure activity (Ambrosio et al., 2002). Animal research studies have demonstrated that carbamazepine exerts its effects by lowering polysynaptic nerve response and inhibiting post-tetanic potentiation, i.e. by an increase in neurotransmitter release after a brief, high-frequency train of action potentials (Ambrosio et al., 2002; Powell and Castillo, 2008).

Carbamazepine is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for treatment of the headshaking syndrome. No alternatives are identified in the current list, and the specific advantage captured is that carbamazepine is acting as an anti-convulsant with sodium channel-blocking effects. It is used mainly for treatment and diagnostic confirmation of trigeminal neuralgia (headshaking syndrome).

It is a second-generation antiseizure available for the symptomatic treatment of seizures in humans (Löscher and Klein, 2021). There are various treatments and management procedures described for the treatment of the headshaking syndrome in horses (Roberts, 2019). The most effective treatment options are percutaneous electrical nerve stimulation (PENS) therapy and surgical platinum coil ablation of the superficial sensory branches of the trigeminal nerves. Treatment with carbamazepine does not result in resolving the condition, particularly since the etiology of head shaking syndrome is not known (Newton et al., 2000; Roberts, 2019). Diazepam, which is proposed to be retained in the list of essential substances, is considered a satisfactory alternative for the same indication with a better clinical profile. Carbamazepine is not considered to bring added clinical benefit compared to any of the treatment options listed above.

Head shaking, if untreated, causes unacceptable suffering of the animal.

Carbamazepine was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any carbamazepine-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance carbamazepine is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indication in animals of the equine species; it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Diazepam is discussed in detail in section 4.12 (substances for nervous system disorders) where it is proposed for premedication and induction of anaesthesia, mild tranquilisation with minimal cardiovascular and respiratory side effects (please refer to section 4.12 for the detailed assessment of the substance). Diazepam is also proposed under this section as short-term anticonvulsant for treatment of seizures. This indication for diazepam is currently included in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013).

Diazepam is a well-recognised sedative/ataractic/neuroleptic substance that has been largely used in equine practice (Shini, 2000). It is a second generation antiseizure medication with a better clinical profile compared to alternatives such as carbamazepine or primidone (Shaniko, 2000; Lacombe, 2015). These alternatives are not proposed for inclusion in the list.

Convulsions, if untreated, are potentially life-threatening and cause unacceptable suffering of the animal.

The substance diazepam is proposed to be qualified as essential because no satisfactory alternative treatments are authorised for food-producing animals of the equine species for the following indication: short-term anti-convulsant for treatment of seizures.

Phenytoin is a hydantoin derivate that is used as a drug for long-term treatment of epilepsy and is also effective in the treatment of cardiac arrhythmias. Phenytoin exerts its antiepileptic effect by stabilizing the function of brain cell membranes and increasing the levels of the inhibitory neurotransmitter serotonin (5-HT) and γ-aminobutyric acid (GABA) in the brain. In cardiac tissue it inhibits the ectopic rhythm of the atrium and ventricle and accelerates the conduction of the atrioventricular node to reduce myocardial autonomy, producing an antiarrhythmic effect (Patocka et al., 2020).

Phenytoin is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) as anti-convulsant therapy in foals, and treatment of rhabdomyolysis and stringhalt. Alternatives identified in the current list include diazepam and primidone(for convulsions). The specific advantages captured are that phenytoin is an essential anti-convulsant in foals, it is generally added to the treatment of seizure control if primidone/phenobarbital does not control the seizures.

Indeed, phenytoin is used for the treatment of convulsions in foals and there are no satisfactory alternatives authorised for food-producing animals of the equine species for these indications.

Convulsions, if untreated, may be potentially life-threatening and cause unacceptable suffering of the animal.

Phenytoin was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any phenytoin-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance phenytoin is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indications: treatment of convulsions in foals. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal. Phenytoin is also proposed for the treatment of rhabdomyolysis, myotonia, hyperkalemic paralysis and stringhalt in adult horses. Please refer to section 4.9 for further details.

Primidone is a first-generation barbiturate type antiepileptic medication for treating seizures, commonly partial and generalized seizures. The mechanism of action is not well defined, but it appears

to bind centrally with voltage-gated sodium channels and inhibits the monotonous firing of action potentials. It also activates the GABA receptor complex with chloride ionophore (Lenkapothula and Cascella, 2023).

Primidone is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) as anti-convulsant therapy in foals. Diazepam and phenytoin are alternatives listed in the current list and the specific advantage is that primidone is indicated as follow-up to diazepam therapy or as an alternative.

Primidone is a first-generation barbiturate type antiepileptic whereas diazepam is a second-generation drug that constitutes a satisfactory alternative and is proposed to be retained in the list of essential substances (Rho and White, 2018). Primidone is not considered to bring added clinical benefit compared to this treatment option.

Convulsions, if untreated, are potentially life-threatening and cause unacceptable suffering of the animal.

Primidone was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any primidone-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance primidone is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indication in animals of the equine species; it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering for the animal.

Knowledge regarding substances for nervous disorders for this assessment was derived from textbooks, and peer-reviewed articles, and case-studies in horses.

B. Considerations regarding consumer safety

Phenytoin is a hydantoin derivative like dantrolene and the oldest non-sedative anticonvulsant drug known. It is primarily used as an antiepileptic drug (Adams, 1995; Plumb, 1999; Tilley, 1997). It also acts as an antiarrhythmic substance and is classified as a class IB antiarrhythmic drug (Adams, 1995). In addition, phenytoin can be used as an effective therapeutic agent in diseases of the locomotor system, such as toxic cockerel tremor (Australian stringhalt), stress-induced rhabdomyolysis and hyperkalemic periodic paralysis (HYPP) in horses (Allen et al., 2005). Phenytoin alters sodium, potassium and calcium conductance across cell membranes thereby altering membrane potentials and amino acid and neurotransmitter concentrations (i.e. norepinephrine (noradrenaline), acetylcholine and GABA). Its major mode of action appears to be the blockade of sodium channels and the inhibition of the generation of repetitive action potentials (membrane stabilization) (Bertone and Horspool, 2004).

Oral absorption occurs in the small intestine and is dose dependent. Total absorption of phenytoin in humans during a 33-hour period was 90% (Davis et al., 1993). Peak blood concentrations occur 2-4 hours after single 100 mg doses but may be delayed by 4–12 hours after a loading dose of 600 mg. Peak levels may not occur for 2-7 days after an oral overdose. The drug is widely distributed in the body and is almost completely protein-bound, primarily to albumin. It easily crosses the blood–brain barrier and the placenta and enters the milk (Mirkin, 1971). Up to 90% of phenytoin is metabolised primarily in the liver by CYP2C9 and CYP2C19 to 5-(4-hydroxyphenyl)-5-phenylhydantoin (4'-HPPH, p-HPPH), which is glucuronidated and excreted. This main metabolite is considered to be inactive (Thorn

et al., 2012). Minor metabolites that are produced include 3,4-dihydrodiol, catechol (3,4-dihyroxyphenyl-phenylhydantoin), and 3-O-methylated catechol (Inselman and Hansen, 2014). Resulting from a saturation of metabolite-inducing enzymes, phenytoin displays non-linear elimination pharmacokinetics (Browne and LeDuc, 1995); the elimination half-life differs with plasma concentration. Strictly speaking, it is inappropriate to refer to a 'half-life', but in practical terms, a mean value of around 22 hours is a useful guide (IARC, 1996). After metabolsm in the liver, the metabolites, which are mainly conjugated by glucuronate, are largely excreted via the kidneys (Tilley, 1997; Plumb, 1999). Only small amounts of phenytoin are excreted unchanged in the urine (2–4%) and feces (5%). The elimination half-life averages 20–30 hours (12–20 hours in children) but may be as long as 60 hours, and as high as 200 hours after overdose, due to saturation of hydroxylation pathways.

There are some species differences in bioavailability after oral administration (Tilley, 1997; Boothe, 2011). In the dog, bioavailability is only 40% (Tilley, 1997; Plumb, 2011). In horses, this is given as 34.5 ± 8.6% (Kowalczyk and Beech, 1983; Bowen et al., 2004; Soma et al., 2001) and its half-life is 8 hours (Kowalczyk and Beech, 1983).

Soma et al. (2001) studied the pharmacokinetics of phenytoin in six horses after single or twice-daily administration for 5 days. Mean (±SD) elimination half-life following a single intravenous or oral administration of 8.8.mg/kg bw was 12.6 ± 2.8 and 13.9 ± 6.3 hours, respectively, and 11.2 ± 4.0 hours following twice-daily administration for 5 days. Significant differences in terminal elimination phase half-life (5.8 to 13 hours) following intravenous administration, compared with the elimination-phase half-life after oral administration were not determined. Values for oral bioavailability ranged from 14.5 to 84.7%.

Mean peak plasma concentration (C_{max}) following single oral administration was 1.8 ± 0.68 µg/ml. Peak plasma concentrations were extremely variable and ranged from a low steady-state value of approximately 1 µg/ml to a high value of 6 µg/ml. This variability in plasma concentrations over a 5-day period reflects the wide range of bioavailability of the drug. Peak plasma concentration was reached at 6.3 ± 2.4 hours (T_{max}) (Soma et al., 2001).

Steady-state plasma concentration following twice-daily administration for 5 days was 4.0 ± 1.8 µg/ml. Out of 12.0 ± 5.4% of the drug excreted during the 36-hour collection period, 0.78 ± 0.39% was the parent drug phenytoin, and 11.2 ± 5.3% was 5-(p-hydroxyphenyl)-5-phenylhydantoin (p- HPPH). Following twice-daily administration for 5 days, phenytoin was quantified in plasma and urine for up to 72 and 96 hours, respectively, and p-HPPH was quantified in urine for up to 144 hours after administration. This excretion pattern was not consistent in all horses (Soma et al., 2001).

Clinical effects after overdose are generally dose related and primarily involve the peripheral and central nervous systems. Symptoms of overdose include sedation, anorexia (Plumb, 1999), ataxia (Plumb, 1999; Adams, 1995), hypermetria (Adams, 1995), and at high blood levels also coma, hypotension and respiratory depression (Plumb, 1999). Toxicity in animals has been similar to that observed in humans (e.g. effects are primarily neurologic).

Oral chronic toxicity trials in dogs over 3-6 months showed that phenytoin at doses up to 110 mg/kg bw/day was well tolerated (Schaefer et al., 2013). The nervous system appears to be the major target of acute and chronic phenytoin toxicity in experimental animals. In addition, decreased weight gain, centrilobular hepatic hypertrophy and focal necrosis of liver cells was observed in mice at 250 mg/kg and higher (Maeda et al., 1988).

In humans, chronic toxic effects are dose related and typically involve cerebellar and vestibular functions (nystagmus, ataxia, lethargy). One of the most common manifestations of chronic phenytoin

ingestion is gingival hyperplasia (Thomason et al., 1992). Chronic hepatitis, which can progress to liver cirrhosis and intrahepatic cholestasis have been reported (Boothe, 2011; Vernaud et al., 2008). Hypersensitivity reactions, referred to as the phenytoin hypersensitivity syndrome, usually appear early (i.e. two to four weeks) after initiation of treatment, and do not exhibit a dose-response relationship (Delattre et al., 1988; Braddock et al., 1992). Stevens-Johnson syndrome (SJS), and its more severe manifestation toxic epidermal necrolysis (TEN), has been associated with chronic phenytoin use (Reynolds, 1993).

In rats, phenytoin sodium caused reversible changes in sperm motility, count, morphology and cytoarchitecture of testes (Rao et al., 2009). Phenytoin is teratogenic in mice, rats and rabbits (Harbison and Becker, 1969; Sullivan and McElhatton, 1975; Harbison and Becker, 1972; McClain and Langhoff, 1980).

In humans, the known potential adverse effects of phenytoin on the fetus include facial clefts, vitamin K and D deficiencies, heart malformations, limb deformities, and neurologic defects (Brewer and Waltman, 2003). According to Adams et al. (1990) there is evidence for the teratogenicity of phenytoin ingested at 100-800 mg/day during the first trimester of gestation.

Regarding mutagenicity, Ames Salmonella tests, mouse lymphoma tests and assays on Chinese hamster ovary cells have been negative, whereas studies of sister chromatid exchange were positive (Inselman and Hansen, 2014). However, further studies of sister chromatid exchange gave equivocal results (Habedank et al., 1982; Hunke and Carpenter, 1978; Kaul and Goyle, 1999; Riedel and Obe, 1984). The IARC summarised the data on the genotoxicity of phenytoin and found some indications of genotoxic effects of phenytoin, including a single finding in Salmonella typhimurium in the presence of a metabolic activation system and some evidence of aneuploidy in a study in primary mouse embryonic fibroblasts in vitro and in human amniotic cells, but not in lymphocytes. The overall results of in vivo micronucleus tests in rodents are inconclusive. Studies of human lymphocytes in vivo showed no induction of micronuclei, chromosomal aberrations or aneuploidy but an increase of polyploidy was found in one study. Neither chromosomal aberrations nor aneuploidy were induced in human bone marrow. It was hypothesized that the metabolism of phenytoin may involve the formation of an arene oxide which has the potential to bind to macromolecules. A more recent review of phenytoin doubts the relevance of the arene oxide formation from phenytoin in vivo and attributes the arene oxide fromation to the experimental conditions in the earlier mutagenicity tests, however, without providing any explanation. The available further genotoxicity data are not discussed (Patocka et al., 2020). Overall, the results for genotoxicity are inconclusive and no firm conclusion on genotoxicity can be drawn. The metabolite 5-(4'-hydroxyphenyl)-5-phenylhydantoin, a metabolite present in horses, was found to be mutagenic in Salmonella typhimurium in the presence of a metabolic activation system but did not induce micronuclei in mouse bone marrow in vivo (IARC, 1996). An independent assessment of the data reviewed by IARC was not carried out. A literature search did not identify any additional or new experimental data.

Phenytoin had no carcinogenic potential in F344 rats after oral administration for 2 years (Jang et al., 1987). Oral administration to rats did not increase the incidence of tumours in two studies. In mice, phenytoin and its sodium salt have been reported to be carcinogenic after oral or intraperitoneal administration, producing lymphomas and leukaemias (IARC, 1977; Krueger and Bedoya, 1978; Windholz, 1983). There was clear evidence of carcinogenic activity of phenytoin in female B6C3F1 mice (increased incidence of hepatocellular neoplasia). Combined perinatal and adult exposure of male mice resulted in increased incidences of hepatocellular neoplasms (hepatocellular carcinomas and multiple adenomas) that were not seen when dietary exposure was limited to the adult exposure period only (NTP, 1993). Taking into account the above findings, IARC (1996) concluded that there is inadequate

evidence for the carcinogenicity of phenytoin in humans, but sufficient evidence in experimental animals for the carcinogenicity of phenytoin, i.e. in mice. Accordingly, phenytoin has been classified as possibly carcinogenic to humans based on animal data (group 2B). It was suggested that liver tumours in mice are induced by a promoting mechanism and the possible genotoxic effects may be due to a reactive intermediate involving formation of an arene oxide (IARC, 1996). The most recent NTP report on carcinogens continues to list phenytoin as "reasonably anticipated to be a human carcinogen" (NTP, 2021).

Phenytoin is carcinogenic in mice and teratogenic in several laboratory animals. Metabolism of phenytoin involves the formation of an arene oxide which can bind to macromolecules. There is some indication of genotoxicity of phenytoin, however the overall evidence is rather inconclusive. The metabolite 5-(4'-hydroxyphenyl)-5-phenylhydantoin did not induce micronuclei in mouse bone marrow in vivo.

Considering that there is evidence for carcinogenicity of phenytoin in mice, the mode of action of which might involve genotoxic effects but is not entirely clear, and therefore, no firm conclusion can be drawn as to whether or not phenytoin should be considered a genotoxic carcinogen, that phenytoin is teratogenic in several laboratory animals and residue depletion data is not available, it can be concluded that phenytoin will pose an unacceptable risk to consumers if used in food-producing animals of the equine species and a six-month withdrawal period is respected.

4.10.3. Assessment of new substances proposed to be added to the list in the stakeholder's survey

A. Considerations on the essentiality of the substance(s)

Alprazolam was mentioned (once) in the survey to stakeholders and it was suggested for addition to the list. The responder referred to the case study by Wong et al. (2015) in which alprazolam was used to facilitate mare-foal bonding in an aggressive post-parturient mare. Alprazolam was well-tolerated in this study and only caused slight sedation and ataxia after administration of a single dose, which became more evident when multiple doses were administered. The responder further stated its use as a sedative and anxiolytic in horses with behavioral disturbances.

Alprazolam is a benzodiazepine with an average half-life of 12 to 15 hours, equivalent to that of oxazepam. It crosses the blood brain barrier and therefore enters the central nervous system where it binds non-selectively to the gamma-amino-butyric acid (GABA)-benzodiazepine receptor complex (Verster and Volkerts, 2004). This results in inhibition of neuronal excitability and slowing of brain activity, thus producing sedation. It has been used as an anxiolytic and tranquilizer in horses. There are other sedatives such as acepromazine and diazepam, which are proposed to be kept on the list of essential substances. Other alternatives include detomidine, romifidine and xylazine, as sedatives, which are included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 and for which there are veterinary medicinal products authorised for use in food-producing animals of the equine species. It is considered that alprazolam does not bring added clinical benefit compared to these.

Excess excitability, if untreated, may cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any alprazolam-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance alprazolam is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indication in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and it does not bring

added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Fluoxetine was mentioned (twice) in the survey to stakeholders and it was suggested for addition to the list for treatment of anxiety and behavioral problems such as stereotypic behavior or stress-induced behavior in horses. This proposal was based on the study by Fontenot et al. (2021) which identified the records of 95 horses that had been prescribed fluoxetine by the equine service of a veterinary teaching hospital between November 2010 and February 2019, and collected data from the medical records.

Fluoxetine is a selective serotonin reuptake inhibitor (SSRI), and it is used as antidepressant and for treatment of obsessive-compulsive and anxiety-related disorders in humans; it is also used in dogs and cats. Anxiety conditions without traumatic causes with purely intrinsic origins are extremely rare in horses and there is limited data and clinical experience in equine medicine regarding the treatment of anxiety and behavioral disorders in horses. Clinical efficacy trials of fluoxetine in horses suffering from stereotypic behaviors or other anxiety-related behavioral disorders have not been reported. The conclusions by Fontenot et al. (2021) are based solely on retrospective collected data obtained from medical records of equine hospital's computerized medical records system, including 95 horses having been treated after surgery and 27 horses with behavioral disorders. All assessments are carried out by horse owners. Limitations of this study include the absence of placebo group and the subjective nature of identifying behavioral problems in horses. The main reason for prescribing fluoxetine in horses is to facilitate stall rest after orthopaedic surgery, rehabilitation for nonsurgical orthopaedical conditions and stall rest following exploratory laparotomy. There are other common pharmacological therapeutic approaches by administration of sedatives such as acepromazine and diazepam, which are proposed to be kept on the list of essential substances. Other alternatives include detomidine, romifidine and xylazine, as sedatives, which are included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 and for which there are veterinary medicinal products authorised for use in food-producing animals of the equine species. It is considered that fluoxetine does not bring added clinical benefit compared to these.

Excess excitability, if untreated, may cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any fluoxetine-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance fluoxetine is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indication in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Phenobarbital was mentioned (once) in the survey to stakeholders and it was suggested for addition to the list for treatment of seizures. As stated by the responder, it is the most common drug for treatment of seizures. No scientific references were provided.

Phenobarbital is a barbiturate that acts by binding and activating the inhibitory GABA(A) receptors in the central nervous system (CNS). The binding of phenobarbital to a domain of the ligand activated GABA(A) receptor leads to the opening of ion channels. Direct activation of the receptor is thought to be responsible for the sedative property of phenobarbital. The antiepileptic effects of phenobarbital are probably based on at least two mechanisms, namely a reduced monosynaptic conduction, from which a

reduced neuronal excitability could follow, and an increase in the electrical excitation threshold of the motor cortex. It is used as an antiepileptic drug predominately in foals (Aleman et al., 2006) but also in adult horses for the treatment of epilepsy (Duran et al., 1987) and seizures (Sakurai et al., 2021). Phenobarbital is further described for treatment of generalized tetanus in foals (Mykkänen et al., 2011).

The use of phenobarbital in horses has been investigated, particularly its kinetics following oral and intravenous administration (Duran et al., 1987; Ravis et al., 1987; Knox et al., 1992). Its clinical efficacy for treatment of seizures is widely recognized, also in the horse. With regards possible alternatives, potassium bromide is listed in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with a 'No MRL required' entry for all food producing species; diazepam is proposed to be retained in the list of essential substances. While both substances could constitute an alternative treatment, the clinical efficacy demonstrated by phenobarbital constitutes an added clinical benefit.

Epilepsy and seizures, if untreated, are potentially life-threatening and cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any phenobarbital-containing veterinary medicinal product authorised for use in equine species. There are veterinary medicinal products authorised for use in species other than the equine (i.e. dog and cat).

The substance phenobarbital is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indications: for treatment of epilepsy and seizures in foals and adult horses; for treatment of generalized tetanus in foals. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Trazodone was mentioned (once) in the survey to stakeholders and it was suggested for addition to the list. The responder stated that the substance is useful for behavioral therapy during periods of reduced exercise such as during fracture repair. No scientific references were provided.

It is a serotonin reuptake inhibitor and used as an anxiolytic and antidepressant drug for behavioral therapy mostly in human medicine and small animal practice, especially in dogs. Anxiety conditions without traumatic causes with purely intrinsic origins are extremely rare in horses. There is limited data and clinical experience in equine medicine regarding the treatment of anxiety and behavioral disorders in horses. Trazodone is generally used an adjunctive treatment in the initial phase of acute laminitis, to encourage recumbency and minimize ambulation (Hobbs et al., 2023). There are other common pharmacological therapeutic approaches by administration of sedatives such as acepromazine and diazepam, which are proposed to be kept on the list of essential substances. Other alternatives include detomidine, romifidine and xylazine, as sedatives, which are included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 and for which there are veterinary medicinal products authorised for use in food-producing animals of the equine species.

Excess excitability, if untreated, may cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any trazodone-containing veterinary medicinal product authorized for use in equine species (neither in other animal species).

The substance trazodone is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do

yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Knowledge regarding substances for nervous disorders for this assessment was derived from textbooks, and peer-reviewed articles, and case-studies in horses.

B. Considerations regarding consumer safety

Phenobarbital is a barbiturate that is used in both human and veterinary medicine as an antiepileptic drug, although it has also been used to treat generalised tetanus in foals and other clinical uses have been described in humans (i.e. insomnia and apprehensiveness). It is a weak acid (pKa 7.3) that is poorly water-soluble and with relatively low lipid solubility (Trinka, 2023).

Pharmacokinetics of phenobarbital has been studied in several species. In humans, phenobarbital is readily absorbed from the gastrointestinal tract, with greater than 95% bioavailability. After intravenous administration, it distributes into the body with volumes of distribution ranging from 0.54 to 0.73 l/kg in adults. Brain penetration is relatively slow. It is 45-60% bound to plasma proteins and is only partly metabolised in the liver to two major metabolites, p-hydroxyphenobarbital (PBOH) and 9-d-glucopyranosylphenobarbital (PNG). Approximately 20-40% of a dose is excreted in the urine unchanged at normal urinary pH. The excretion is considerably higher when the pH of the urine is alkaline. The plasma half-life is about 50-160 hours in adults. The elimination follows first-order kinetics. The therapeutic range of plasma-phenobarbital has been quoted as 15 to 40 µg/ml. It crosses the placental barrier and considerable interindividual variation in phenobarbital kinetics has been described (Anderson, 2002; Johannessen, 2004; Sweetman, 2009). Brain phenobarbital concentrations were significantly less than in other organs including liver, kidney, spleen, pancreas, and lungs in pediatric patients; the authors concluded that estimating the serum phenobarbital concentration during clinical pediatric practice gives a good indication of the brain concentration (Onishi et al., 1984).

The pharmacokinetics were evaluated after a 2.6 mg/kg intravenous and a 2.9 mg/kg oral doses; half-lives were 5.8 and 5.1 days for the intravenous and oral route, respectively, and volume of distribution was 0.60 l/kg after the intravenous dose. The adjusted absolute availability of phenobarbital from the oral tablets studied was 94.9% (Nelson et al., 1982). A loading dose of phenobarbital of 15 mg/kg as an intravenous infusion and maintenance dose of 5 mg/kg at 24 and 48 hours later were investigated in children with cerebral malaria. Mean half-life was 82.9 hours with a volume of distribution of 0.8 l/kg (Kokwaro et al., 2003).

Aging has been associated with a significant decrease in phenobarbital clearance, which might be related to a reduction in glomerular filtration rate or diminished drug-metabolising capacity in the liver, or both (Messina et al., 2005).

The pharmacokinetics of phenobarbital have also been studied in horses, dogs and goats. In horses, a single oral dose of 26 mg/kg and repeated doses of 13 mg/kg administered orally for 42 days were studied. Elimination half-lives were 24.2 ± 4.7 and 11.2 ± 2.3 hours, after single and repeated doses, respectively. The volume of distribution was 0.96 ± 0.06 and 0.914 ± 0.119 l/kg, and clearance 28.2 ± 5.1 and 57.3 ± 9.6 ml/h/kg. The authors concluded the significant decrease in half-life after repeated dosing might be indicative of enzyme induction (Knox et al., 1992). Other authors reported half-life of 19.0 ± 4.4 hours after a single oral dose of 5.5 mg/kg. The steady-state volume of distribution and the total body clearance were 0.753 ± 0.115 l/kg and 27.9 ± 9.2 ml/h/kg, respectively. The oral absorption ranged from 76 to 124% among the six horses from this study (Ravis et al., 1987).

In dogs, after both intravenous and oral doses of 10 mg/kg with an interval of 4 weeks, half-lives ranging from 42 to 72 hours were observed. Bioavailability was in the range of 86-96% (Al-Tahan and Frey, 1985). Other authors reported terminal elimination half-lives from 40-74 hours after single oral doses. After longer durations of administration, the elimination half-life of phenobarbital in an individual will decline due to induction of the patient's hepatic microsomal enzymes. Maximum concentrations are observed 4-8 hours after oral administration, although food delays peak concentrations by 2-4 hours (Brown, 1988).

Some differences have been observed in goats. Oral bioavailability is poor (24.9%), and the half-life was very short due to a high clearance (3.8-4 hours) (Yates et al., 2020).

Regarding toxicity, the principal acute toxicity of phenobarbital is drowsiness or sedation and the chronic adverse effects due to prolonged therapy include elevated serum activities of ALT, ALP and glutamate dehydrogenase (Brown, 1988). Furthermore, Eze et al. (2009) studied the histology of the liver and the brain of Wistar rats after a daily administration of 31.25, 37.50, 43.75 and 50 mg/kg for a period of 3 weeks; they observed that high doses of phenobarbital or discrete doses administered for long periods had adverse effects on the liver and that neurodegeneration increases as phenobarbital dosage increases (Eze et al., 2009).

Phenobarbital is a teratogen and a developmental neurotoxicant in humans and experimental animals (IARC, 2001). Various types of cardiovascular malformations were detected in pups from rats administered with 80 or 120 mg/kg phenobarbital by gavage on two consecutive gestational days (Okuda and Nagao, 2006). Yanai et al. (1979) evaluated the effects on the neuronal number and size in the offspring when phenobarbital was administered to pregnant mice. They concluded that the prenatally forming large neurons were affected by phenobarbital administration. Rogel-Fuchs et al. (1992) studied hippocampal cholinergic alterations and related behavioral deficits after prenatal and neonatal exposure to phenobarbital in mice. The results suggested that early exposure induces alterations in postsynaptic components of the hippocampal cholinergic system and concomitantly in hippocampus-related behavior. Endocrine effects have also been described when administering phenobarbital to pregnant rats (Gupta et al., 1980; Gupta and Yaffe, 1981). Waters et al. (1994) concluded that phenobarbital is associated with an increased risk of fetal death and anomalies.

Neither phenobarbital nor its sodium salt induced sister chromatid exchanges, chromosomal aberrations, micronuclei or sperm abnormalities in mice treated in vivo. Phenobarbital induced chromosomal aberrations and mutation but not sister chromatid exchanges in cultured human cells. Both positive and negative results were obtained for transformation in rodent cells in vitro. Phenobarbital enhanced transformation of virus infected rat embryo cells initiated with 3-methylcholanthrene in a two-stage transformation assay. It induced sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster cells, but not in cultured rat liver cells; micronuclei and aneuploidy were not induced in Chinese hamster cells. Phenobarbital induced mutation in Chinese hamster cells, but conflicting or negative results were obtained in other rodent cells. Phenobarbital and its sodium salt did not induce DNA strand breaks, and phenobarbital did not induce unscheduled DNA synthesis, in cultured rodent cells. Phenobarbital inhibited intercellular communication in human hepatoma cells and both phenobarbital and its sodium salt did so in rodent systems. Phenobarbital induced neither somatic mutation nor recombination in Drosophila; the sodium salt did not induce sex-linked recessive lethal mutations. Phenobarbital induced aneuploidy but not mutation or gene conversion in fungi. Conflicting results were obtained concerning the mutagenicity of these compounds in bacteria (IARC, 2001).

The inconsistency of the results, the absence of any direct evidence of an Interaction with DNA and the generally negative in-vivo data lead to the conclusion that phenobarbital is not genotoxic, and

genotoxicity does not appear to play a role in its hepatocarcinogenicity. Phenobarbital is a microsomal enzyme inducer and has been studied extensively for its ability to promote hepatic tumours. There is evidence that the microsomal enzyme induction is correlated with hepatic tumour promotion by phenobarbital. The potency for CYP induction in mice and rats also correlates with the degree of tumour promotion. Phenobarbital does not induce these enzymes in hamsters and does not produce liver tumours in this species. The enhancement of hepatic tumorigenesis was shown to be due to tumour promotion. Although the mechanisms of tumour promotion are not completely known, effects on the control of cell proliferation appear to play a role. Long-term exposure of rodents to phenobarbital produces hepatomegaly and hepatocellular hypertrophy and hyperplasia. In initiation-promotion models, phenobarbital selectively increased the labelling index in foci as compared with normal surrounding liver. The foci progress to the stage of hepatocellular adenoma, in which cellular proliferation no longer depends on the presence of phenobarbital. The mitogenic and tumour-promoting effects of phenobarbital appear to involve changes in growth factors, intracellular communication, gene expression and cell cycle signal transduction. Overall, the experimental evidence supports the conclusion that the mode of action of phenobarbital in the production of hepatic tumours is non-genotoxic and involves tumour promotion (IARC, 2001).

There is inadequate evidence in humans for the carcinogenicity of phenobarbital and sufficient evidence in experimental animals for the carcinogenicity of phenobarbital. The carcinogenicity of phenobarbital has been investigated by oral administration in multiple studies in mice and rats; phenobarbital consistently produced hepatocellular adenomas and carcinomas in mice. Hepatocellular adenomas were produced in rats after lifetime exposure in one study. Phenobarbital promoted thyroid follicular-cell tumours in one study in mice and in several studies in rats. Phenobarbital is classified as possibly carcinogenic to humans (group 2B) (IARC, 2001).

Considering that phenobarbital is a teratogen and a developmental neurotoxicant in humans and experimental animals, the inconsistency of the results for genotoxicity, the carcinogenicity data in animals and the lack of residue depletion data, it can be concluded that phenobarbital will pose an unacceptable risk for consumers when used in food-producing animals of the equine species and a six-month withdrawal period is respected.

4.10.4. Conclusion

Based on the above assessment and justifications, the following recommendations are proposed:

1. The following active substance, currently in Regulation (EU) 1950/2006 as amended by Regulation (EU) 122/2013, is proposed to be retained in the list, either without modification or with an amendment of the current entry as shown below:

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Diazepam[48]	short-term anti-convulsant for treatment of seizures	none identified	second-generation antiseizure

2. The following active substances, currently in Regulation (EU) 1950/2006 as amended by Regulation (EU) 122/2013, are proposed to be removed from the list: carbamazepine, phenytoin, primidone.

3. The following active substances, suggested for addition to the list in the survey to stakeholders, are not proposed for inclusion: alprazolam, fluoxetine, phenobarbital, trazodone.

[48] This substance is discussed in detail in section 4.12 (substances for sedation and premedication (and antagonism)).

4.11. Substances for ophthalmology

4.11.1. Overview

	Active substance (ATCvet code)
Substances in Regulation (EU) 1950/2006 **proposed to be retained**, with or without amendment of the current entry	Cyclosporine A (QL04AD01); Phenylephrine (QS01FB01); Timolol maleate (QS01ED01); Triamcinolone acetonide[49] (QS01BA05); Tropicamide (QS01FA06)
Substances in Regulation (EU) 1950/2006 **proposed to be removed**	Dorzolamide (QS01EC03); Latanoprost (QS01EE01)
Substances from stakeholders' survey **proposed for inclusion**	Acetazolamide (QS01EC01); Cyclopentolate (QS01FA04); Synephrine (QS01GA06); Tetryzoline (QS01GA02)
Substances from stakeholders' survey **not proposed for inclusion**	Brinzolamide (QS01EC04); Pilocarpine (QS01EB01); Tacrolimus (QL04AD02)

4.11.2. Review of the existing entries in Regulation (EU) 1950/2006, as amended by Regulation (EU) 122/2013, considering the survey results

A. Considerations on the essentiality of the substance(s)

Cyclosporine A is an immunosuppressant used for prophylaxis of transplant rejection (kidney, liver, heart, lung, pancreas, bone marrow) and therapy of autoimmune diseases; it is a lipophilic cyclic peptide consisting of 11 amino acids.

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), as an immunosuppressive used for the treatment of autoimmune diseases of the eye. No alternatives are identified in the current list, which is considered its specific advantage.

Topical cyclosporin A is used in recurrent uveitis and immune-mediated keratitis in horses (Padjasek et al., 2022; Gilger et al., 2005). The mechanism of action involves modulation of the immune system by inhibiting T-lymphocyte proliferation and reducing cytokine gene expression (Padjasek et al., 2022). As immunosuppressants, topical steroids, some of which have an entry in Table 1 of Commission Regulation (EU) No 37/2010 could qualify as an alternative treatment option (e.g. dexamethasone, for which there are veterinary medicinal products authorised for food-producing animals of the equine species). However, it is recognised the added clinical benefit of cyclosporine A.

Autoimmune diseases of the eye, if untreated, might cause unacceptable suffering for the animal.

Cyclosporine A was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any cyclosporine-A-containing veterinary medicinal product authorised for use in equine species. There are veterinary medicinal products authorised for use in species other than the equine (i.e. dog and cat) and for different topical uses in the eye (i.e. conjunctival, subconjunctival, ocular, and intraocular) and oral use.

[49] This substance is discussed in detail in section 4.2 (analgesics).

The substance cyclosporine A is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: for the treatment of autoimmune diseases of the eye. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Dorzolamide is a sulfur- and nitrogen-containing chiral heterocyclic chemical compound derived from thiophene. Due to its efficacy as a carbonic anhydrase inhibitor, the pure isomer is used as a drug in eye drops to lower intraocular pressure.

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), for treatment of glaucoma and topical use. Latanoprost and timolol maleate are identified as its alternatives, and the specific advantage captured is its specific mode of action as a carbonic anhydrase inhibitor.

Its clinical use in humans for the reduction of intraocular pressure (IOP) in patients with either open-angle glaucoma or ocular hypertension is well established (Balfour and Wilde, 1997; Martens-Lobenhoffer and Banditt, 2002; Loftsson et al., 2012). It is commonly used in combination with timolol since both have different mechanisms of action and their effects are additive when administered together (Ormrod and McClellan, 2000; Konstas et al., 2021). Use of dorzolamide in equine topical glaucoma therapy is also well established (Willis et al., 2002), generally in combination with timolol for an enhanced clinical performance (Willis et al., 2001b; Tofflemire et al., 2015). Other carbonic anhydrase inhibitors, e.g. acetazolamide (for systemic therapy) or brinzolamide are alternatives, though dorzolamide is the drug of choice.

Glaucoma, if untreated, might cause unacceptable suffering for the animal.

Dorzolamide was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database retrieves at least one dorzolamide-containing veterinary medicinal product authorised for use in equine species (non-food-producing horses) containing dorzolamide for ocular use (also indicated for use in dogs and cats).

The substance dorzolamide is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: treatment of glaucoma, topical use. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Latanoprost is used in human medicine for topical treatment of elevated intraocular pressure due to (open-angle or closed-angle) glaucoma. Latanoprost is a prostaglandin F2α analogue that acts as a selective agonist at the prostaglandin F receptor.

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), for treatment of glaucoma and topical use. Dorzolamide and timolol maleate are identified as its alternative and the specific advantage captured is its specific mode of action as a prostaglandin F2α analogue.

Prostaglandins increase the sclera’s permeability to aqueous fluid. By giving latanoprost outflow of aqueous fluid is increased thus lowering intraocular pressure; however, it is unclear if latanoprost provides a useful clinical effect in horses. A latanoprost ophthalmic solution was evaluated in two studies, with eight horses per study. Davidson et al. (2002) found latanoprost had no significant effect on intraocular pressure or pupillary diameter in normal horse eyes compared with control eyes, for up to 5 days. However, in a second non-controlled study, Tofflemire et al. (2017) found latanoprost induced a significant reduction in intraocular pressure and vertical pupil diameter in normal horse eyes;

however, this was followed by adverse events, including blepharospasm, blepharoedema, epiphora, and conjunctival hyperemia. In another study involving 20 clinically normal adult horses, latanoprost reduced intraocular pressure when dosed every 24-hours; however, the frequency of prostaglandin-induced adverse events was high (conjunctival hyperemia, epiphora, blepharospasm, and blepharedema) (Willis et al., 2001a). Since recurrent uveitis appears to be a risk factor for glaucoma in horses, topical administration of latanoprost may potentiate prostaglandin-mediated inflammatory disease in affected horses. On the contrary, timolol is an efficacious alternative which is retained in the list. Clinical evidence from the survey results supports this proposal (see below).

Glaucoma, if untreated, might cause unacceptable suffering of the animal.

Latanoprost was mentioned (five times) in the survey to stakeholders and it was proposed to be removed from the list due to lack of efficacy and existence of available alternatives with better efficacy profile.

A search in the veterinary medicines database does not retrieve any latanoprost-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance latanoprost is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indication in animals of the equine species; it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Phenylephrine belongs to the group of direct-acting sympathomimetics. It acts as an agonist at the α1-adrenoceptor with a partially indirect effect. Phenylephrine is structurally identical to adrenaline except for a missing 4-hydroxy group and is used in the EU mainly as a local vasoconstrictor.

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), for treatment of glaucoma, epiphora, nasal oedema and splenic entrapment. Tropicamide (for the treatment of glaucoma) is identified as its only alternative. The specific advantage captured is that both, phenylephrine and tropicamide, have been shown to be equally effective in the treatment of glaucoma.

Phenylephrine hydrochloride is a potent, effective, relatively safe drug with few ocular side effects (Meyer and Fraunfelder, 1980). The use of phenylephrine (alone or in combination with other drugs) in patients with glaucoma is well known in human medicine (Vandewalle et al., 2013). Published scientific evidence for the same use in horses is old and scarce (Hacker et al., 1987); however, expertise within the expert group preparing this scientific advice confirmed the clinical relevance of phenylephrine for the treatment of glaucoma. It is used as an alternative to atropine for pupil dilation and can be used for equine recurrent uveitis (ERU) management; atropine can cause elevated intra ocular pressure, whereas phenylephrine either does not or only slightly increase it (Bizrah and Corbett, 2019; Kazemi et al., 2021). In eyedrops, it is used as a diagnostic test in equine grass sickness (Hahn and Mayhew, 2000).

Glaucoma and epiphora, if untreated, might be life-threatening and cause unacceptable suffering of the animal.

Phenylephrine was mentioned (three times) in the survey to stakeholders, proposing to add it to the list, though this substance is already included in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013).

A search in the veterinary medicines database does not retrieve any phenylephrine-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance phenylephrine is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: treatment of glaucoma and epiphora. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal. Phenylephrine is also proposed for the treatment of nasal oedema (under respiratory disorders), grass sickness diagnosis (under diagnostic procedures), and treatment of splenic entrapment (under gastrointestinal disorders). Please refer to sections 4.4, 4.6 and 4.7, respectively, for further details.

Timolol maleate is a non-selective beta-adrenergic receptor blocking agent that does not have significant intrinsic sympathomimetic, direct myocardial depressant, or local anaesthetic activity. It combines reversibly with the beta-adrenergic receptor and inhibits the usual biologic response that would occur with stimulation of that receptor (Negri et al., 2019). This specific competitive antagonism blocks stimulation of the beta-adrenergic (agonist) stimulating activity, whether originating from an endogenous or exogenous source. Reversal of this blockade can be accomplished by increasing the concentration of the agonist which will restore the usual biological response. Timolol maleate is used primarily in eye drops for the treatment of open-angle glaucoma (Willis et al., 2002; Tofflemire et al., 2015; Konstas et al., 2021).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), for treatment of glaucoma and topical use. Dorzolamide and latanoprost are identified as its alternatives and the specific advantage captured is its specific mode of action as a non-selective beta-adrenergic receptor blocking agent that causes vasoconstriction, which in turns leads to decrease of the aqueous humour.

Use of timolol in equine topical glaucoma therapy is also well established, generally in combination with dorzolamide (or other substances) for an enhanced clinical performance, as indicated previously (Willis et al., 2001b; Tofflemire et al., 2015). Its specific mode of action as a non-selective beta-adrenergic receptor blocking agent, provides for an important therapeutic choice in the treatment of glaucoma. It has a better clinical profile than latanoprost.

Glaucoma, if untreated, might cause unacceptable suffering of the animal.

Timolol maleate was mentioned (once) in the survey to stakeholders, proposing to add it to the list, though this substance is already included in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013).

A search in the veterinary medicines database does not retrieve any timolol-maleate-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance timolol maleate is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: treatment of glaucoma, topical use. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Triamcinolone acetonide is discussed in detail in section 4.2. (analgesics) where it is proposed for the treatment of joint inflammation (please refer to section 4.2 for the detailed assessment of the substance). Triamcinolone acetonide is also proposed under this section for the treatment of recurrent uveitis in cases that are refractory to other treatments.

Recent research shows that an additional indication for triamcinolone use in equines is for treatment of recurrent uveitis via the suprachoroidal administration (Gagnon et al., 2021). There is increasing interest in this alternative as a potentially effective, low morbidity treatment in cases that are

refractory to other treatments. It is thus considered to bring added clinical benefit compared to the alternatives listed for the treatment of recurrent uveitis (i.e. tropicamide, atropine).

Recurrent uveitis, if untreated, may cause unacceptable suffering for the animal.

The substance triamcinolone acetonide is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: for the treatment of recurrent uveitis in cases that are refractory to other treatments. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering for the animal.

Tropicamide is a synthetic pupil dilating drug used in the form of eye drops for diagnostic purposes in ophthalmology.

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), for treatment of glaucoma and topical use. Phenylephrine is identified as its alternative, and the specific advantage captured is that both, phenylephrine and tropicamide, have been shown to be equally effective in the treatment of glaucoma.

In humans, tropicamide is considered safe mydriatic agent, even in people with chronic glaucoma (Pandit and Taylor, 2000). Due to its rapid onset of action it is considered a viable alternative to other mydriatic agents such as cyclopentolate, especially in situations where the clinical findings are variable or there is no consistency between the examination results and the clinical manifestations (Yazdani et al., 2018). In combination with phenylephrine and lidocaine (injectable solution) is an emerging option for mydriasis/anaesthesia in adults undergoing cataract surgery (Deeks, 2019). In horses, maximal pupil dilation was achieved approximately 40- to 45-min following the application of topical tropicamide 1% (McMullen et al., 2014). These results are consistent with those obtained in rats, with effect lasting approximately 6 hours (Pumphrey et al., 2021). A single dose of topical 1% tropicamide resulted in a statistically significant reduction in Schirmer tear test values in clinically normal horses (Selk Ghaffari et al., 2009). It is used in the treatment of equine recurrent uveitis, an important ocular disease and the most common cause for blindness in horses (Spiess, 1997; Curling, 2011a, 2011b; Gerding and Gilger, 2016). Atropine could be considered as an alternative treatment for equine recurrent uveitis included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010, with an entry that covers food-producing horses and veterinary medicinal products authorised for food-producing animals of the equine species; however, tropicamide offers clinical advantages as previously indicated.

Recurrent uveitis, if untreated, might cause unacceptable suffering of the animal.

Tropicamide was mentioned (twice) in the survey to stakeholders, proposing a modification to the entry as presented in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013). Comments received are in support of the substance being considered essential; it is necessary for complete ophthalmic exams in the clinical setting (it allows fundoscopy and complete lens assessment). However, the current indication being glaucoma is not aligned with current evidence-based medicine (it is contraindicated in glaucoma). No scientific evidence is provided to support this statement. On the contrary, published data in humans seem to indicate that the risk of inducing acute glaucoma following mydriasis with tropicamide can be disregarded, and that the presence of chronic glaucoma constitutes no additional risk (Pandit and Taylor, 2000).

A search in the veterinary medicines database retrieves at least one tropicamide-containing veterinary medicinal product authorised for use in equine species (non-food-producing horses) for ocular use (also indicated for use in dogs and cats).

The substance tropicamide is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: treatment of recurrent uveitis, topical use. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Knowledge regarding use of ophthalmic medicines in horses for this assessment was derived from textbooks, review articles, retrospective studies, and clinical trials.

B. Considerations regarding consumer safety

Cyclosporine A is an immunosuppressant used in humans for prophylaxis of transplant rejection (kidney, liver, heart, lung, pancreas, bone marrow) and therapy of autoimmune diseases. It is on the EU market as a human medicinal product.

In humans, its absorption is poor after oral administration; this occurs mainly in the intestine (Freeman, 1991; Faulds et al., 1993; Forsythe and Paterson, 2014). The bioavailability is about 30%. Maximum blood concentrations are usually observed 1 to 8 hours after oral administration, with a second peak appearing only in some individuals. It is then rapidly distributed, approximately within 10 minutes, and drug tissue concentrations appear to corelate positively with lipid levels in tissues. Cyclosporine A does not cross the blood-brain barrier or enter the cerebrospinal fluid to any significant extent but may cross the blood-retinal barrier when it is compromised, i.e. in patients with severe ocular disease. It is predominantly metabolised in the liver but there is no single major metabolite pathway and more than 30 metabolites have been observed. Main metabolites show only 10-20% of the immunosuppressive activity of the parent compound. It exhibits linear elimination, with clearance rate of 0.38 to 3 l/h/kg. Elimination half-life is approximately 19 hours. Following oral administration, 90% of the dose is excreted in the bile and 6% in urine; less than 1% unchanged in both cases (Faulds et al., 1993).

From the product information of human medicinal products (HMPs) it can be assumed that though animal studies might have shown reproductive toxicity following systemic, such exposures were considered sufficiently in excess compared to the maximum human exposure assessed, indicating little relevance to its safety profile. Some other HMPs provide LD50 values in laboratory species following oral and intravenous administration, which are not considered applicable for this assessment. It should be noted that animal studies have shown reproductive toxicity in rats and rabbits, and that cyclosporine is classified as pertaining to group 1 by the IARC (carcinogenic to humans).

However, considering primarily the proposed use of the substance (i.e. topically applied to the eye for diagnostic purposes) and its pharmacokinetic characteristics in humans described above, despite being classified as IARC group 1, it can be exceptionally accepted that cyclosporine A 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.

Dorzolamide is marketed in the EU as both human and veterinary medicinal products. In humans, dorzolamide is known as a powerful inhibitor of carbonic anhydrase II (CA-II) that penetrates the sclera and cornea to reach the ciliary process and lowers formation of HCO3- and aqueous humor.

Like for other drugs that are topically applied into the eye, dorzolamide can enter the systematic circulation (Balfour and Wilde, 1997). The absorption rate was suggested at 33% (possibly lower), with free circulating dorzolamide binding only moderately to plasma proteins and leaving 68 to 76%

unbound dorzolamide according to a study in rats (Martens-Lobenhoffer and Banditt, 2002). Dorzolamide has a logPow 1.7 at pH 7.4 (Loftson et al., 2012). Data from human medicinal products suggests, from studies performed in rabbits, that dorzolamide penetrates well into ocular tissues and fluids, with concentrations of > 5 mg/kg or mg/L being found in the cornea, iris/ciliary body, retina and aqueous humour after instillation of 2% dorzolamide. Dorzolamide (as other carbonic anhydrase inhibitors) is known to reach systemic circulation, possibly causing adverse events (Loftson et al., 2012).

Once in the systemic circulation, dorzolamide and its active metabolite (N-desethyldorzolamide) compete for accumulating in erythrocytes by binding to the carbonic anhydrase (Biollaz et al., 1995). In rats treated at human doses, the formation of the main metabolite and the proportion of free dorzolamide remained low (Wong et al., 1996; Martens-Lobenhoffer and Banditt, 2002). Dorzolamide concentrations in erythrocytes reached steady state after approximately 8 days administering 2% dorzolamide, 3 times a day, to both eyes in healthy volunteers (Balfour and Wilde, 1997). However, other studies seem to indicate that steady state might be reached later and be dose-dependent (Martens-Lobenhoffer and Banditt, 2002; Matuszewski and Constanzer, 1992).

According to Biollaz et al. (1995) and Balfour and Wilde (1997), dorzolamide has a terminal half-life ≥ 120 days. The substance washes out from the red blood cells in a non-linear fashion, with a rapid initial decline followed by a slower elimination phase. Human case studies revealed analytical findings of dorzolamide in urine six months after red blood cell transfusion from a blood donor treated with dorzolamide (Kintz et al., 2022); similarly it could be detected in doping controls several months after its use (Pokrywka et al., 2021).

Maren et al. (1997) applied a common human dose for the treatment of glaucoma, which is 1 drop (30 µL of 2% solution) every 8 hours per treated eye, equivalent to a total daily dose of 4 mg. On this regime, the red cells accumulated drug over a period of 8 days, reaching a value of 20-25 µM, which corresponds to the concentration of carbonic anhydrase II (CA-II) in human red cells. This drug concentration persisted throughout the 18 months of application. The plasma concentration was 0.034 µM, or 1/700 that of the red cells. This plasma concentration corresponds to that calculated from the dilution of administered drug into body water. The data are well fitted into the equilibrium expression for KI of dorzolamide against CA II at 37°C, as 8 × 10−9 M. The red cells also contained a small amount (5 µM) of the N-des-ethyl metabolite, probably reflecting its modest binding to CA I. In the initial 8-day drug period, virtually no drug appeared in the urine since CA II sites were being filled. At steady state, renal excretion was 1.3 mg/day and the renal clearance 90 ml/min. These excretion numbers include the small (20%) amount of the des-ethyl metabolite of dorzolamide. The relation of these data to its effect in lowering of intraocular pressure is not disputed.

Dorzolamide is primarily excreted in the urine, mainly unchanged, though its main metabolite is also found and accounts one third of the dose (Maren et al., 1997; Martens-Lobenhoffer and Banditt, 2002). Data from human medicinal products indicates that (some) elderly patients with renal impairment (i.e. with an estimated CrCl 30-60 ml/min) had higher metabolite concentrations in red blood cells, but no meaningful differences in carbonic anhydrase inhibition and no clinically significant systemic side effects directly attributable to this finding.

Similar data from human medicinal products indicates that in rabbits given maternotoxic doses of dorzolamide associated with metabolic acidosis, malformation of the vertebral bodies was observed; in lactating rats, a decrease in body weight gain of the offspring was observed; no adverse effects upon fertility were observed in male and female rats given dorzolamide prior to and throughout mating. In clinical studies with human patients, they did not develop signs of metabolic acidosis or serum electrolyte changes indicative of systemic CA inhibition; therefore, it is not expected that the effects

noted in animal studies would be observed in patients receiving therapeutic doses of dorzolamide. Data in animals has shown excretion of dorzolamide and its metabolites in milk; a risk to the newborns or infants cannot be excluded.

Toxicological data is reported in the summary of product characteristics of human medicinal products. Developmental studies in laboratory animals (rabbits and rats) retained the same NOAEL of 1 mg/kg bw day due to vertebral malformations and decreased foetal body weight in rabbits, and reduced birth weight, reduced weight gain, and a slight delay in postnatal development in rats. The lowest NOAEL for maternal toxicity derived from a developmental toxicity study in rats was 0.1 mg/kg bw/day, due to reduced body weight gain.

No pharmacokinetic information or depletion studies are available in horses. The available knowledge from humans indicates that dorzolamide accumulates in the red blood cells and can produce side effects, and that even low concentrations in plasma have a long elimination half-life close to the proposed six-month withdrawal period in the horse. Under the assumption of an even distribution of residues in the tissues and a worst-case exposure scenario an estimation of the possible presence of residues after the six-month withdrawal period was conducted (see section 7.2.2 in the annex for further details). Several assumptions had to be made due to the lack of data in horses, noting the estimate presented considers the treatment of a single eye. Monoliteral glaucoma is, according to the clinical experts consulted, the most common presentation of glaucoma; however, bilateral glaucoma cannot be excluded which would double the exposure estimate, thus reducing the margin of exposure calculated. Different toxicity reference values were considered, either from the available developmental toxicity studies in rats and rabbits or from human therapeutic doses, considering those ensuring an absence of side effects. Similarly, different absorption factors were considered. The overall estimation results in a MoE (around 30) that is not sufficient to exclude a risk for consumers due to the presence of residues that could inhibit CAII in red blood cells after a six-month withdrawal period in the horse.

Considering the available information and the long half-life (≥ 120 days) in humans, in the absence of pharmacokinetic information or depletion data in horses and using a toxicological reference value (TRV) based on the human therapeutic use (see proposed worst-case scenario in the annex), not enough margin of safety (MoE) is achieved for dorzolamide when used as proposed. The resulting MoE only just reaches the required MoE and an underestimation of the exposure proposed cannot be excluded, even when based on a worst-case residue depletion with available data so far. Therefore, it can be concluded that dorzolamide can pose an unacceptable risk for consumers when used in food-producing animals of the equine species and a six-month withdrawal period is respected.

Phenylephrine is an alpha-1 adrenergic receptor agonist used in human medicine to treat hypotension, dilate the pupil and induce local vasoconstriction (NCBI, 2023a). When in ophthalmic formulations, it is indicated to induce mydriasis for ocular diagnostic purposes and therapeutic procedures. Side effects from topical instillation are uncommon but include severe systemic cardiovascular effects with elevated blood pressure and stroke (Meyer and Fraunfelder, 1980).

In humans it is absorbed through the mucosa, so systemic effects may follow the application to the eyes (or nasal mucosa); phenylephrine 10% eye drops can have powerful systemic effects (Scriba, 2009). It has been described that the maximum plasma level of phenylephrine is usually achieved during the first 20 min following topical application (Kumar et al., 1986).

When administered orally, the bioavailability is low (approximately 38%) due to irregular absorption and first-pass metabolism, i.e. sulphate conjugation by monoamine oxidase in the intestinal wall and liver (Scriba, 2009). Another major route of metabolism is deamination by monoamine oxidase; glucuronidation also occurs to a lesser extent. The half-life is relatively short, 2 to 3 hours following

oral or intravenous administration. Phenylephrine and its metabolites are excreted mainly in urine and about 86% of the dose is recovered in urine (Kanfer et al., 1993). Following oral or intravenous administration, 80 to 86% of the dose is excreted in urine within 48 hours (NCBI, 2023a).

Regarding toxicity, toxicological studies in rats and mice have been performed. As a vasoconstrictor, phenylephrine can increase systolic and diastolic blood pressure, and thus cause reflex bradycardia. However, some authors report no significant changes after instillation of 2.5% phenylephrine; other authors, though, report a transient increase in blood pressure after topical drops of 2.5% phenylephrine, that returns to baseline levels in less than 20 minutes (Kazemi et al., 2021). No evidence of mutagenicity or carcinogenicity has been observed in studies in vitro and in vivo performed in these species (NTP, 1987)

Data in horses is scarce (Hinchcliff et al., 1991). However, a rapid elimination as that described in humans could be expected.

Considering its expected rapid elimination and the low oral bioavailability described in humans, it can be accepted that phenylephrine 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.

Timolol maleate is a nonselective beta-adrenergic blocking agent with associated intrinsic sympathomimetic activity and without significant membrane-stabilizing activity. The substance is on the EU market as a human medicinal product, alone or in combination with other active substances.

Like other topically applied ophthalmic agents, timolol is absorbed systemically in humans. It has been reported that approximately 80% of a dose topically administered in the eye is systemically absorbed (Volotinen et al., 2011). In one study with healthy human volunteers the systemic bioavailability of the ophthalmic eyedrops was 78.0 ± 24.5% indicating that caution must be observed when this drug is administered as it may be significantly absorbed causing systemic effects in the respiratory, cardiovascular, and central nervous systems, as well as in the gastrointestinal tract or even dermatologic effects (Korte et al., 2002; Volotinen et al., 2011). Peak plasma concentration after ophthalmic administration was 1.14 ± 0.34 ng/ml, achieved within 15 minutes following the administration. The mean area under the curve (AUC) was 6.46 ng/ml per hour after intravenous injection and 4.78 ng/ml per hour following ophthalmic administration. Mean of half-life elimination from plasma after ophthalmic administration was 3.11 ± 0.84 hours, noting in aged individuals the elimination half-life is increased (Korte et al., 2002).

The metabolism of timolol has not been extensively studied. Earlier investigators have suggested that, in humans, at least two major metabolites are formed by cleavage of the morpholine ring. Four different metabolites were produced by human liver homogenate (Volotinen et al., 2011). According to Tocco et al. (1975) timolol maleate is extensively metabolized in the liver by hydrolytic cleavage of the morpholino ring with subsequent oxidation; following oral dose, 80% is metabolized and 20% is eliminated in urine as parent compound.

The product information of human medicinal products indicates that due to its beta-adrenergic component similar adverse reactions as those seen with systemic beta-adrenergic blocking agents may occur. There are no adequate data for the use of timolol maleate in pregnant women; epidemiological studies have not revealed effects in reproduction but show a risk for intra uterine growth retardation when beta-blockers are administered by the oral route. Timolol is not considered as carcinogenic to humans according to the IARC classification.

Data regarding residue depletion in horse tissues are not available.

Considering the proposed use of the substance (i.e. topically applied to the eye) and its main pharmacokinetic characteristics as described above, it can be accepted that tropicamide 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.

Tropicamide is currently used in human medicine as topical formulations for pupillary dilation, either for diagnostic purposes or for any other ocular procedure. It works by non-selectively blocking muscarinic receptors to cause mydriasis and cycloplegia (NCBI, 2023b).

Tropicamide was observed to be rapidly absorbed systemically from the eye; peak tropicamide concentrations varied between 1.3 and 5.2 ng/ml in eight individual human patients 5-30 minutes after the last drop application. Mean peak concentration was 2.8 ± 1.7 ng/ml, detected 5 minutes after the application. Although no data on the elimination half-life in plasma is available, Vuori et al. (1994) reported that tropicamide disappears rapidly from the systemic circulation, with a concentration of 0.46 ± 0.51 ng/ml after 60 minutes, and below the detection limit at 120 minutes. Other authors report that tropicamide is a short-acting muscarinic receptor antagonist with a plasma half-life of approximately 30 minutes (Kilic et al., 2017).

The literature seems to agree in that tropicamide in the form of eye drops rarely causes adverse systemic reactions (Vuori et al., 1994; van Minderhout et al., 2015; Major et al., 2020).

Regarding toxicity studies, the acute toxicity by oral, intraperitoneal and subcutaneous administration in rodent species has been studied showing little toxicity, although there is limited information on tropicamide overdose (NCBI, 2023b).

Therefore, considering the proposed use of the substance (i.e. topically applied to the eye), the rapid absorption and elimination reported and the low systemic toxicity profile, it can be accepted that tropicamide 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.

4.11.3. Assessment of new substances proposed to be added to the list in the stakeholder's survey

A. Considerations on the essentiality of the substance(s)

Acetazolamide was mentioned (once) in the survey to stakeholders and it was suggested for addition to the list. No specific indication was mentioned by the responder.

It is a potent diuretic and carbonic anhydrase inhibitor whose indications include, in humans, glaucoma, idiopathic intracranial hypertension, congestive heart failure, altitude sickness, periodic paralysis, and epilepsy (Alberts et al., 2000; Farzam and Abdullah, 2023). In horses, it has been recommended as an ophthalmic medicine in animals with hyperkalemic periodic paralysis (Beech and Lindborg, 1995).

Published scientific literature indeed indicates that acetazolamide can be used for the treatment of hyperkalemic periodic paralysis in horses; however, results are somehow contradictory (Spier et al., 1990; Robertson et al., 1992). Its effect on fluid flux from the pulmonary vasculature in horses is well described in the literature (Vengust et al., 2006, 2010, 2013). With regards the use of acetazolamide in the treatment of glaucoma in horses, Tolar and Labelle (2013) indicated the efficacy of this drug in decreasing intraocular pressure is unknown; however, it is available as an oral formulation, which makes it advantageous for use in patients in which topical administration of a solution is not possible. However, data from current evidence in humans indicates that the use of locally acting carboanhydrase inhibitors has largely replaced use of systemic drugs; additive effects are not to be expected following

the simultaneous use of systemic and local carbonic anhydrase inhibitors. In addition, long-term use of acetazolamide is limited by adverse effects (e.g. electrolyte disturbances, paraesthesias, confusion in excessive diuresis) (Aslam and Gupta, 2023). Such evidence can be considered equally applicable to horses.

Glaucoma, if untreated, can be life-threatening and cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any acetazolamide-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substances dorzolamide and brinzolamide are better carbonic anhydrase inhibitor alternatives for the treatment of glaucoma. However, in view of their safety, these two cannot be proposed for inclusion in the list. Therefore, the substance acetazolamide is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: treatment of glaucoma, oral use. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Brinzolamide was mentioned (once) in the survey to stakeholders and it was suggested for addition to the list. No specific indication was mentioned by the responder. It is a carbonic anhydrase inhibitor with a well-established use for the treatment of open-angle glaucoma and ocular hypertension (Cvetkovic and Perry, 2003; Iester, 2008; Holló et al., 2015), both in humans and horses.

For the topical treatment of glaucoma in horses, published evidence suggests it exerts greater efficacy when compared to other carbonic anhydrase inhibitors (e.g. dorzolamide); it is often used in combination with other drugs, such as timolol (Germann et al., 2008; Holló et al., 2015). A meta-analysis of human data assessed the efficacy and safety of brinzolamide as add-on to prostaglandin analogues (PGAs) or β-blocker in treating patients with glaucoma or ocular hypertension who fail to adequately control intraocular pressure. Brinzolamide significantly decreased intraocular pressure of patients with refractory glaucoma or ocular hypertension and the adverse events were tolerable (Liu et al., 2019); brinzolamide and timolol were not significantly different and was as effective as dorzolamide in lowering intraocular pressure.

Glaucoma, if untreated, may cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any brinzolamide-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance brinzolamide is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: treatment of glaucoma, topical use. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Cyclopentolate was mentioned (once) in the survey to stakeholders and it was suggested for addition to the list. No specific indication was mentioned by the responder.

Cyclopentolate is an anticholinergic agent (a muscarinic receptor antagonist) commonly used for diagnosing and treating certain eye conditions, e.g. treatment of corneal ulcers and anterior uveitis, due to its cycloplegic and mydriatic effect (Contreras-Salinas et al., 2022); it has proven to have benefits over other available cycloplegic and mydriatic agents.

For mydriasis, atropine could be considered as an alternative treatment that it is included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010, with an entry that covers food-producing

horses and for which there are veterinary medicinal products authorised for food-producing species of the equine species. However, cyclopentolate use in horses (alone or combined with phenylephrine) has proved to induce significant mydriasis without affecting tear production, intraocular pressure, digestive function (i.e. gut motility and faeces production), or heart rate (HR) (Bessonnat and Vanone, 2021). This is considered to bring added clinical benefit.

Eye conditions, if untreated, may cause unacceptable suffering of the animal.

A search in the veterinary medicines database retrieves at least one cyclopentolate-containing veterinary medicinal product authorised for use in equine species (non-food-producing horses) containing cyclopentolate as hydrochloride for ocular use (also indicated for use in dogs and cats).

The substance cyclopentolate is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: as a mydriatic agent. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Pilocarpine was mentioned (once) in the survey to stakeholders and it was suggested for addition to the list. No specific indication was mentioned by the responder; no scientific references were provided.

Pilocarpine is a muscarinic acetylcholine agonist that induces both full and partial agonism of the muscarinic M3 receptor (Panarese and Moshirfar, 2023). In human medicine, it is used for the treatment and management of acute angle-closure glaucoma; in patients with open-angle glaucoma or ocular hypertension, although not a first-line treatment, it is useful as an adjunct medication in the form of ophthalmic drops for the reduction of elevated intraocular pressure (IOP). Despite contraindications and adverse effects, if used appropriately, pilocarpine is known to reduce intraocular pressure in glaucoma by 20 to 25%.

Though scarce, there are reports of its use for the reduction of IOP in horses (Smith et al., 1986; Van der Woerdt et al., 1998). However, alternatives with a better clinical profile (or with less side effects) have been proposed for addition to the list (i.e. phenylephrine and timolol maleate), and pilocarpine is not considered to bring added clinical benefit when compared to these.

Intraocular pressure, if untreated, causes unacceptable suffering of the animal.

A search in the veterinary medicines database retrieves at least three pilocarpine-containing veterinary medicinal products authorised for use in equine species (non-food-producing horses) containing pilocarpine as hydrochloride as diuretic. There are veterinary medicinal products authorised for use in species other than the equine (i.e. dog and cat).

The substance pilocarpine is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Synephrine was mentioned (twelve times) in the survey to stakeholders and it was suggested for addition to the list. No scientific references were provided. The ANSES opinion dated March 2016 was referred in support of this proposal, as well as a position paper from FEEVA and FVE (Federation of European Equine Veterinary Associations and Federation of Veterinarians of Europe) in support of the ANSES opinion. The responders stated this substance brings added clinical benefit due to its local, faster and more efficient effect compared to other anti-inflammatory systemic therapy options; it was

also stated that it provides synergistic effects with local antimicrobial therapy (due to enhances penetration of the drug in the local site).

Synephrine has three different isomeric forms, i.e. p-synephrine, m-synephrine, and o-synephrine; the major component of adrenergic agonists effect is p-synephrine, that is known for its longer acting effect compared to norepinephrine (Drugbank, 2024). It has to be noted that p-synephrine naturally occurs in citrus plants from the *Rutaceae* family, with the highest content being found in the fruitlets and fruit maturation resulting, generally, in a gradual decline in the p-synephrine content. It can also be found as an ingredient of herbal and nutritional supplements, and commonly found in the peel extract of bitter orange, which is used as a flavouring agent (Roman et al., 2007).

In human medicine, synephrine is used as a sympathomimetic agent for, among others, topical eye treatment due to its vasoconstrictor and decongestant properties. In horses it has similar use, though scientific reports supporting it are scarce. Despite the little literature available there seems to be sufficient clinical consensus regarding the added clinical benefit of synephrine for the treatment of the mucous membranes of the eye as a decongestant. Phenylephrine and tetryzoline are proposed for addition to the list. However, it is considered that synephrine brings added clinical benefit.

Congestive eye, if untreated, causes unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any synephrine-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance synephrine is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: for the treatment of the mucous membranes of the eye as a decongestant. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Tacrolimus was mentioned once in the survey to stakeholders and it was suggested for addition to the list for the treatment of immune mediated eye keratitis. The responder stated that tacrolimus' mechanism of action is similar to cyclosporin A, but more potent. No scientific references were provided.

Tacrolimus is a macrolide antibiotic with immunosuppressive properties. It has a similar mode of action to cyclosporin A and acts as a calcineurin inhibitor, exerting its effects through impairment of gene expression in target cells (Thomson et al., 1995). The overall effect is to inhibit cell-mediated immune response. It has a number of reported indications, mainly in transplant medicine, but topical application for treatment of conditions such as atopic dermatitis is also described. Its use in horses to treat canon hyperkeratosis (Hilton et al., 2008) and as an ophthalmic preparation (Gilger and Michau, 2004; González-Medina, 2018) has been reported. Cyclosporine A is an alternative treatment option already included in the list, so would be topical steroids, some of which have an entry in Table 1 of Commission Regulation (EU) No 37/2010 (e.g. dexamethasone, for which there are veterinary medicinal products authorised for food-producing equine species). The claimed additional potency of tacrolimus over cyclosporine A cannot be currently supported.

Immune-mediated keratitis, if untreated, may cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any tacrolimus-containing veterinary medicinal product authorised for use in equine (neither in other animal species).

The substance tacrolimus is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; satisfactory alternative

treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Tetryzoline was mentioned (thirteen times) in the survey to stakeholders and it was suggested for addition to the list for the local treatment of eye inflammation. No scientific references were provided. The ANSES opinion dated March 2016 was referred in support of this proposal, as well as a position paper from FEEVA and FVE (Federation of European Equine Veterinary Associations and Federation of Veterinarians of Europe) in support of the ANSES opinion.

Also known as tetrahydrozoline, is a derivative of imidazoline with central and peripheral alpha (α)-adrenergic properties (Daggy et al., 2003). It is a vasoconstrictor with decongestant effect used for antiseptic decongestant treatment of the mucous membranes of the eyes. It is also known to be used in horses due to its decongestant effect, thought scientific literature available is scarce (Wong et al., 2018). It is an effective alternative to phenylephrine for allergic alterations of the eye surface due to its fast local effect, which is considered an added clinical benefit.

Congestive eye, if untreated, causes unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any tetryzoline-containing veterinary medicinal product authorised for use in equine species. There are veterinary medicinal products authorised for use in species other than the equine (i.e. dog and cat).

The substance tetryzoline is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: for the treatment of the mucous membranes of the eye as a decongestant. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Knowledge regarding use of ophthalmic medicines in horses for this assessment was derived from textbooks and review articles; no clinical trials with horses were located.

B. Considerations regarding consumer safety

Acetazolamide is a sulfonamide active substance from the group of carbonic anhydrase inhibitors. Acetazolamide is used systemically as oral therapy for acute glaucoma in horses (acetazolamide is commonly available as tablets). Under certain circumstances, also intravenous administration may be used in horses. Topical application of the formulation without a special delivery system is not suitable due to the low corneal permeability of acetazolamide.

In humans, the systemic bioavailability seems to reach 100% after oral administration. The apparent volume of distribution is reported as 0.2 L/kg, the extent of protein binding in plasma is 93%, the plasma elimination half-life 3.5 hours and the fraction unchanged in urine 0.9 (Ritschel et al., 1998a). The results of an oral human study showed in the baseline group (N=12) peak plasma/whole blood concentrations after about 1-3 hours, 64.5 ± 8.2% binding to erythrocytes, an apparent volume of distribution (plasma) of 0.39 ± 0.06 L/kg (blood 0.3 ± 0.12 L/kg) and an elimination half-life in plasma of 6.0 ± 0.9 hours (blood 12.0 ± 3.0 hours) (Ritschel et al., 1998a). Reported also by other authors is: a rapid absorption (Tsikas, 2024) and a high protein binding (Chapron et al., 1985; Yano et al., 1998; Tsikas, 2024); half-lives of 2.5 hours in plasma/blood after single intravenous administration (Chapron et al., 1985; cited by Tsikas, 2024), of 4- 10 hours in plasma/blood after single oral administration

(Yakatan et al., 1978; Hossie et al., 1980; Straughn et al., 1982; Yano et al., 1998; Shukralla et al., 2022; cited by Tsikas, 2024) and up to 24 hours in plasma/blood after single oral administration (Hampson et al., 2016); elimination of virtually unchanged acetazolamide via urine (Shukralla et al., 2022; Tsikas, 2024); and a low apparent volume of distribution (Yano et al., 1998).

After ingestion of a 250 mg acetazolamide-containing tablet the concentrations in urine were stable at least up to five hours in one healthy volunteer, which is in accordance with other reports that showed a long-lasting excretion of acetazolamide (Begou et al., 2020). The human therapeutic dose in the circulation is also reported to be rather stable for at least up to 10 hours in a dose study with 17 patients (Yano et al., 1998). A mean residence time in the body was calculated to be 19.6 ± 4.4 hours (whole blood) or 9.8 ± 1.1 hours (plasma) in a baseline group (Ritschel et al., 1998a). When Ritschel et al. (1998b) followed the urinary excretion for 36 hours, they revised their previous result of half-life of 3.5 hours, i.e. determined it to be rather 9 to 12 hours (in urine). Also, other authors reported half-lives in urine after oral ingestion between 2 to 9 hours (Chapron et al., 1985; Campíns-Falcó et al., 1994; Busardò et al., 2022; cited by Tsikas, 2024) and up to 16 hours (Hampson et al, 2016; cited by Tsikas, 2024).

Only recently, metabolites were detected in plasma and urine such as from hepatocyte incubations and after glutathione conjugation, glucuronidation, and N-acetylation. Metabolites were detected in plasma 1.5 h after intake and in urine from 0.25 to 24 h after intake (Busardò et al., 2022; cited by Tsikas, 2024).

In erythrocytes, higher concentrations of acetazolamide were measured compared to plasma after oral or intravenous administration (Chapron and White, 1984; Ritschel et al., 1998a). The longest half-life in plasma is reported to be 24.5 ± 5.6 hours in 10 volunteers that received 15 mg oral acetazolamide for 4 consecutive days. Only in this study, the erythrocyte sequestration (i.e. binding to erythrocytes resulting in an acetazolamide microdose), was considered; it was reported as the cause for the observed longer plasma half-life compared to other studies using a similar therapeutic dose (i.e. single 250 mg acetazolamide tablet). This phenomenon was particularly evident when the cumulative red blood cell exposure on day 5 was compared with that after residual subtraction (133,107 h*ng/mL blood versus 49,555 h*ng/mL blood). The elimination half-life from the red blood cells was 50.2 ± 18.5 hours (N = 6) (Hampson et al., 2016). However, it has to be noted that plasma protein binding may be lowered in aged individuals with stronger erythrocyte accumulation of acetazolamide. Advanced renal failure can be followed by plasma half-lives prolonged in the range of 26 hours (Roy et al., 1992; Kassamali and Sica, 2011). Overall, although acetazolamide readily binds to the erythrocytes, this binding did not appear to be very persistent because the half-lives in humans from red blood cells, plasma and blood were in the magnitude of hours and of maximum a few days, respectively, and there were no signs of prolonged accumulation in the human body.

No residue depletion data were found for acetazolamide administrated in horses or in closely related species of the *Equidae* family nor in other species.

Acetazolamide is suspected of damaging the unborn child based on skeletal malformations observed in rats (Layton and Hallesy, 1965; Wilson et al., 1968). In an oral study with a diet with acetazolamide at concentrations of 0, 0.2, 0.4, or 0.6% acetazolamide (0, 115, 208, 284 mg/kg bw/d) during pregnancy until parturition, out of 187 offspring of mothers in the groups with 0.4 or 0.6% acetazolamide, 45 showed a right-sided forelimb deformity (Layton and Hallesy, 1965). The developmental NOAEL for malformations in this study could have been reported as 115 mg/kg bw/d. The same malformations were observed by Wilson et al. (1986) at 0.3% or 0.6% acetazolamide fed in diet, but this study lacking a lower dosage group and control. The malformations observed could be confirmed by Dodo et al. (2010). Pregnant rats (N=8/group) were orally administered by gavage with 0, 200, 400,

800 mg/kg bw/d during gestation days 10 and 11. No maternal deaths or abnormal clinical signs, and no effects on post implantation loss or the number of live fetuses at any dose were observed; body weight gains were suppressed in dams and fetuses. Treatment with acetazolamide at 400 and 800 mg/kg bw/d increased (on a dose-dependent fashion) the numbers of fetuses with external malformations, i.e. ectrodactyly and polydactyly, mainly in the right hand. Acetazolamide was also considered to potentially induce rib malformations (Dodo et al., 2010). Thus, the developmental NOAEL for malformations in this study could have been reported as 200 mg/kg bw/d. Overall, the dose levels at which malformations were observed are noted to be extremely higher than those of human clinical use. Further, acetazolamide was used in recent studies examining its potential effects and its mechanism in several animal species (e.g. recently by Cappon et al. (2005)). For human medicinal products the use during pregnancy is not permitted, especially during the first trimester as the products can affect the fetus. An effect on male fertility was observed in animal studies.

Considering its pharmacokinetics in humans it can be considered that residues in horse would be unlikely after six months; therefore, it can be accepted that acetazolamide 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.

Brinzolamide is a carbonic anhydrase inhibitor with a well-established use for the treatment of open-angle glaucoma and ocular hypertension (Cvetkovic and Perry, 2003; Iester, 2008; Holló et al., 2015), both in humans and horses.

Studies in rabbits, monkeys and humans have shown that topically applied brinzolamide is absorbed from the eye into the systemic circulation, with a 70% absorption reported in rats (Hall et al., 1999). After topical ocular administration in rabbits, brinzolamide has shown to distribute to all eye tissues. Peak concentrations of brinzolamide in anterior segment tissues was reached within 0.5 – 2 h. Clearance from the aqueous humour and cornea took place with half-lives of 3 and 5 h, respectively, with the cornea acting as a reservoir (sustained release to the ciliary processes) (DeSantis, 2000).

Brinzolamide also extensively distributes to blood; Hall et al. (1999) reported several µg/ml after repeated dosing. It binds to carbonic anhydrase (CA) in the erythrocytes, generally resulting in low plasma levels but a long systemic half-life (Iester, 2008). This has been demonstrated in 15 normal volunteers receiving a 3% brinzolamide ophthalmic suspension three-times daily to both eyes for 14 days. Upon discontinuation of treatment, the mean terminal half-life in whole blood was approximately 111 days (Shoji, 2007). The information in human medicinal products state an average half-life of around 24 weeks (168 days) for brinzolamide.

Since binding to erythrocyte CA is saturable, whole-blood pharmacokinetics of brinzolamide (in contrast to plasma) are nonlinear (Hall et al., 1999). Naageshwaran et al. (2021) showed ratios of about 1:31:1196 for mean concentrations of brinzolamide in aqueous humor and blood plasma (<2 ng/ml, respectively) versus mean concentrations in the iris-ciliary body (ICB; 78,7 ng/g) versus mean concentrations in whole blood (2990 ng/ml) at 336 h after intravenous application of 0.75 mg/kg in rabbits; the plasma elimination half-life was > two weeks. Suzuki et al. (2021) found a T_{max} of 24 h, a C_{max} of 1.62. ± 0.36 µg/ml, and an $AUC_{0\text{-}672}$ of 589 ± 101 µg/ml/h in blood following single ocular application of 30 µl 1.0 % brinzolamide suspension in rabbits using a human formulation. In vitro protein-binding in human plasma was in the range of 59-63% (Hall et al., 1999); in rabbits it was 47-57% (Naageshwaran et al., 2021).

Excretion of the majority of absorbed brinzolamide takes place unchanged via urine. Metabolism is primarily hepatic via oxidative o- and n-dealkylation. Three active metabolites have been documented in rats: n-desmethoxypropyl-brinzolamide, o-desmethyl-brinzolamide and n-desmethyl-brinzolamide.

N-desethyl-brinzolamide and o-desmethyl-brinzolamide are found in cynomolgus monkeys' whole blood after topical application; in humans, n-desethyl-brinzolamide were seen to accumulate in erythrocytes to steady-state levels (range of 5-25 µM), while n-desmethoxypropyl-brinzolamide and o-desmethyl-brinzolamide were present in urine (DeSantis, 2000).

Tissue distribution of brinzolamide has been described in rats (1 mg/kg 14C-brinzolamide PO), with prolonged retention in tissues containing CA (the concentration of radioactivity in tissues was higher than in plasma). Highest radioactivity concentrations (C_{max} values) were observed in tissues with high content of CA (liver, kidneys, spleen, stomach, small intestine, lungs, and salivary glands), while levels in fat, skeletal muscle, skin, eyes, and testes were low. Tissue half-life ranged up to about 33 days and were generally similar to those of blood (approximately 20 days) (Hall et al., 1999). Whole-body autoradiography in rats showed that low levels of radioactivity crossed the blood-brain barrier (Alcon Research Ltd., cited by DeSantis, 2000).

Germann et al. (2008) monitored the effect of topical administration of 1% brinzolamide on the intraocular pressure in clinically normal horses. IOP decrease was noted in both the treated and the contralateral untreated eye (as it has been previously described for dorzolamide in horses and for MK-927 in dogs) (King et al., 1991; Willis et al., 2001). This confirms systemic absorption of brinzolamide also takes place in horses, even if no analysis of blood levels or urinary excretion is available further describing the systemic distribution of the drug (Germann et al., 2008).

No studies on systemic kinetics of brinzolamide following ocular application in horses were found. No residue depletion data for horses could be found either.

The most common ocular side effects in humans were summarized by March and Ochsner (2000), and included (among others) blurred vision, pain, discomfort, hyperemia, discharge, dry eye, blepharitis and keratitis, while the most common non-ocular event being taste perversion. As complete saturation of CA in erythrocytes is not achieved (due to low plasma levels of brinzolamide despite the high degree of systemic bioavailability) systemic acidosis or other common side effects associated with oral CAIs are not expected to occur (DeSantis, 2000). Brinzolamide forms an aqueous suspension at pH 7.4, leading to less ocular discomfort than, e.g. dorzolamide (acidic pH) (Eller et al., 1985; March and Ochsner, 2000). The minimum dose with a significant pharmacological effect assumed to be without side effects was reported to be a 0.3 % solution (Silver, 2000)

Published peer-reviewed literature on brinzolamide toxicity is scarce. From the SPC of human medicinal products it is stated that brinzolamide showed no hazard from single and repeated dose toxicity studies, genotoxicity, carcinogenicity, developmental toxicity and teratogenicity and topical ocular irritation studies. According to the classification provided by companies to ECHA in CLP notifications brinzolamide is harmful if swallowed (ECHA, 2024). Brinzolamide is not listed by the IARC or NTP. Its non-mutagenicity is consistent with that reported for other carbonic anhydrase inhibitors.

From the available data is worth noting that in a chronic carcinogenicity study in mice, tumours and dose-related proliferation changes in the urinary bladder were deemed not relevant for humans and considered unique to mice (Chandra and Frith, 1991; Friedrich and Olejniczak, 2011). And with regards TRVs, data from human medicinal products retained 2 mg/kg bw/day as maternal and foetal NOAELs from an embryofetal development study in pregnant rats (dose-related decreases in foetal weights were observed). In rabbits, however, the lowest maternal NOAEL was set at 1 mg/kg bw/day due to weight loss in the developmental study (mortality, emaciation and abortions were seen at the highest dose tested, i.e. 6 mg/kg bw/day).

Similarly to dorzolamide, an estimation of the possible presence of residues after the six-month withdrawal period was conducted (see section 7.2.3 in the annex for further details). No

pharmacokinetic information or depletion studies are available in horses. The available knowledge from humans indicates that brinzolamide accumulates in the red blood cells and can produce side effects, and that even low concentrations in plasma have a long elimination half-life close to the proposed six-month withdrawal period in the horse. The estimation was conducted under the assumption of an even distribution of residues in the tissues and considering a worst-case exposure scenario. Several assumptions had to be made due to the lack of data in horses, noting the estimate presented considers the treatment of a single eye (monoliteral glaucoma) since it is the most common presentation of glaucoma according to the clinical experts consulted; however, bilateral glaucoma cannot be excluded which would double the exposure estimate, thus reducing the margin of exposure calculated. Different toxicity reference values were considered, either from the available developmental toxicity studies in rats and rabbits reported above or from human therapeutic doses, considering those ensuring an absence of side effects. Similarly, different absorption factors were considered. The overall estimation results in a MoE (around 30) that is not sufficient to exclude a risk for consumers due to the presence of residues that could inhibit CAII in red blood cells after a six-month withdrawal period in the horse.

Considering the available information and the long half-life (≥ 168 days) in humans, in the absence of pharmacokinetic information or depletion data in horses and using the lowest toxicological reference value (TRV) based on the human therapeutic use (see proposed worst-case scenario in the annex) not enough margin of safety (MoE) is achieved for brinzolamide when used as proposed. The resulting MoE only just reaches the required MoE and an underestimation of the exposure proposed cannot be excluded, even when based on a worst-case residue depletion with available data so far. Therefore, it can be concluded that brinzolamide can pose an unacceptable risk for consumers when used in food-producing animals of the equine species and a six-month withdrawal period is respected.

Cyclopentolate is an anticholinergic agent (a muscarinic receptor antagonist) commonly used for diagnosing and treating certain eye conditions. It has an octanol/water coefficient of logP 2.4 and a water solubility of 1.5 mg/mL (Drugbank, 2024).

This substance is generally used as cyclopentolate hydrochlorid in medicinal products. Is rapidly absorbed into the systemic circulation following ophthalmic administration (Salminen, 1990; Drugbank, 2024). Studies in human adults and infants are available and show variable peak plasma concentrations after administration of one or two 1% cyclopentolate drops (equal to 30-35 µl per drop); concentrations in adults ranged 2-15.5 ng/ml up to 30 minutes after administration (Kaila et al., 1989; Lahdes et al., 1993; Haaga et al., 1998) and were higher in infants, reaching 53 ng/ml (Lahdes et al., 1990; Mitchell et al., 2016). The elimination half-life observed in a study involving eight adults ranged from 49-266 min, with a mean of 111 min (Lahdes et al., 1993).

In neonatal mice, following administration of 3 µl 0.5% cyclopentolate, serum concentration of cyclopentolate reached 86 ng/ml 30 minutes after administration, 60 ng/ml one hour after administration, and 6.2 ng/ml 24 hours after administration (Rozette et al., 2014). No pharmacokinetic data in the equine species is available.

According to the classification provided by companies to ECHA in REACH registrations this substance is harmful if swallowed, is harmful in contact with skin and is harmful if inhaled. There is data lacking for other endpoint's classification (ECHA, 2024). The publicly available toxicity data is scarce. Rozette et al. (2014) noted in an animal model (neonatal mice) reduced weight gains after a single ophthalmic dose of one drop of 0.5% cyclopentolate (approximately 3 µl) on days 3 and 7. From human medicinal products, a risk of serious systemic side effects in young children due to overdosing is noted, mainly affecting the cardiopulmonary and central nervous systems. In fact, Mitchell et al. (2016) observed high systemic levels in preterm infants. Cyclopentolate has not been assessed by IARC for its carcinogenicity classification (IARC, 2024).

No residue depletion studies in horses or other species are available in the public literature.

Considering the rapid elimination noted from human data, it can be accepted that cyclopentolate 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.

Synephrine (p-syneprhine) is a naturally occurring proto alkaloid that has structural similarities to ephedrine and adrenaline. It is widely used as an ingredient of dietary supplements (DS) and specialized foodstuffs (SF) intended for weight loss and fitness improvement in humans. Along with thermogenic and lipolytic effects, synephrine can cause cardiovascular side effects, especially when combined with caffeine and physical activity (BfR, 2012; Perova et al., 2021; EFSA, 2021).

Synephrine is a weak adrenergic agonist, acting primarily through β3-adrenergic receptors (which stimulates lipolysis). Its adrenergic effect is caused by the presence of R-(-)-p synephrine, that can make up about 90% or more of the total proto alkaloids in e.g. bitter orange.

The consumption of this naturally occurring proto alkaloid is regulated in some regions (e.g. Russia), with permissible levels up to 30 mg per day (Perova et al., 2021). In the EU no exposure data are available on the intake of dietary or supplemental synephrine. French consumption data showed that the dietary p-synephrine intakes are 6.2 mg/day on average and 20.0 mg/day at the 95^{th} percentile for the maximum levels (ANSES, 2014). German consumption data revealed that the dietary synephrine intakes are 0.88-6.73 mg per day for average consumers and 6.62-25.82 mg per day for high consumers (BfR, 2012).

In their opinion from 2012, the BfR (Bundesinstitut fur Risikobewertung, German Federal Institute for Risk Assessment) recommends that no more than 6.7 milligrams per day should be consumed in the form of a synephrine food supplement. This quantity represents the average intake via conventional foods with maximum contents of synephrine and would ensure, even for frequent consumers, that their total intake of synephrine from both conventional foods and food supplements does not exceed 25.7 milligrams.

The toxicological data on synephrine are limited. Data on chronic toxicity are lacking.

No information is available on pharmacokinetics or residue depletion studies in horses. Considering the proposed used (topical ophthalmic application), a worst-case treatment schedule of 2 eye drops, 6 times a day for 10 days, and the average amount of synephrine (as tartrate) contained in ophthalmic preparations (generally 1.2 mg synephrine per ml), it is anticipated that the amount of substance to which a consumer could be exposed if the whole dose was to be ingested would still be in the order or micrograms, whereas the daily limit stablished by BfR is in the order of milligrams.

Considering the proposed use in horses, noting the substance is a naturally occurring proto alkaloid used as food supplement in humans, it can be concluded that synephrine 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.

Tetryzoline (or tetrahydrozoline) is a selective alpha-1 adrenergic receptor agonist that plays an important pharmacological role as nasal drops in the symptomatic treatment of rhinitis in humans (Hosten and Snyder, 2020). It is authorized for use in human medicinal products in the EU.

When consumed orally, tetryzoline is rapidly absorbed from the gastrointestinal tract and can cross the blood-brain barrier (Stillwell and Saady, 2012). Following ocular administration of 0.05% tetryzoline, the mean serum half-life was approximately 6 hours. Systemic absorption varied among subjects, with the maximum serum concentrations ranging from 0.068 to 0.380 ng/ml. At 24 hours, all patients had

detectable urine concentrations of tetrahydrozoline at a range 13 – 210 ng/ml (Carr et al., 2011). A case of accidental paediatric oral exposure with resultant neurological and cardiovascular complications is described in the literature (Daggy et al., 2003).

Regarding toxicity, toxicological studies in rats and mice have been performed (Strey, 2020). Tetryzoline is not considered as carcinogenic to humans according to the IARC classification.

No pharmacological data in horses are available. The opinion of the ANSES (French Agency for Food, Environmental and Occupational Health & Safety) was noted, where a worst-case consumer exposure estimation is proposed (ANSES, 2016). While the proposed estimation does not account for the six-month withdrawal period proposed for substances in the list, it concludes that a risk for consumers is not to be anticipated.

Considering the use of the substance in horses (i.e. topically applied to the eye), the pharmacological and toxicological data available from humans, and that a withdrawal period of six months will have to for substances included in the list, it can be accepted that tetryzoline 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.

4.11.4. Conclusion

Based on the above assessment and justifications, the following recommendations are proposed:

1. The following active substances, currently in Regulation (EU) 1950/2006 as amended by Regulation (EU) 122/2013, are proposed to be retained in the list, either without modification or with an amendment of the current entry as shown below:

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Cyclosporine A	for the treatment of autoimmune diseases of the eye	topical steroids	immunosuppressive effect by inhibiting T-lymphocyte proliferation and reducing cytokine gene expression
Phenylephrine	treatment of glaucoma and epiphora	atropine and tropicamide	it does not (or only slightly) increase intra ocular pressure
Timolol maleate	treatment of glaucoma, topical use	acetazolamide	its specific mode of action as a non-selective beta-adrenergic receptor blocking agent, provides for an important therapeutic choice in the treatment of glaucoma
Triamcinolone acetonide[50]	treatment of recurrent uveitis in cases that are refractory to other treatments	atropine, tropicamide	effective, low morbidity treatment in cases refractory to other treatments
Tropicamide	treatment of recurrent uveitis	atropine, cyclopentolate, triamcinolone acetonide	rapid onset of action

2. The following active substances, currently in Regulation (EU) 1950/2006 as amended by Regulation (EU) 122/2013, are proposed to be removed from the list: dorzolamide, latanoprost.

3. The following active substances, suggested for addition to the list in the survey to stakeholders, are proposed to be added to the list with an entry as shown below:

[50] This substance is discussed in detail in section 4.2 (analgesics).

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Acetazolamide	treatment of glaucoma, oral use	phenylephrine	its mechanism of action as carbonic anhydrase inhibitor
Cyclopentolate	mydriatic agent	atropine, phenylephrine	induces significant mydriasis without affecting tear production, intraocular pressure, digestive function (i.e. gut motility and faeces production), or heart rate
Synephrine	for the treatment of the mucous membranes of the eye as a decongestant	phenylephrine, tetryzoline	fast local effect; may enhances penetration local therapy, providing synergistic effects with e.g. local antimicrobial therapy
Tetryzoline	for the treatment of the mucous membranes of the eye as a decongestant	phenylephrine, synephrine	fast local effect

4. The following active substances, suggested for addition to the list in the survey to stakeholders, are not proposed for inclusion: brinzolamide, pilocarpine, tacrolimus.

4.12. Substances for sedation and premedication (and antagonism)

4.12.1. Overview

	Active substance (ATCvet code)
Substances in Regulation (EU) 1950/2006 **proposed to be retained**, with or without amendment of the current entry	Acepromazine (QN05AA04); Atipamezole (QV03AB90); Diazepam (QN05BA01); Flumazenil (QV03AB25); Naloxone (QV03AB15); Propofol (QN01AX10)
Substances in Regulation (EU) 1950/2006 **proposed to be removed**	Midazolam (QN05CD08); Sarmazenil (QV03AB91); Tiletamine (QN01AX99); Zolazepam (QN01AX99)
Substances from stakeholders' survey **proposed for inclusion**	Dexmedetomidine (QN05CM18)
Substances from stakeholders' survey **not proposed for inclusion**	Medetomidine (QN05CM91); Vatinoxan (no ATCvet code identified)

4.12.2. Review of the existing entries in Regulation (EU) 1950/2006, as amended by Regulation (EU) 122/2013, considering the survey results

A. Considerations on the essentiality of the substance(s)

Acepromazine is a phenothiazine derivative psychotropic drug with strong antiadrenergic activity. Acepromazine acts as an antagonist on different postsynaptic receptors: on dopaminergic-receptors (subtypes D1, D2, D3 and D4) and on serotonergic-receptors (5-HT1 and 5-HT2). It is a potent neuroleptic and sedative with antiemetic, anticholinergic, slight antihistaminergic and antiserotonergic properties (Alef and Oechtering, 2003). It also causes spasmolytic effects on smooth muscles and intestines (Hall et al., 2001).

It is listed In Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for premedication prior to general anaesthesia and mild sedation. Alternatives that are identified in the current list include detomidine, romifidine, xylazine, diazepam, and midazolam. The specific advantages captured are that acepromazine has consistently been shown to reduce risk of anaesthetic

death, and that its mode of action (on limbic system) and unique quality of sedation cannot be produced by the alpha-2 agonist sedatives (detomidine, romifidine and xylazine) or benzodiazepines (diazepam, midazolam).

With regards to the above-mentioned alternatives, it is noted that detomidine, romifidine and xylazine have an MRL assessment and are included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010, whereas diazepam and midazolam are not; there are veterinary medicinal products authorised for use in food-producing animals of the equine species containing detomidine, romifidine and xylazine, as sedatives, among other indications. However, it is agreed that the mode of action of acepromazine and its unique quality of sedation cannot be produced by alpha-2 agonist sedatives; it is an anxiolytic that is used as part of premedication for general anaesthesia, rather than as sedative. Thus, it is proposed to adjust the indication for a multimodal approach for sedation and premedication in combination with other sedatives.

The substance is not intended for the treatment of a specific condition. However, failure to adequately anesthetise the animal could cause unacceptable suffering of the animal and may be life-threatening.

Acepromazine was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database retrieves several acepromazine-containing veterinary medicinal products authorised for use in equine species (non-food-producing horses. There are veterinary medicinal products authorised for use in species other than the equine (i.e. cat and dog).

The substance acepromazine is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indications: for a multimodal approach for tranquilisation and premedication in combination with other sedatives. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Atipamezole is a synthetic α2-adrenoceptor antagonist used to reverse the sedative and analgesic effects of medetomidine, dexmedetomidine and xylazine (Hall et al., 2001). It cancels sedative and analgesic effects and adverse cardiovascular reactions (Vähä-Vahe, 1990).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for reversal of α-2 agonists. No alternatives are identified in the current list, and the specific advantages captured for the substance are that it is the only treatment for hypersensitive individuals and overdose, and that it is an emergency medicine and specifically used in cases of respiratory depression.

Atipamezole is indeed the only substance authorised as veterinary medicinal product (for species other than horses) to antagonize sedation with medetomidine, dexmedetomidine and xylazine.

The substance is not intended for the treatment of a specific condition. However, failure to adequately reverse the effects of α2 agonists may be life-threatening and cause unacceptable suffering of the animal.

Atipamezole was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any atipamezole-containing veterinary medicinal product authorised for use in equine species. There are veterinary medicinal products authorised for use in species other than the equine (i.e. dog and cat).

The substance atipamezole is proposed to be qualified as essential because no satisfactory alternative substances are authorised for food-producing animals of the equine species for the following indication: for reversal of α-2 agonists.

Diazepam is a benzodiazepine with anxiolytic, anticonvulsant, sedative, hypnotic, and muscle relaxant properties as well as a long duration of action and few cardiopulmonary adverse effects (Riebold et al., 1995). Its actions are mediated by enhancement of gamma-aminobutyric acid (GABA) activity by increasing its inhibitory action at its receptor site (GABA) (Mason, 2004).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for premedication and induction of anaesthesia, for mild (benzodiazepine) tranquilisation with minimal cardiovascular and respiratory side effects, and as anti-convulsant, essential for treatment of seizures. Alternatives that are identified in the current list include acepromazine, detomidine, romifidine, xylazine, midazolam,(for premedication and induction of anaesthesia and mild tranquilisation). The specific advantages captured are as follows: in modern medicinal standards it is an essential component of anaesthetic induction protocols with very considerable equine experience; it is used with ketamine for induction of anaesthesia, producing essential relaxation that allows smooth induction and intubation; its mode of action (acts at GABA receptor) and unique tranquilisation without cardiorespiratory depression cannot be produced by the α-2 agonist sedatives (detomidine, romifidine and xylazine) or acepromazine.

Diazepam is largely used in equine practice in combination with ketamine and xylazine hydrochloride for induction of anaesthesia and alone as a sedative/ataractic/neuroleptic (Shini, 2000). As stated above, there are alternatives available in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with an entry for *Equidae* and veterinary medicinal products authorised for food-producing animals of the equine species; however, its mode of action at the GABA receptor level and unique tranquilisation without cardiorespiratory depression cannot be produced by alternatives, particularly α-2 agonists.

The substance is not intended for the treatment of a specific condition. However, failure to adequately anesthetise the animal could cause unacceptable suffering of the animal and may be life-threatening .

Diazepam was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any diazepam-containing veterinary medicinal product authorised for use in equine species. There are veterinary medicinal products authorised for use in species other than equines (i.e. dog and cat).

The substance diazepam is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: for premedication and induction of anaesthesia, mild tranquilisation with minimal cardiovascular and respiratory side effects. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal. Diazepam is also proposed as a short-term anticonvulsant for treatment of seizures. Please refer to section 4.11 for further details.

Flumazenil is a benzodiazepine antagonist that is used for the complete or partial reversal of the sedative effects caused by benzodiazepines (e.g. diazepam) in various clinical settings, such as induced general anaesthesia for diagnostic and therapeutic procedures (Plumb, 2002; Muir, 1991).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) as an intravenous reversal agent for benzodiazepines and reversal of benzodiazepine effect during recovery from Total Intravenous Anaesthesia (TIVA) techniques. The only alternative identified in the current list is sarmazenil, and the specific advantages for the substance are its differing mode of action as compared to sarmazenil providing additional means of benzodiazepine reversal at the end of TIVA techniques; sarmazenil is a partial inverse agonist of benzodiazepine receptors whereas flumazenil is an antagonist competitively inhibiting the benzodiazepine binding site at the GABA receptor.

Before the introduction of flumazenil, compounds such as theophylline derivatives and anticholinesterases were used in an attempt to reverse benzodiazepine's effects. Flumazenil does result in benzodiazepine reversal at the end of e.g. TIVA techniques and provides a better means of benzodiazepine reversal by competitively inhibiting the benzodiazepine binding site at the GABA receptor, when compared to sarmazenil. In addition, the comment on sarmazenil from the survey to stakeholders is noted (see 'sarmazenil' below). It should be noted that TIVA involves more active substances than benzodiazepines, and thus flumazenil is only a reversal agent for the benzodiazepine portion of the TIVA.

The substance is not intended for the treatment of a specific condition. However, failure to adequately reverse the effects caused by benzodiazepines could cause unacceptable suffering of the animal and may be life-threatening.

Flumazenil was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any flumazenil-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance flumazenil is proposed to be qualified as essential because no satisfactory alternative treatments are authorised for food-producing animals of the equine species for the following indication: intravenous reversal agent for benzodiazepine effect during recovery from Total Intravenous Anaesthesia (TIVA) techniques.

Midazolam 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).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for premedication and induction of anaesthesia, mild (benzodiazepine) tranquilisation with minimal cardiovascular and respiratory side effects, and as anti-convulsant for treatment of seizures, particularly in adult horses with tetanus. Alternatives that are identified in the current list include acepromazine, detomidine, romifidine, xylazine, diazepam, primidone, and phenytoin. The specific advantages listed are as follows: it is similar to diazepam, but water soluble, and thus suitable for intravenous injection and essential for intravenous infusion in combination with anaesthetics; it is shorter acting than diazepam and more suitable than diazepam for foals; as an anti-convulsant for treatment of seizures, particularly in adult horses with tetanus, it is better than diazepam for use over several days due to its water solubility; it is used with ketamine for induction of anaesthesia, producing essential relaxation that allows smooth induction and intubation; its mode of action (acts at GABA receptor) and unique tranquilisation without cardiorespiratory depression cannot be produced by the α-2 agonist sedatives (detomidine, romifidine and xylazine) or acepromazine.

These advantages for midazolam have been recently discussed in the literature with results differing from those cited above (Jarrett et al., 2018). Data from clinical trials suggests that for horses undergoing short (i.e. 60 minutes) periods of general anaesthesia, recovery quality may be better following induction with propofol and ketamine, compared with midazolam and ketamine. It is noted that there are alternatives available for premedication, sedation, or as anti-convulsant for food-producing horses (see below).

The substance is not intended for the treatment of a specific condition. However, failure to adequately anesthetise the animal or treat seizures could cause unacceptable suffering of the animal and may be life-threatening.

Midazolam was mentioned (once) in the survey to stakeholders, proposing a modification to the entry as presented in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013). The responder suggests the indications be premedication, induction and maintenance of anaesthesia, muscle relaxation and treatment of seizures. For the information on use and purpose two are proposed: (i) for mild (benzodiazepine) tranquilisation with minimal cardiovascular and respiratory side effects, and (ii) as anti-convulsant, for treatment of seizures, particularly in adult horses with tetanus. With regards to alternative treatments, guaifenesin is mentioned. For the specific advantages, it is proposed to add that it can be given intramuscularly as replacement of guaifenesin in injection anaesthesia protocols.

A search in the veterinary medicines database retrieves one midazolam-containing veterinary medicinal product authorized for use in equine species (non-food-producing horses) containing midazolam (neither in other animal species). Alternative active substances for premedication and/or induction of anaesthesia, or treatment of seizures, e.g. detomidine and xylazine, are listed in Table 1 of Regulation (EC) No 37/2010, with MRL entries for *Equidae* and veterinary medicinal products authorised for food-producing animals of the equine species. These substances are considered satisfactory alternative treatments for food-producing animals of the equine species. Moreover, it is 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.

The substance midazolam is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Naloxone acts as a competitive antagonist by binding to μ, δ, and κ opioid receptors, with its greatest affinity to μ receptors (Freise et al., 2012). As an opioid receptor antagonist, it rapidly blocks or reverses the effects of opioid drugs (Lynn and Galinkin, 2018).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) as an opioid-antidote in emergency medicine. No alternatives are identified in the current list; the lack of alternatives is indicated as the specific advantage of this substance.

Naloxone is the only substance available to antagonize the effects of opioid drugs.

The substance is not intended for the treatment of a specific condition. Failure to adequately reverse the effects of opioids may be life-threatening and cause unacceptable suffering of the animal.

Naloxone was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any naloxone-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance naloxone is proposed to be qualified as essential because no satisfactory alternative treatments are authorised for food-producing animals of the equine species for the following indication: for the reversal of opioid effects during emergencies.

Propofol belongs to the group of alkyl phenols. It is a short acting hypnotic drug, with little or no analgesic properties and is not related to any other anaesthetics (Reves et al., 2000; Plumb, 2002). Its mechanism of action is not understood completely, but propofol interacts with the GABA receptor chloride ionophore complex (Reinhold et al., 1998). Propofol is thought to enhance the effect of the

natural ligand GABA on its receptor via allosteric binding to the β subunit of chloride channels. Thus, postsynaptic influx of chloride is increased causing hyperpolarization and reduction of excitability of neurons (Thurmon et al., 1996).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) as an intravenous anaesthetic, for induction of anaesthesia in foals. Alternatives that are identified in the current list include sevoflurane or isoflurane. The specific advantages listed are that it is a rapidly cleared injectable anaesthetic, and that reports had demonstrated a vast improvement in cardiovascular stability and quality of recovery over inhalation anaesthesia.

Propofol boluses are effective as an adjunct for maintaining a surgical plane of anaesthesia or recumbency with a quick recovery and minimal ataxia (Bidwell, 2012). Propofol appears to be safe as an anaesthetic adjunct in healthy foals (Bidwell, 2012).

The substance is not intended for the treatment of a specific condition. However, failure to adequately anesthetise the animal could cause unacceptable suffering of the animal and may be life-threatening.

Propofol was mentioned (once) in the survey to stakeholders, proposing a modification to the entry as presented in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013). The responder suggests the specific mention to induction "in foals" be removed (i.e. proposing a general indication for induction of anaesthesia). However, there are other options for the induction of anaesthesia in equines, other than foals, listed in Table 1 of Regulation (EC) No 37/2010 with an MRL entry for *Equidae* and veterinary medicinal products authorised for food-producing animals of the equine species (e.g. isoflurane); the amendment of the indication as proposed it is not considered appropriate – propofol brings added clinical benefit in foals.

A search in the veterinary medicines database does not retrieve any propofol-containing veterinary medicinal product authorised for use in equine species. There are veterinary medicinal products authorised for use in species other than the equine (i.e. dog and cat).

The substance propofol is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: as an intravenous anaesthetic, for induction of anaesthesia in foals. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Sarmazenil is a benzodiazepine antagonist. Its effects are generally compared to those of flumazenil; however, sarmazenil does not exert any inherent activity (Kaegi, 1990; Erhardt 2004). It competitively blocks the action of benzodiazepines on receptors at the level of the central nervous system (López-Romero et al., 1998).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) as a benzodiazepine antagonist. Flumazenil is the only alternative identified in the current list. The specific advantages captured for sarmazenil are that it produces a clean reversal of benzodiazepine sedation, required after infusion during total intravenous anaesthesia, and that there is wider clinical experience with sarmazenil compared to other potential candidates for essential substances.

However, the available scientific evidence does not suggest that sarmazenil brings added clinical benefit compared to other treatment options, i.e. flumazenil, which provides for benzodiazepine reversal by competitively inhibiting the binding site at the GABA receptor. In addition, the below comment from the survey to stakeholders is noted.

The substance is not intended for the treatment of a specific condition. However, failure to adequately reverse the effects caused by benzodiazepines could cause unacceptable suffering of the animal and may be life-threatening.

Sarmazenil was mentioned (once) in the survey to stakeholders. The responder proposed to remove this substance from the list since (as indicated in the response), it is not produced anymore.

A search in the veterinary medicines database does not retrieve any sarmazenil-containing veterinary medicinal product authorised for use in equine species (neither in other animal species). In fact, there seems to be no authorised veterinary or human medicinal product containing this substance in the EU (according to a search ran by ATC code in the MRI and VMRI product indexes[51]).

The substance sarmazenil is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species (i.e. benzodiazepine antagonist). It does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Tiletamine and **Zolazepam** are discussed together since these two substances are used in combination for analgesia, minor surgery, or anaesthesia, especially for chemical immobilization in wildlife fauna (Kreeger et al., 2002).

Tiletamine is a dissociative anaesthetic agent that falls under the drug category of NMDA receptor antagonists. It is chemically similar to another dissociative anaesthetic, ketamine, and its mode of action compares to ketamine as well (Carroll and Hartsfield, 1996). It is used in combination with zolazepam, and its mode of action depends on the combination of these two substances. Depending on the dosage, the combination can result in sedation or anaesthesia with less convulsions and better muscle relaxation as compared to tiletamine only (Pablo and Bailey, 1999). It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) as a dissociative anaesthetic similar to ketamine, especially used for field anaesthesia; it is used in combination with zolazepam. The only alternative identified in the current list is ketamine. Specific advantages captured in the current list are that the use in combination with zolazepam is essential in cases when there is no access to inhalation anaesthesia e.g. for field anaesthesia; that this combination is also essential where anaesthesia with ketamine combinations is too short; and that typical applications are castrations, laryngotomies, periosteal stripping, cyst or lump excisions, repair of facial fractures, cast applications and umbilical hernia repairs.

Zolazepam is a pyrazolodiazepinone derivative structurally related to benzodiazepines, used as an anaesthetic. Its action at the GABAA receptor site increases the inhibitory effect of the neurotransmitter GABA (Pawson and Forsyth, 2002). It is typically used in combination with the NMDA antagonist tiletamine improving muscle relaxation and reducing convulsions (Mason, 2004). It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for benzodiazepine tranquilisation especially used for field anaesthesia in combination with tiletamine. Alternatives that are identified in the current list include diazepam or midazolam. The specific advantages captured in the current list are that it is a benzodiazepine tranquiliser, which is longer acting than either diazepam or midazolam. The use with tiletamine is essential in cases when there is no access to inhalation anaesthesia e.g. for field anaesthesia. This combination is essential where anaesthesia with ketamine combinations is too short. Typical applications are castrations, laryngotomies, periosteal stripping, cyst or lump excisions, repair of facial fractures, cast applications and umbilical hernia repairs.

[51] Mutual Recognition Information product index (MRI, for human medicinal products) and Veterinary Mutual Recognition Information product index (VMRI, for veterinary medicinal products)

Tiletamine and zolazepam are indeed used in combination for analgesia, minor surgery, or anaesthesia, as stated above (Kreeger et al., 2002), and in animals of the equine species. However, for these purposes there are alternatives available, including ketamine, which is listed in Table 1 of Regulation (EC) No 37/2010, with an MRL entry that includes *Equidae*. For undertaking any of the above-mentioned procedures in *Equidae* (e.g. castrations, cast applications, or umbilical hernia repairs, even under field conditions), and for the induction of anaesthesia, there are satisfactory alternative treatments for food-producing animals of the equine species (e.g. combinations of ketamine and detomidine, xylazine or romifidine).

These substances are not intended for the treatment of a specific condition. However, failure to adequately anaesthetise the animal could cause unacceptable suffering of the animal and may be life-threatening.

Tiletamine was mentioned (once) in the survey to stakeholders, proposing a modification to the entry as presented in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013). The responder suggests the indication be modified to remove that it is "especially used for field anaesthesia"). However, as indicated above, there are alternatives for induction of anaesthesia in equines and it is not considered appropriate to modify the indication as proposed.

Zolazepam was mentioned (once) in the survey to stakeholders, proposing a modification to the entry as presented in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013). The responder suggests the indication be modified to include "benzodiazepine for tranquilization to be used in combination during anesthesia with tiletamine". Similarly, whereas it is acknowledged that zolazepam is used in combination with tiletamine, the proposed amendment is not considered appropriate in light of the alternative options available for anaesthesia in equines.

A search in the veterinary medicines database does not retrieve any tiletamine- or zolazepam-containing veterinary medicinal product authorised for use in equine species. There are veterinary medicinal products authorised for use in species other than the equine (i.e. dog, cat and wild animals).

The substances tiletamine and zolazepam are not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and tiletamine and zolazepam do not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Knowledge regarding sedation and premedication in horses for this assessment was derived from textbooks, review articles, retrospective and prospective studies.

B. Considerations regarding consumer safety

Acepromazine is a phenothiazine derivative that is used in veterinary medicine for small animals and in non-food producing horses. It is administered orally or parenterally.

The pharmacokinetics of acepromazine have been described for horses. Acepromazine is widely distributed within the horse and binds extensively to plasma proteins (Ballard et al., 1982). It is characterized by a short elimination half-life, which is largely attributable to rapid metabolism to 2-(1-hydroxyethyl) promazine (HEP) and 2-(1-hydroxyethyl) promazine sulfoxide (HEPS). The terminal half-life observed was approximately 5.16, 6.70 and 4.39 hours after intravenous, sublingual and oral administration of 0.09 mg/kg acepromazine, respectively. The volume of distribution at steady state

was 16.2 L/kg and the total serum clearance 131.7 ml/min kg after intravenous administration of the same dose. In the same study, two metabolites were also evaluated (HEP and HEPS). The terminal half-life was longer for HEP, being approximately 8.26, 6.37 and 11.3 hours, after intravenous, oral and sublingual administration, respectively (Knych et al., 2018). The metabolite HEPS was quantifiable for up to 24 h in plasma and 144 h in urine, after intravenous administration of 30 mg of acepromazine, while acepromazine was quantifiable in plasma for up to 3 h with relatively little found in urine unmodified (Scheneiders et al., 2012).

Other authors report acepromazine half-lives of 6.04 and 2.6 h after 0.5 mg/kg oral administration and 0.1 mg/kg intravenous administration, respectively (Hashem et al., 1993).

The bioavailability has been described as approximately 55% after oral and sublingual administration in horses (Hashem et al., 1993; Knych et al., 2018) and averaged 20% in the dog (Hashem et al., 1992).

Regarding toxicity, acute and chronic toxicity studies have shown a low order of toxicity. Clinically, cases of overdose in humans manifest toxicity similar in appearance to other phenothiazines (CNS depression, respiratory depression, hypotension). However, in this case, the CNS depression resolves more quickly than with other cases of phenothiazine toxicity, which could be explained by the short elimination half-life. Acepromazine is not listed by the IARC.

Considering its pharmacokinetic behavior in horses, it can be accepted that acepromazine 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.

Atipamezole is used in veterinary medicine as a selective α2-adrenergic receptor antagonist, with an imidazole structure used to reverse the sedative effects of α2 agonists. Blockade of α2-adrenoceptors, particularly presynaptic auto receptors in noradrenergic neurons, promotes the release of norepinephrine. It rapidly reverses sedation/anaesthesia induced by α2-adrenoceptor agonists (Pertovaara et al., 2005). There are currently several veterinary medicinal products authorized for dogs and cats.

Atipamezole is rapidly absorbed and distributed from the periphery to the central nervous system. Intravenous administration of 10, 20, 30 mg in humans resulted in linearly dose-related concentrations in plasma. The elimination half-life observed was 1.7-2 h and the plasma clearance was 1.1-1.5 l/h Kg. No atipamezole could be detected in plasma after oral dosing, since there appears to be poor oral bioavailability. The 10 and 30 mg doses were well tolerated. The largest dose, 100 mg, caused some sympathomimetic-like subjective effects (coldness or sweating of limbs, cold shivers, tension or restlessness, irritability, tremor, increased salivation) (Karhuvaara et al., 1990).

In another study in humans, atipamezole was applied on the buccal mucosa. Peak concentrations were measured at 30 and 60 minutes after administration. Mean elimination half-lives were approximately 1 ½ hours after treatment. Bioavailability of 33% was calculated for buccal administration, whereas systemic availability after an oral dose was <2% (Huupponen, et al., 1995).

Atipamezole is well tolerated in humans and rodents. In rats, the elimination half-life is 1.3 hours after subcutaneous administration. Atipamezole undergoes extensive first-pass metabolism (Pertovaara et al., 2005). It is rapidly and completely metabolized. The metabolites are mainly excreted in urine with a small amount excreted in faeces.

In veterinary practice, it can be concluded that atipamezole has proved to be useful in rapidly reversing the anaesthesia and side effects induced by α2-adrenoceptor agonists alone or in combination with other anaesthetics. Considering its pharmacokinetic characteristics, it can be

accepted that atipamezole 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.

Diazepam is a long-acting benzodiazepine with anti-convulsant, anxiolytic, sedative, muscle relaxant and amnestic properties. It is commonly used in veterinary and human medicine. It can be administered orally, rectally and parenterally in human medicine. Diazepam exerts its principal action on GABA receptors in the central nervous system (CNS), both within the brain and spinal cord. Drowsiness, sedation, muscle weakness and ataxia are the most frequent adverse effects. Overdosage can produce CNS depression and coma or paradoxical excitation.

Pharmacokinetics of diazepam have been well described in the literature. It is readily and completely absorbed from the gastrointestinal tract with peak plasma concentrations occurring within 30 to 90 minutes, following oral doses. Absorption may be erratic after intramuscular injection and lower peak plasma concentrations may be obtained compared with those after oral doses. Diazepam has a biphasic half-life with an initial rapid distribution phase and a prolonged terminal elimination phase of 1 or 2 days; its action is further prolonged by the even longer half-life of 2 to 5 days of its principal active metabolite, desmethyldiazepam (nordiazepam). Both accumulate with repeated dosage. Diazepam is extensively metabolized in the liver, notably via cytochrome P450 and in addition to desmethyldiazepam, its active metabolites include oxazepam and temazepam. It is excreted in the urine. Diazepam is 98 to 99% bound to plasma proteins (Scriba, 2009).

In the horse, diazepam is also extensively metabolized to nordiazepam, temazepam and oxazepam. Nordiazepam was measured in urine out to a collection time of 53-55 hours, oxazepam 121 hours and temazepam 77-79 hours. Diazepam and nordiazepam were measured in equine serum out to collection times of 6 and 54 hours, respectively, whereas oxazepam and temazepam were not detected (Marland et al., 1999).

Cytochrome P450-dependent hydroxylation and demethylation as well as glucuronidation by means of uridine diphosphate glucuronosyltransferase has been reported for horses. In one study, 0.2 mg/kg was administered intravenously to horses in order to describe the fate of the three main metabolites in plasma and urine. The mean elimination half-life of diazepam was 19.9 h. Nordiazepam was the main metabolite in plasma detected up to 16 days post-administration (<LOD, 0.01 ng/ml) and oxazepam was the longest detectable in urine (up to 26 days, <LOD, 0.2 ng/ml) (Schenk et al., 2021).

Regarding toxicity, a number of repeated dose studies have been carried out. In general, toxic effects have not been remarkable (Inchem, 1998). Furthermore, diazepam is currently not classifiable as to its carcinogenicity to humans (group 3 IARC).

Considering all the information mentioned above about the pharmacokinetic behavior of diazepam and its metabolites, it can be accepted that it 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.

Flumazenil is a benzodiazepine antagonist that is used in human medicine via the intravenous route for the reversal of the sedative effects caused by benzodiazepines.

In humans, flumazenil is well absorbed from the gastrointestinal tract, but undergoes extensive first-pass hepatic metabolism and has a systemic bioavailability of approximately 16-20%. It is about 40-50% bound to plasma proteins. After intravenous administration, it is extensively metabolized in the liver to the inactive carboxylic acid form, which is excreted mainly in the urine. Small amounts of the unchanged drug have been observed in the urine. Elimination half-life is approximately 40 to 80 minutes and high total plasma or blood clearances have been described (520 to 1300 ml/min) (Klotz and Kanto, 1988; Scriba, 2009).

A short duration of antagonism of flumazenil was observed from a study in which six volunteers were administered 10 mg of flumazenil intravenously over 10 minutes; the volume of distribution at steady-state was 64.8 ± 12.5 L, the total body clearance was 53.8 ± 1.2 L/h and the elimination half-life reported was 70.2 ±9.9 mins (Breimer et al., 1991). In children administered a bolus of flumazenil 10 µg/kg followed by an infusion of flumazenil 5 µg/kg/min, the mean terminal elimination half-life was 35.3 ± 13.8 min, the total plasma clearance was 20.6 ± 6.9 ml/min/kg and the apparent volume of distribution at steady state was 1.0 ± 0.2 L/kg. From this study, 5.8-13.8 % of the flumazenil dose was recovered unchanged in the urine (Jones et al., 1993). In chronic liver disease, an increased plasma-half-life up to 7-times normal and up to a 7-fold decrease in the plasma clearance rate was observed by Van der Rijt et al. (1991).

No pharmacokinetic data is available in horses.

Regarding toxicity studies, systemic tolerance was good in rats and dogs administered flumazenil intravenously at dosages up to 10 mg/kg per day, for 4 weeks. In 13-week oral studies, 80 mg/kg/day were well tolerated in dogs and, after 125 mg/kg/day in rats, no untoward compound-related findings were seen apart from a 10-15% increase in liver weights in females. Embryotoxicity and teratogenicity studies done on rats (between the 7^{th} and 16^{th} day of gestation) and rabbits (between the 7^{th} and 19^{th} day of gestation) revealed no signs of embryotoxicity at dosages of 15, 50 and 150 mg/kg per day, by oral gavage. No drug-related effects were observed in a perinatal and postnatal study in rats orally administered 5, 25 or 125 mg/kg from day 16 of gestation to day 22 of lactation. Flumazenil was not mutagenic in the Ames test or micronucleus test or in tests using *Saccharomyces cerevisiae* or Chinese hamster V79 cells. An in vivo DNA repair test using germ cells of male mice did not yield DNA-damaging activities (Inchem, 1993). No carcinogenicity data is available.

As it has been mentioned, no information about the pharmacokinetics of flumazenil in horses is available. However, considering the fast elimination described in humans, the low oral bioavailability, the lack of genotoxicity or teratogenic potential reported in animals and the limited use of flumazenil (as an intravenous reversal agent of benzodiazepines), it can be accepted that flumazenil 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.

Naloxone is a semisynthetic, lipophilic, competitive, non-selective opioid receptor antagonist that reverses and blocks opioid-induced effects (e.g. analgesia, respiratory depression). It is used in human and veterinary medicine.

Naloxone can be administered via various routes. It is usually given intravenously for a rapid onset of action, which occurs within two minutes. The onset of action is only slightly less rapid when it is given intramuscularly or subcutaneously. It is metabolized in the liver, mainly by glucuronide conjugation, and excreted in the urine. It has a plasma half-life of approximately one hour after parenteral administration (Scriba, 2009).

One study reported that, following oral administration, the bioavailability of naloxone was very low, with mean absolute bioavailabilities (based on AUC_t) of 0.9-2%, over a dose range of 5-120 mg administered orally as prolonged release tablets (Smith et al., 2012).

Time to maximum plasma concentration (T_{max}) is approximately 2-3 min when administered intravenously, 10-20 min when administered intramuscularly, and 15-30 min when administered intranasally. The elimination half-life is 1-2 hours when administered intramuscularly or intranasal. The short half-life of naloxone relative to many opioid receptor agonists may lead to the recurrence of respiratory depression during an opioid overdose event and require multiple doses of naloxone to be administered. Naloxone is generally considered safe and effective (Britch and Walsh, 2022).

In dogs, naloxone is rapidly absorbed after intravenous and intramuscular administration and has an apparent wide margin of safety. Following intranasal administration in dogs, naloxone was rapidly absorbed, with a short lag time of 2.3 minutes to detection of naloxone in plasma samples (Wahler et al., 2019). These results are comparable to those reported in humans. Some differences in terminal half-life were observed between humans and dogs (1.7-2.2 hours in humans vs 0.8 hours in dogs) which may be attributable to interspecies differences in the metabolism and clearance of naloxone. From the same study, mean terminal half-life after intranasal and intravenous administration was 47.4 and 37 minutes, respectively.

Pharmacokinetics and pharmacodynamics of intranasal and intramuscular naloxone were investigated in dogs, exposed to fentanyl. Maximum plasma concentration was lower after intranasal administration; median C_{max} was 11.7 (2.8-18.8) ng/ml for intranasal and 36.7 (22.1-56.4) ng/ml for intramuscular administration. However, T_{max} was not significantly different between both groups (median 0.5 hours) (Barr et al., 2023).

Naloxone is an opioid receptor antagonist with no intrinsic efficacy following receptor binding. Previous studies of humans, who had received no other medications, showed minimal pharmacodynamic impact, with no notable changes in heart rate and minimal changes in blood pressure, following naloxone administration (Wahler et al., 2019).

Acute toxicity studies with naloxone have been performed in mice, rats and dogs. The maximum nontoxic subcutaneous dose in rats was found to be of the order of 50 mg/kg. Daily doses of 0.2 mg/kg given intravenously to dogs for 16 days and 5 mg/kg given subcutaneously to monkeys for 30 days caused no toxicity. However, a subcutaneous dose of 20 mg/kg resulted in lethargy and tremor in monkeys (Inchem, 1993).

Considering all the information summarised above, it can be accepted that naloxone 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.

Propofol is a short-acting anaesthetic given intravenously for the induction and maintenance of general anaesthesia. It is also used for sedation in adult patients undergoing diagnostic procedures, in patients undergoing surgery with local or regional anaesthesia and in ventilated adult patients under intensive care. It is used in human and veterinary medicine.

The pharmacokinetic properties of propofol are unique and contribute to its favorable clinical properties. It is characterized by a rapid onset of action and fast metabolic clearance.

The pharmacokinetics of propofol are best described by a 3-compartment model in humans. After a single bolus dose, two distribution phases are seen. The first phase has a half-life of 2 to 4 minutes. This is followed by a slow distribution phase with a half-life of 30 to 60 minutes. Significant metabolism of propofol occurs during the second phase. Propofol is over 95% bound to plasma proteins. Terminal half-life of 3 to 12 hours have been described (Scriba, 2009).

Metabolism is predominantly performed in the liver, with water-soluble compounds excreted via the kidneys. Less than 1% is excreted unchanged in urine and 2% in faeces. Extra-hepatic elimination has been proposed for propofol. The product information of some veterinary medicinal products (VMPs) suggests that the lungs and kidney play a role in extra-hepatic metabolism. In dogs, no accumulation of blood levels has been observed after repeated daily dosing. In rats, the highest level of propofol was observed in the liver. Recovery after administration is rapid due to the high clearance level of the active substance.

De Vries et al. (2013) described the pharmacokinetics of propofol in horses as similar to those in humans. After a bolus of 0.3 mg/kg and an infusion of 0.16 mg/kg/min, for the first thirty minutes in ponies, propofol clearance and volume of distribution were 31.4 ± 6.1 ml/min/kg and 220.7 (132.0) ml/kg. During the infusion, blood propofol concentrations ranged between 1.52 and 7.65 µg/ml. Furthermore, in this study, all recoveries were scored as excellent and maximum whole blood concentration was 1.40 µg/ml at regaining standing position.

Boscan et al. (2010) reported a plasma elimination half-life of 44.8 ± 21.3 min and a clearance of 45.8 ± 6.5 ml/min/kg after a single intravenous dose of 2 mg/kg in horses. During recovery, the plasma concentrations ranged between 0.16 and 0.65 µg/ml at first movement and 0.03 and 0.20 µg/ml when the horses stood.

Regarding toxicity no evidence of adverse foetal effects have been noted in rats and rabbits at doses of 5-15 mg/kg/day. Propofol is not a teratogen at therapeutic doses in humans and is not considered to be a carcinogen.

Considering the general pharmacokinetic characteristics of propofol and those in horses, it can be accepted that it 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.

4.12.3. Assessment of new substances proposed to be added to the list in the stakeholders survey

A. Considerations on the essentiality of the substance(s)

Dexmedetomidine was mentioned (five times) in the survey to stakeholders and it was suggested for addition to the list for peri-anaesthetic use, PIVA (partial intravenous anaesthesia technique) and sedation. The responders stated it is superior to detomidine, providing improved recovery quality, with high specificity for alpha2-adrenergic receptors which decreases general anaesthetic requirements; for PIVA technique, increasing evidence of its clinical benefit in recent years was mentioned. No scientific references were provided.

Dexmedetomidine is a potent alpha2-adrenergic receptor agonist and is the most selective alpha2 agonist. It is used in horses for sedation, as part of general anaesthesia protocols (i.e. premedication, partial or total intravenous anaesthesia protocols). It is of increasing interest for equine practice, due to its perceived benefits, including a short half-life and a rapid distribution, which particularly favours its use for continuous rate infusion (Gozalo-Marcilla et al., 2018).

The substance is not intended for the treatment of a specific condition. However, failure to adequately sedate the animal could cause unacceptable suffering of the animal and may be life-threatening.

A search in the veterinary medicines database does not retrieve any dexmedetomidine-containing veterinary medicinal product authorised for use in equine species. There are veterinary medicinal products authorised for use in species other than the equine (i.e. dog, cat).

The substance dexmedetomidine is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: sedation or general anaesthesia as part of partial or total intravenous anaesthesia protocols. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Medetomidine was mentioned (three times) in the survey to stakeholders and it was suggested for addition to the list for peri-anaesthetic use. The responders stated it is superior to detomidine,

providing improved recovery quality, with high specificity for alpha2-adrenergic receptors which decreases general anaesthetic requirements; however, no scientific references were provided.

Medetomidine is a potent alpha2-adrenergic receptor agonist and is used in horses for sedation, as part of general anaesthesia protocols (premedication, partial or total intravenous anaesthesia protocols) and has comparable effects to other commonly used alpha2 agonists such as xylazine, romifine and detomidine (Bettschart-Wolfensberger et al., 1999, 2005a; Bueno et al., 1999; Grimsrud et al., 2015). Suggested advantages of medetomidine over other alpha 2 agonists are difficult to determine as such effects will be dependent on the method of administration (single bolus vs continuous rate infusion) and dosing regimen. In one recent study (Hollis et al., 2020) an infusion of medetomidine (at the dose investigated) was found to have fewer sedative effects than the compared dose of detomidine.

The substance is not intended for the treatment of a specific condition. However, failure to adequately sedate the animal could cause unacceptable suffering of the animal and may be life-threatening.

A search in the veterinary medicines database does not retrieve any medetomidine-containing veterinary medicinal product authorised for use in equine species. There are veterinary medicinal products authorised for use in species other than the equine (i.e. dog, cat).

The substance medetomidine is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indication (peri-anaesthetic use) in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Vatinoxan was mentioned (once) in the survey to stakeholders and it was suggested for addition to the list as α2-adrenoceptor antagonist used for preventing and alleviating the peripheral effects of α2 agonists without reversing their sedative and antinociceptive effects. According to the responder, there are no alternatives identified. It is specifically used for prevention and treatment of hypertension, bradycardia, hyperglycaemia and reduced gastrointestinal motility, caused by activation of peripherally located α2-adrenoceptors, in horses sedated with α2 agonists.

It is used as an α2 adrenolytic agent for the treatment of intestinal diseases in connection with ischemia and tissue injury (Verhaar et al., 2023) and to improve tissue oxygenation and perfusion (Neudeck et al., 2021). It is also used for preventing and alleviating the peripheral effects of α2 agonists without reversing their sedative and antinociceptive effects. Specifically used for prevention and treatment of hypertension, bradycardia, hyperglycemia and reduced gastrointestinal motility in horses sedated with α2 agonists. Vatinoxan poorly penetrates the blood-brain barrier. Atipamezole is a better clinical alternative for an equivalent indication, and it is retained in the listed of essential substances. A search in the current literature did not identify any added clinical benefit of vatinoxan compared to atipamezole.

The substance is not intended for the treatment of a specific condition. However, failure to adequately reverse the effects of α-2 agonists may be life-threatening and cause unacceptable suffering for the animal.

A search in the veterinary medicines database does not retrieve any vatinoxan-containing veterinary medicinal product authorised for use in equine species. There are vatinoxan-containing veterinary medicinal products authorised for use in species other than the equine (i.e. dog).

The substance vatinoxan is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indication in animals of the equine species; it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering for the animal.

Knowledge regarding sedation and premedication in horses for this assessment was derived from textbooks and peer-reviewed articles.

B. Considerations regarding consumer safety

Dexmedetomidine is a selective alpha2-adrenergic receptor agonist with anxiolytic, analgesic and sedative properties. As mentioned above, it is the active d-isomer of medetomidine.

The pharmacokinetics of dexmedetomidine have been described in humans. It is a highly plasma protein-bound drug, with 94% of dexmedetomidine bound to albumin and α1-glycoprotein (Weerink et al., 2017). Its bioavailability was determined after oral, buccal and intramuscular administration, resulting in 16, 82 and 104%, respectively; Chamadia et al. (2020) described an oral bioavailability of 7.2%. Therefore, dexmedetomidine appears to be well absorbed through oral mucosa; however, after oral administration dexmedetomidine is likely subject to an extensive first-pass effect (Anttila et al., 2003).

After IV administration of 2 µg/kg, the elimination half-life was 2.17 ± 0.42 hours, the clearance was 0.64 ± 0.14 l/kg and the apparent volume of distribution, at steady state, was 1.61 ± 0.26 l/kg. Elimination half-life was 2.5 ± 0.6 h when the same dose was administered intramuscularly (Anttila et al., 2003). As described in human medicinal products, dexmedetomidine is eliminated mainly through biotransformation by the liver with less than 1% excreted unchanged, with the urine being the major route of excretion.

In horses, continuous-rate-infusion (CRI) (initial bolus at 5 µg/kg followed by a CRI at 10 µg/kg/h for 15 min and 5 µg/kg/h for 60 min) and an IM group (10 µg/kg, followed by 5 µg/kg in 30-min intervals for 60 min) were investigated. Clearances and elimination half-life were 134 ± 67.4 ml/kg/min and 44.63 ± 26.3 min, respectively in the CRI group and 412 ±306 ml/kg/min and 38.9 ± 18.6 min, respectively, in the IM group (Shane et al., 2021). Ranheim et al., (2014) described a mean plasma elimination half-life of 20.9 ± 5.1 min, a clearance of 0.3 ± 0.2 L/min/kg and a volume of distribution at steady state of 13.7 ± 7.9 L/kg after a CRI of dexmedetomidine at 8 µg/kg/h for 150 min. A faster elimination was described after an intravenous bolus of 5 µg/kg (mean elimination half-life was 8.03 mins, volume of distribution was 1053.52 ml/kg and clearance 78.62 ml/kg/min) (Rezende et al., 2015). In another study, dexmedetomidine plasma levels declined rapidly in ponies administered intravenously with 3.5 µg/kg and fell beyond limit of quantification (LOQ, 0.05 ng/ml) within 60-90 min (Bettschart-Wolfensberger et al., 2005b).

Some studies have been performed using rats and dogs. In these species, dexmedetomidine is also biotransformed in the liver and the metabolites obtained are without pharmacodynamic activity, according to the product information of human medicinal products. For rats, values of the elimination half-life of dexmedetomidine (56.2 and 57.4 min) were calculated from a study with two different intravenous infusion protocols (Bol et al., 1997). Dexmedetomidine was readily absorbed following subcutaneous or intramuscular administration in rats and dogs. The radioactive labelled drug was rapidly and widely distributed throughout the body. Radioactivity in plasma and most tissues decreased

substantially within 72 hours, with the exception of adrenals. Low binding to red blood cells was observed and dexmedetomidine crossed the placenta.

Dexmedetomidine toxicity has been well studied in rats and dogs. In repeat dose toxicity studies, the main clinical findings are consistent with the pharmacology of the substance. A common observation was corneal keratitis/opacity. Drug related histopathological changes in the adrenal glands, lungs, eyes, liver and injection site have also been observed, according to data in human medicinal products.

Effects of dexmedetomidine on foetal development have been investigated in rats treated subcutaneously with 5, 10 and 20 µg/kg daily from gestation day 7 to day 19. No adverse effects on perinatal morphology of pups, birth weight and length, physical growth and postnatal behavioral performances were observed (Tariq et al., 2008). Dexmedetomidine had no effect on male and female rats and female rabbits' fertility at doses up to 54 µg/kg. Maternal and foetal toxicity were observed but no teratogenic effects were observed. In rats, 200 µg/kg subcutaneously caused an increase in embryofetal death and reduced the foetal weight, which was associated with maternal toxicity. Reduced fetal body weight also was noted in rats at a dose 18 µg/kg/day subcutaneously and was accompanied with delayed ossification at dose 54 µg/kg/day (evidence in human medicinal products).

Regarding the mutagenicity and clastogenicity of dexmedetomidine, the standard battery of in vitro and in vivo tests showed negative results, for all the studies performed, according to the product information of human medicinal products. No carcinogenicity studies are available.

Considering the rapid elimination of dexmedetomidine, the low oral bioavailability, the extensive metabolism, the toxicity profile and its limited use for surgical procedures, it can be accepted that medetomidine 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.

4.12.4. Conclusion

Based on the above assessment and justifications, the following recommendations are proposed:

1. The following active substances, currently in Regulation (EU) 1950/2006 as amended by Regulation (EU) 122/2013, are proposed to be retained in the list, either without modification or with an amendment of the current entry as shown below:

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Acepromazine	for a multimodal approach for tranquilisation and premedication in combination with other sedatives	detomidine, romifidine, xylazine, diazepam	the mode of action of acepromazine and its unique quality of sedation cannot be produced by alpha-2 agonist sedatives or benzodiazepines
Atipamezole	for reversal of α-2 agonists	none identified	reverses sedative and analgesic effects and adverse cardiovascular reactions
Diazepam	for premedication and induction of anaesthesia, mild tranquilisation with minimal cardiovascular and respiratory side effects	acepromazine, detomidine, romifidine, xylazine	the mode of action (at GABA receptor) provides unique tranquilisation without cardiorespiratory depression that cannot be produced by α-2 agonist sedatives (detomidine, romifidine and xylazine) or acepromazine
Flumazenil	intravenous reversal agent for benzodiazepine effect during recovery	none identified	antagonist that competitively inhibits the

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
	from Total Intravenous Anaesthesia (TIVA) techniques		benzodiazepine binding site at the GABA receptor
Naloxone	reversal of opioid effects during emergencies	none identified	no alternatives available
Propofol	as an intravenous anaesthetic, for induction of anaesthesia in foals	isoflurane	improvement in cardiovascular stability and quality of recovery over inhalation anaesthesia in foals

2. The following active substances, currently in Regulation (EU) 1950/2006 as amended by Regulation (EU) 122/2013, are proposed to be removed from the list: midazolam, sarmazenil, tiletamine, zolazepam.

3. The following active substance, suggested for addition to the list in the survey to stakeholders, is proposed to be added to the list with an entry as shown below:

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Dexmedetomidine	sedation or general anaesthesia as part of partial or total intravenous anaesthesia protocols	detomidine, romifidine, xylazine, diazepam	the most selective alpha2 agonist; short half-life and rapid redistribution, which particularly favour its use as a continuous rate infusion

4. The following active substances, suggested for addition to the list in the survey to stakeholders, are not proposed for inclusion: medetomidine, vatinoxan.

4.13. Substances for systemic disorders

4.13.1. Overview

	Active substance (ATCvet code)
Substances in Regulation (EU) 1950/2006 **proposed to be retained**, with or without amendment of the current entry	Allopurinol (M04AA01); Dobutamine (QC01CA07); Dopamine (QC01CA04); Ephedrine (QC01CA26); Glycopyrrolate (QA03AB02); Noradrenaline/norepinephrine (QC01CA03) ; Vasopressin (QH01BA01)
Substances in Regulation (EU) 1950/2006 **proposed to be removed**	Hydroxyethyl-starch (QB05AA07); Pentoxifylline (C04AD03)
Substances from stakeholders' survey **proposed for inclusion**	Dalteparin (QB01AB04); Gelatinpolysuccinate (B05AA06)
Substances from stakeholders' survey **not proposed for inclusion**	Aminocaproic acid (QB02AA01); Clopidogrel (QB01AC04); Enoxaparin (QB01AB05); Nadroparin (QB01AB06); Tranexamic acid (QB02AA02)

4.13.2. Review of the existing entries in Regulation (EU) 1950/2006, as amended by Regulation (EU) 122/2013, considering the survey results

A. Considerations on the essentiality of the substance(s)

Allopurinol is a structural analogue of the natural purine base, hypoxanthine. It inhibits xanthine oxidase enzyme, the enzyme that converts hypoxanthine to xanthine to uric acid.

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), for treatment of neonatal ischaemia-reperfusion injury. Vitamin E is the only identified alternative and the specific advantage listed is its different mode of action for reperfusion injury; allopurinol is a xanthine oxidase inhibitor inhibiting free radical production during reperfusion following ischaemia.

As stated in the current list, the advantage of allopurinol compared to vitamin E for the treatment of reperfusion injury is its different mode of action in inhibiting the formation of reactive oxygen species (ROS). Inhibition of xanthine oxidase by allopurinol decreases the formation of ROS and thus reduces oxidative stress, as shown in 6 horses during intense exercise (Mills et al., 1997). Vitamin E can exert its efforts as an antioxidant as well as a regulator of signal transduction, including inhibition of NF-κB, which plays a role in ROS-mediated inflammation and cellular damage during reperfusion following ischemic events (Medling et al., 2010). There are several vitamin E-containing veterinary medicinal products authorised for food-producing animals of the equine species. However, allopurinol's different mode of action than vitamin E in the formation of ROS is considered to bring added clinical benefit for the treatment of ischaemia reperfusion injury.

Neonatal ischaemia, if untreated, is life-threatening and causes unacceptable suffering of the animal.

Allopurinol was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any allopurinol-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance allopurinol is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: neonatal ischaemia reperfusion injury. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Dobutamine is a synthetic catecholamine. It is primarily a β1 receptor agonist, whereby higher doses can also produce balanced α1 and β2 effects (Schauvliege and Gasthuys, 2013). The use of dobutamine as a positive inotrope for horses under general anaesthesia has been well documented (Schier et al., 2016; Loughran et al., 2017). Hypotension is a well-known complication of general anaesthesia in horses (esp. Draft horses) and significantly increases the risk of poor anaesthetic recoveries and outcomes. Dobutamine has been shown to be effective at increasing both blood pressure and cardiac output in anaesthetised horses (Schauvliege and Gasthuys, 2013).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), for treatment of hypotension during anaesthesia. The identified alternative in the current list is dopamine, and the specific advantages are as follows: positive inotrope therapy, probably more used than dopamine but preferences vary; horses usually develop hypotension during anaesthesia, and maintenance of normal blood pressure has been shown to reduce the incidence of serious post-operative rhabdomyolysis; dobutamine is invaluable during volatile anaesthesia in horses.

Hypotension is common in horses undergoing general anaesthesia with rates of 42% and 88% reported in horses for elective and abdominal surgeries, respectively (Deutsch and Taylor, 2022).

Hypotension under general anaesthesia can lead to prolonged recoveries and is implicated in several potentially fatal anaesthetic complications such as myopathy or neuropathy. Therefore, medications to treat and or prevent hypotension in horses under general anaesthesia are necessary.

Dobutamine is considered to be a more potent inotrope than dopamine, and as such is generally the first-choice medication for the treatment of hypotension in adult equines under general anaesthesia (Schauvliege and Gasthuys, 2013; Dancker et al., 2018). However, it is still considered necessary to have an available alternative to dobutamine. Depending on the overall health status of the animal under anaesthesia, and comorbidities present, the effect of any inotrope may be variable. For example, there are some situations where dobutamine is less effective – such as when animals are endotoxic and have a low systemic vascular resistance – and in these cases ephedrine can be more effective (Schauvliege and Gasthuys, 2013).

Hypotension, if untreated, is life-threatening and causes unacceptable suffering of the animal.

Dobutamine was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any dobutamine-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance dobutamine is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: to manage hypotension under general anaesthesia. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Dopamine is a noradrenaline precursor that binds to presynaptic dopamine-2 (DA2) and α2 receptors as well as postsynaptic dopamine-1 (DA1), α1, α2, and β1 receptors. It can also affect the release and prevent reuptake of noradrenaline, and mainly through the release of noradrenaline, then dopamine has positive inotropic and chronotropic effects and has been shown to increase cardiac output and potentially arterial blood pressure in anaesthetised horses (Schauvliege and Gasthuys, 2013).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), for treatment of hypotension during anaesthesia. Dobutamine is the only identified alternative and the specific advantage captured is that dopamine is required in horses that do not respond to dobutamine; in foals dopamine is used in preference to dobutamine; additionally required for treatment of intraoperative brady-dysrhythmias that are resistant to atropine.

Dopamine's efficacy for the management of hypotension during anaesthesia in horses is well described (Schauvliege and Gasthuys, 2013). However, efficacy is not consistent, and indirect through the release of noradrenaline. Different horse breeds have varying sensitivity to noradrenaline, ponies being the most sensitive. Release of noradrenaline in ponies also represents an increased risk of hyperlipemia. Several alternatives for hypotension during anaesthesia are available, e.g. dobutamine or ephedrine, which puts into question its essentiality as per the criteria given.

The use of low-dose dopamine in horses as part of a treatment protocol for acute kidney injury/renal failure, on the contrary, is considered essential. Low doses of dopamine, within a specific timeframe, have been shown to result in renal vasodilation, increased renal blood flow, and increased urine production without systemic cardiovascular effects in conscious healthy horses (Trim et al., 1989), and there are sporadic reports in the literature of its use for the treatment of oliguric renal failure in horse (Matsuda et al., 2021; Divers, 2022).

Acute kidney injury/renal failure, if untreated, is life-threatening and causes unacceptable suffering of the animal.

Dopamine was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any dopamine-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance dopamine is proposed to be qualified as essential because no satisfactory alternative treatments are authorised for food-producing animals of the equine species for the following indication: as part of a treatment protocol for only acute kidney injury/renal failure.

Ephedrine is an adrenergic agonist with α1 and β1 effects (Schauvliege and Gasthuys, 2013; Garcia-Filho et al., 2023). It has been shown to increase cardiac output, stroke volume and arterial blood pressure in anaesthetised horses (Schauvliege and Gasthuys, 2013).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), for treatment of hypotension during anaesthesia. Dobutamine and dopamine are the identified alternatives and the specific advantage captured are as follows: required where dopamine and dobutamine are ineffective; a unique sympathomimetic agent, which is structurally similar to adrenaline; it is impossible to use the action of catecholamines on specific receptors in the body to the benefit of equine patients without recourse to the use of a number of catecholamines, each active at a different receptor profile – hence ephedrine, which causes noradrenaline release at the nerve endings, thereby increasing cardiac contractility and obtunding hypotension, is used when dobutamine and dopamine are ineffective; ephedrine lasts minutes to hours and is effective after a single intravenous injection, whereas dobutamine and dopamine last only a few seconds or minutes and must be given by infusion.

As indicated for dobutamine, it is still considered necessary to have available alternatives since depending on the overall health status of the animal under anaesthesia, and comorbidities present, the effect of any inotrope may be variable – in some cases, ephedrine can be more effective (Schauvliege and Gasthuys, 2013).

Hypotension, if untreated, is life-threatening and causes unacceptable suffering of the animal.

Ephedrine was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any ephedrine-containing veterinary medicinal product authorised for use in equine species. There are veterinary medicinal products authorised for use in species other than the equine (e.g. dog).

The substance ephedrine is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: to manage hypotension under general anaesthesia. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Glycopyrrolate is a muscarinic receptor antagonist which antagonises acetylcholine's muscarinic effects without affecting nicotinic receptors at neuromuscular junctions (Schauvliege and Gasthuys, 2013). It has been shown to have a positive chronotropic effect on anaesthetised horses which improves cardiac output and arterial blood pressure (Neto et al., 2004).

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), for prevention of bradycardia. The identified alternative in the current list is atropine and the specific advantage

captured is that glycopyrrolate has limited central nervous system (CNS) effects and is more suitable in conscious horses (before and after anaesthesia) than atropine.

It is noted that atropine sulphate can be accessed in licensed veterinary medicinal products that can be used as an alternative to glycopyrrolate for the treatment and prevention of bradycardia in horses. However, glycopyrrolate is considered to be more appropriate as an anticholinergic agent than atropine for the equine species, as it does not cross the blood brain barrier, therefore producing fewer CNS signs such as excitement and sedation. It's effects on gastrointestinal motility are also considered to be of shorter duration than those of atropine (Singh et al., 1997), whose effects can last up to 12 hours (Ducharme and Fubini, 1983). Due to long lasting effects of atropine in horses, atropine use represents a risk for colic.

Bradycardia, if untreated, is life-threatening and causes unacceptable suffering of the animal.

Glycopyrrolate was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any glycopyrrolate-containing veterinary medicinal product authorised for use in equine species. There are veterinary medicinal products authorised for use in species other than the equine (i.e. cat and dog).

The substance glycopyrrolate is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: to treat and prevent bradycardia. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Hydroxyethyl-starch (HES) is a non-ionic starch derivative; synthetic colloids commonly used for fluid resuscitation to replace intravascular volume. HES is a general term and can be sub-classified according to average molecular weight, molar substitution, concentration, C2/C6 ratio. It is used as intravenous solution to prevent shock following severe blood loss caused by trauma, surgery, or other issues. When given intravenously, molecules smaller than the renal threshold (60,000-70,000 daltons) are readily and rapidly excreted in the urine, while molecules with higher molecular weights are metabolized by plasma α-amylase prior to excretion via the renal route (Mutter et al., 2013).

Hydroxyethyl-starch is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for colloidal volume substitution. No alternatives are identified in the current list, and the specific advantage captured for the substance is that it is practical and readily available as an alternative to blood or plasma.

Hydroxyethyl-starch has been associated with renal damage and fatalities. In humans, evidence suggests a dose-dependent pattern between HES administration and postoperative kidney injury in adult patients undergoing major noncardiac surgery (Kashy et al., 2015). Another study revealed that all HES products elevate the risk of acute kidney injury and the need for renal replacement therapy across various patient groups (Mutter et al., 2013). All human medicinal products containing HES have been suspended in 2022 in the EU (European Commission, 2022). These findings have led to investigation into the safety of HES solutions in veterinary species. Administration of HES did not appear to increase the risk of acute kidney injury in cats (Sigrist et al., 2017a), but two studies in dogs have divergent outcomes (Hayes et al., 2016; Sigrist et al., 2017b). Insufficient data are available on HES induced acute kidney injury or mortality in horses (Van Galen and Hallowell, 2019). Hypertonic saline could be used as a satisfactory alternative treatment option for acute recovering blood pressure lasting for 20 to 30 minutes. In addition, gelatinpolysuccinat (a colloid solution) is proposed to be added to the list. Due to available alternatives and considering its known negative effects on the kidney, HES cannot be considered essential in fluid resuscitation.

Hypovolemia, if untreated, is potentially life-threatening and causes unacceptable suffering of the animal.

Hydroxyethyl-starch was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any hydroxyethyl-starch-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance hydroxyethyl-starch is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Noradrenaline/norepinephrine is a naturally occurring adrenergic agonist with very potent α1 and α2 activity, with some β1 adrenoceptor activity (Corley, 2002; Schauvliege and Gasthuys, 2013). It has a sympathomimetic effect by causing vasoconstriction. In horses under general anaesthesia, noradrenaline is used, where it is necessary, to increase perfusion pressures, or to counteract vasodilatory effects of other drugs, but used alone it may decrease cardiac output (Schauvliege and Gasthuys, 2013). Noradrenaline has also been used in combination with dobutamine to treat hypotension in critically ill neonatal foals that were refractory to treatment with dobutamine and dopamine, alone (Corley, 2002).

It Is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), for treatment of cardiovascular failure in foals. There are no identified alternatives in the current list. The specific advantages captured in the current list are as follows: the animal's catecholamine receptor profile responds precisely to medicines acting at different sites – hence a range of catecholamines acting more or less exclusively on different types of adrenergic receptors is used to produce a precise effect; noradrenaline acts primarily on α1 receptors to vasoconstrict arterioles, thereby increasing blood pressure and maintaining central circulation; in foals, noradrenaline is commonly the only catecholamine effective in the treatment of hypotension.

Noradrenaline can be effective for treatment of hyperdynamic (early septic) shock, where the animal is not responsive to dobutamine and/or dopamine; it has been shown to be effective in horses (Corley, 2002).

Cardiovascular failure and/or hypovolemic shock, if untreated, is life-threatening and causes unacceptable suffering of the animal.

Noradrenaline/norepinephrine was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any noradrenaline/norepinephrine-containing veterinary medicinal product authorised for use in equine species. There are veterinary medicinal products authorised for use in species other than the equine (i.e. dog and cat).

The substance noradrenaline/norepinephrine is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: for treatment of early septic shock; for support of cardiovascular function in critically ill foals. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Pentoxifylline is a methyl xanthine derivative used primarily to improve blood flow by reducing the viscosity (thickness) of blood and enhancing its ability to flow through small blood vessels. The mechanism of action of pentoxifylline is not fully understood. It is a hemorheologic agent with primary

actions that include increasing erythrocyte flexibility, reducing blood viscosity and increasing microcirculatory flow and tissue perfusion (Aviado and Porter, 1984). Pentoxifylline has been used in horses as part of treatment protocols for a wide range of varying conditions, including SIRS (systemic inflammatory response syndrome) from endotoxemia, laminitis, navicular disease and placentitis (Carr, 2015). Evidence for efficacy of these varying conditions is marginal. One study showed that pentoxifylline administration did not result in an increase in blood flow to the digit or dorsal laminae in healthy horses (Ingle-Fehr and Baxter, 1999), which undermines its potential benefits for the treatment of laminitis, although how these findings apply to the clinical disease is unclear.

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013), for treatment of endotoxaemia and laminitis. Flunixin and acepromazine are identified as alternatives and several reasons are given as specific advantages for (i) endotoxaemia: different mode of action (methylated xanthine derivative phosphodiesterase inhibitor) and different clinical effects to alternative (flunixin); decreases endotoxin- mediated release of pro-inflammatory cytokines and leukotrienes from macrophages and neutrophils, reduces systemic response to endotoxins; and (ii) laminitis: different mode of action of improving blood flow to the digit than alternative (acepromazine); reduces blood viscosity and improves blood flow to the digit.

To a large extent, the current listing of pentoxifylline is based on theories of the pathogenesis of these diseases that suggested abnormalities in either blood flow/viscosity or micro-blood clots were responsible for severity of these conditions. However, knowledge of pathogenesis has improved, where abnormalities in either blood flow/viscosity or micro-blood clots are no longer understood as related to the pathogenesis of these conditions.

As previously indicated, despite common use, evidence for its efficacy in horses is marginal. There are alternative treatment options with well-established efficacy profiles for the current indications (i.e. endotoxaemia and laminitis). Alternatives include e.g. acepromazine, proposed for inclusion in the list or NSAIDs e.g. flunixin, listed in Table 1 of the Annex to Commission Regulation (EU) 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin with MRLs set for tissues of *Equidae*.

Endotoxaemia and laminitis, if untreated, are potentially life-threatening and cause unacceptable suffering of the animal.

Pentoxifylline was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any pentoxifylline-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance pentoxifylline is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Vasopressin is a peptide hormone, used to increase blood pressure in patients with vasodilatory shock who are resistant to fluid and catecholamine therapy. It increases vasoconstriction and renal fluid reuptake by acting through vasopressin receptors.

It is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for treatment of circulatory collapse in foals and adult horses. The identified alternatives in the current list are dopamine/dobutamine and epinephrine and the specific advantages are as follows: specific agonist

acting via V1 receptors; has a different mode of action to the other authorised substances which regulate blood pressure: epinephrine (an adrenergic receptor agonist) and dopamine/dobutamine (D1-5 receptors regulating cardiac output and blood vessel tone). Used in situations when dopamine/dobutamine and epinephrine have been unsuccessful, and an alternative pharmacological approach is needed.

Vasopressin can restore vascular tone in refractory vasodilatory shock states due to activation of V1 vascular receptors, modulation of ATP-sensitive potassium channels (K+ATP), modulation of nitric oxide (NO) and potentiation of adrenergic and other vasoconstrictor agents (Holmes et al., 2004). From the alternatives listed in the current list, epinephrine is included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with a 'No MRL required' status for all food-producing species and both dopamine and dobutamine are retained in the list of essential substances. However, it is considered necessary to have vasopressin as an available alternative in cases where standard catecholamine therapies like dopamine/dobutamine and epinephrine are ineffective or require potentiation to restore vascular tone in refractory vasodilatory shock states.

Circulatory collapse, if untreated, is life-threatening and causes unacceptable suffering of the animal.

Vasopressin was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any vasopressin-containing veterinary medicinal products authorised for use in equine species (neither in other animal species).

The substance vasopressin is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: treatment of circulatory collapse in foals and adult horses. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Knowledge regarding hypotension or respiratory medicines during anaesthesia in horses for this assessment was derived from textbooks, review articles, and retrospective studies. No clinical trials could be identified in horses.

B. Considerations regarding consumer safety

Allopurinol and its metabolite, oxypurinol, are inhibitors of the enzyme xanthine oxidase which is responsible for the conversion of oxypurines to uric acid. Other metabolites of allopurinol include allopurinol-riboside and oxipurinol-7-riboside (Reiter et al., 1983). Allopurinol is authorised in the EU as human medicinal products.

In humans, approximately 90% is absorbed from the gastrointestinal tract after oral dosing. Peak levels after oral allopurinol administration occur at 1.5 and 4.5 hours for allopurinol and oxypurinol, respectively (Plumb, 2015). Approximately 20% of the oral dose is excreted in faeces. Elimination of allopurinol occurs mainly by metabolic conversion to oxypurinol by xanthine oxidase and aldehyde oxidase, with less than 10% of the unchanged allopurinol excreted in the urine. Allopurinol has a plasma half-life of about 0.5 to 1.5 hours. Oxypurinol is a less potent inhibitor of xanthine oxidase than allopurinol, but the plasma half-life of oxypurinol is far more prolonged; estimates range from 13 to 30 hours in humans (Plumb, 2015). Therefore, effective inhibition of xanthine oxidase is maintained over a 24-hour period with a single daily dose of allopurinol. Patients with normal renal function will gradually accumulate oxypurinol until a steady-state plasma oxypurinol concentration is reached. Such patients, taking e.g. 300 mg allopurinol/day will generally have plasma oxypurinol concentrations of 5-

10 mg/l. Oxypurinol is eliminated unchanged in the urine but has a long elimination half-life because it undergoes tubular reabsorption. Reported values for the elimination half-life range from 13.6 hours to 29 hours. The product information of human medicinal products states that the large discrepancies in these values may be accounted for by variations in study design and/or creatinine clearance in human patients.

In dogs (Dalmatians) absorption rates were variable between subjects. Peak levels occur within 1-3 hours after oral dosing. Elimination half-life is about 2.7 hours. The serum half-life is 2 and 4 hours for allopurinol and oxypurinol, respectively (Papich, 2021).

In horses, oral bioavailability of allopurinol is low, approximately 15%. Allopurinol is rapidly converted to oxypurinol; the elimination half-life of allopurinol is approximately 5-6 minutes. Oxypurinol has an elimination half-life of about 1.1 hours (Plumb, 2015).

Allopurinol is distributed in total body tissue water. Levels in the central nervous system (CNS) are only about 50% of those found elsewhere. Neither allopurinol nor oxypurinol are bound to plasma proteins, but both drugs are excreted in milk. Plumb (2015) indicates that highest allopurinol concentrations in animals are found in blood, liver, intestine and heart, with the lowest levels noted in brain and lung tissues.

While the safe use of allopurinol during pregnancy has not been established, dosages of up to 20 times the normal dose in rodents have not demonstrated decrease in fertility. Infertility in male humans has been reported with the drug, but a causal effect has not been firmly established. In humans, the FDA categorizes this drug as category C for use during pregnancy i.e. animal studies have shown an adverse effect on the foetus, but there are no adequate studies in humans; or there are no animal reproduction studies and no adequate studies in humans (Plumb, 2015).

The product information of human medicinal products states that animal reproductive toxicity studies have shown conflicting results. One study in mice receiving intraperitoneal doses of allopurinol of 50 or 100 mg/kg on days 10 or 13 of gestation resulted in foetal abnormalities, however in a similar study in rats at 120 mg/kg on day 12 of gestation, no abnormalities were observed. Extensive studies of high oral doses of allopurinol in mice up to 100 mg/kg/day, in rats up to 200 mg/kg/day and in rabbits up to 150 mg/kg/day, during days 8 to 16 of gestation, produced no teratogenic effects. An in vitro study using foetal mouse salivary glands in culture to detect embryotoxicity, results indicated that allopurinol would not be expected to cause embryotoxicity.

Regarding mutagenicity, the product information of human medicinal products states that cytogenetic studies show that allopurinol does not induce chromosome aberrations in human blood cells in vitro at concentrations up to 100 µg/ml and in vivo at doses up to 600 mg/day for a mean period of 40 months. Allopurinol does not produce nitroso compounds in vitro or affect lymphocyte transformation in vitro. Evidence from biochemical and other cytological investigations strongly suggests that allopurinol has no deleterious effects on DNA at any stage of the cell cycle and is not mutagenic. No evidence of carcinogenicity has been found in mice and rats treated with allopurinol for up to 2 years. Allopurinol is not listed by the IARC.

Residue depletion data for horses is not available.

Considering that the substance is intended for treatment of ischemia-reperfusion damage in newborn foals, its low oral bioavailability (around 15%) and the rapid elimination described in horses, it can be accepted that allopurinol 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.

Dobutamine is a direct-acting inotropic agent whose primary activity results from stimulation of the beta-adrenoceptors of the heart. It acts primarily on beta-1 adrenergic receptors, with negligible effects on beta-2 or alpha receptors. It does not cause the release of endogenous norepinephrine, as does dopamine. It is indicated in humans to increase the contractility of the heart in acute heart failure, as occurs in cardiogenic shock and myocardial infarction; it is also used in septic shock, cardiac surgery, and positive end-expiratory pressure ventilation.

As a result of enzymatic degradation in the gut and first-pass metabolism in the liver, dobutamine has a poor oral bioavailability. It has a half-life of about 2 minutes in humans (Scriba, 2009; Patel et al., 2012).

Its pharmacokinetics has been evaluated in neonates since it is one of the most commonly used sympathomimetic inotropes to treat hypotension in neonates. It has a rapid onset of action and short half-life. The mean volume of distribution in children is 1.14 l/kg, which is higher than that observed in adults (0.202 l/kg). Wide ranges of dobutamine clearance have been reported. Mahoney et al. (2016) conducted a literature review and concluded that the majority of studies appear to show that dobutamine displays first-order kinetics with regards its elimination; however, they also admit there is a wide inter-patient spread with regards to its pharmacokinetics.

Data from a 9-year-old child with heart failure showed that approximately 80% of dobutamine administered intravenously at steady state was detected in the urine; forty-seven percent of infused dobutamine was identified as 3-O-methyldobutamine and its acid-hydrolysed derivatives, indicating that the formation of 3-O-methyldobutamine constitutes a major pathway of dobutamine metabolism in humans; small amounts are eliminated in the faeces (Yan et al., 2002; Scriba, 2009).

Dobutamine has inotropic effects. In humans, toxicity is rare, and symptoms are due to sympathetic overstimulation and can include chest pain, palpitations, headaches, tremors, shortness of breath, nausea and vomiting (Ashkar et al., 2023). Dobutamine is not classified by the IARC.

It is acknowledged that no pharmacokinetic or residue data are available for horses.

Considering the very short half-life observed in humans, the small volume of distribution and the poor oral bioavailability, it can be accepted that dobutamine 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.

Dopamine is a catecholamine sympathomimetic that is used by intravenous infusion in human and veterinary medicine.

A two-compartment model was described for dopamine pharmacokinetics in humans (Le Corre et al., 1993; MacGregor et al., 2000). However, variability in pharmacokinetics of dopamine have been observed. Biotransformation of dopamine proceeds rapidly to yield 3-4-dihydroxy-phenylacetic acid (DOPAC) and 3-methoxy-4-hydroxy-phenylacetic acid (homovanillic acid, HVA). It has been reported that about 80% is excreted in the urine within 24 hours, primarily as HVA and its sulfate and glucuronide conjugates and as DOPAC. A small portion is excreted unchanged (Drugbank, 2023).

In one study the plasma concentrations of dopamine measured varied widely after a continuous infusion of 10 µg/kg/min for 10 mins and 3 µg/kg/min for 90 mins. Dopamine is known to increase cardiac output, which, in turn, may affect its own clearance; thus, rates of clearance and other pharmacokinetic parameters may change as plasma dopamine concentrations change. Micro rates constants were observed: initial and terminal half-lives were 0.5 and 12.3 mins, respectively; this was in accordance with the immediate increase and decrease of dopamine concentrations before and after infusion (MacGregor et al., 2000).

The steady-state volume of distribution and the apparent terminal elimination half-life have been described to increase with the dose: 0.78 to 1.58 L/kg and 22.1 to 37.9 mins, after infusions of 3 and 6 µg/kg/min, respectively (Le Corre et al., 1993). Intravenously administered dopamine was sulfoconjugated rapidly and to a great extent. An initial half-life of elimination of 4.8 mins was observed in healthy subjects. However, persistent elevations of free and conjugated dopamine were observed even 18 hours after cessation of an infusion of 2 µg/kg/min (Onasch et al., 2000). The apparent volume of distribution was 2952 ml/kg in children, while the elimination half-life was approximately 26 mins (Eldadah et al., 1991). Pharmacokinetics of intravenous and oral dopamine have also been studied in dogs. Biological half-lives of dopamine, dopamine-3-O-sulfate and DOPAC were calculated to be 10.8, 38.4 and 24.6 mins, respectively, after intravenous administration. After oral administration, the absolute bioavailability was approximately 3%. Dopamine was well absorbed from the intestine, so the low bioavailability was described to be mainly due to the extensive metabolism during the absorption process (Murata et al., 1988).

As sympathomimetic agent, dopamine may have adverse effects relating to both its alpha and beta agonist properties. Dopamine has a short duration of action and most adverse effects respond to discontinuing or reducing its infusion rate. It has been reported that dopamine does not penetrate the blood-brain barrier.

Regarding toxicity studies, a subacute rat toxicity study concluded that at doses of 100 mg/kg per day or less there were no consistent pathological findings attributable to the drug (Zarolisnki et al., 1977). The product information of human medicinal products suggests that animal reproduction studies in rats and rabbits at doses up to 6 mg/kg/day intravenously during organogenesis produced no detectable teratogenic or embryotoxic effects, although maternal toxicity was observed in rats. Administration of 10 mg/kg to pregnant rats throughout gestation or for 5 days starting on gestation day 10 or 15 resulted in decreased body weight gain, increased mortality, and slight increase in cataract formation among the offspring. Dopamine is not listed by the IARC.

No information on pharmacokinetics in the horse is available and the available data in humans describe a high variability between individuals. Based on the pharmacokinetic data in humans and dogs, and considering the short duration of the effects, the low oral bioavailability because of the first-pass metabolism, it can be accepted that dopamine 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.

Ephedrine is a sympathomimetic amine that exhibits several adrenaline actions; it is used in both human and veterinary medicine for different indications/purposes. It raises the blood pressure by increasing cardiac output and by inducing peripheral vasoconstriction. Ephedrine also causes bronchodilatation, reduces intestinal tone and motility, relaxes the bladder wall while contracting the sphincter muscle and relaxing the detrusor muscle of the bladder and usually reduces the activity of the uterus (Scriba, 2009).

The pharmacokinetics of ephedrine have been described in humans, especially after oral administration. It is readily and completely absorbed from the gastrointestinal tract; volumes of distribution of 180-215 litres have been described (Pickup et al., 1976; Csajka et al., 2005). The major route for elimination in humans is urinary excretion. Following an oral dose of 0.35 mg/kg, 88% of the dose was excreted in the urine within the first 24 hours, reaching 97% within 48 hours. Unchanged drug was the major urinary excretory product (53-74%), with N-demethylation occurring to a variable extent (8-20%) (Sever et al., 1975). Ephedrine has been reported to have a plasma half-life ranging from 3 to 6 hours (Scriba, 2009; Pickup et al., 1976; Csajka et al., 2005; Welling et al., 1971). Clearances of 0.34 l/min have been described (Csajka et al., 2005).

In rats, the elimination half-life of pure ephedrine was 93.9 mins (Gad et al., 2021). The metabolism was also investigated in the rabbit by administering radiolabelled D(-)-ephedrine and L(+)-ephedrine. The majority of total radioactivity (71-91%) was excreted within 24 hours. The analysis of the urine revealed that 47-50% of the urinary 14C was attributable to acidic metabolites; it was concluded that the major pathway for the biotransformation involves N-demethylation and oxidative deamination of the side chain (Feller and Malspeis, 1977). A minor binding affinity to albumin (5-10%) has been reported (Gad et al., 2021).

Regarding toxicity studies, fourteen-day and 13-week toxicity studies performed in rats and mice revealed a compound-related reduced weight gain, but no deaths were attributable to compound-related toxicity. During 2-year studies in rats and mice performed with 125 or 250 ppm, the ingestion of ephedrine was also associated with reduced weight gain, while no evidence of compound-related carcinogenicity was observed. Ephedrine was not mutagenic in four strains of *Salmonella typhimuriym* and no induced sister-chromatid exchange or chromosomal aberrations were observed either (NTP, 1986).

Ephedrine has both alpha- and beta-agonist effects and its most common adverse effects are tachycardia, anxiety, restlessness and insomnia; tremor, dry mouth, impaired circulation to the extremities, hypertension, and cardiac arrhythmias may also occur. Clinical cases of acute hepatitis and liver failure after taking ephedrine have been reported, as well as addiction problems when used over an extended period of months. Due to the amphiphilic nature of ephedrine, it can readily cross the blood-brain barrier. Ephedrine may even induce toxic psychosis, but neurotoxicity has been related to its prolonged use. Furthermore, in some psychiatric events in humans, up to 50 times the recommended dose was ingested (Gad et al., 2021; Maglione et al., 2005).

No Information on the pharmacokinetic of ephedrine in the horse is available; it is noted that several toxic effects have been described for ephedrine in humans, related especially to its chronic use. However, human pharmacokinetic data suggests that although ephedrine distributes widely through the body, it is eliminated rapidly in the urine after oral administration. It should be also noted that ephedrine, for the proposed used in *Equidae*, is to be administered as a single dose during anaesthesia to increase cardiac output, stroke volume and arterial blood pressure.

Considering all the above, it can be accepted that ephedrine 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.

Glycopyrrolate is an anticholinergic medication commonly used in human medicine. It is used perioperatively as a muscarinic receptor antagonist to inhibit salivary gland and respiratory secretions. The most frequent reasons for administering anticholinergics include producing an antisialagogue effect, creating a sedative and amnesic effect, and preventing reflex bradycardia (Gallanosa et al., 2023). In veterinary practice it is used for pharmacological reversal of vagally mediated bradycardia in domestic large and small animals (Rumpler et al., 2011).

Pharmacokinetic data has been obtained in both horses and humans. Plasma pharmacokinetics of glycopyrrolate in the horse following a single intravenous dose can be characterized by a three-compartment model. A wide distribution from the central compartment, rapid clearance and prolonged terminal half-life were observed in different studies. The median values for the volume of distribution at steady state observed after intravenous administration of 1.72-1.93 µg/kg, 1.96-2.25 µg/kg and infusion of 8 µg/kg to horses were 1.43, 3.69 and 0.78 l/kg, respectively. Median values for plasma clearance were 20.1, 16.7 and 13.6 ml/min/kg while the median terminal half-lives were 7.40, 18.9 and 13.2 hours, respectively. Concentrations above the LOQ of the method were obtained for up to

168 hours in one study. The fraction bound to plasma protein over a range of plasma drug concentrations (0.1-25 ng/ml) was determined as 37-44% (Rumpler et al., 2011, 2014a, 2014b).

In the horse, the total amount of glycopyrrolate excreted in the urine through 24 hours was characterized by a median of 0.140 mg or 14% of the total administered dose; median renal and nonrenal clearances were estimated to be 2.654 and 14.1 ml/min/kg, respectively. It was hypothesized that the majority of nonrenal clearance is hepatic clearance and, further, the majority of total clearance is attributed to hepatic clearance. This observation contrasts with pharmacokinetic studies in humans that have reported that over 50-80% of the intramuscularly administered dose was excreted unchanged in the urine. It was therefore concluded that in horses the drug is highly extracted by the liver (Ali-Melkkilä et al., 1993; Rumpler et al., 2014b).

In children, following a single oral (50 µg/kg) and intravenous (5 µg/kg) administration of glycopyrrolate, a negligible and variable oral bioavailability was found (3.3%, 1.3-13.3%) (Ali-Melkkilä et al., 1993; Rautakorpi et al., 1998). Inhaled glycopyrrolate has a bioavailability of 57% with a terminal elimination half-life of 52.5 hours (versus 6.2 hours noted following intravenous administration) (Tashkin and Gross, 2018).

When compared with other anticholinergic medications, glycopyrrolate is less likely to cause CNS toxicity because it does not cross the blood-brain barrier. Toxicity studies have been performed in rodent species (PubChem, 2023a).

Pharmacokinetic data on glycopyrrolate in the horse suggests that it is rapidly eliminated from plasma; furthermore, oral bioavailability is low. Considering this, it can be accepted that glycopyrrolate 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.

Noradrenaline/norepinephrine is a sympathomimetic used in human and veterinary medicine. It is used intravenously for the control of blood pressure and as an adjunct treatment during cardiac arrest.

Norepinephrine pharmacokinetics have been described in humans. Like adrenaline, it is inactive when given orally and it is rapidly inactivated in the body. When given intravenously it is extensively metabolized and only small amounts are excreted unchanged in the urine. It has been described to be rapidly eliminated from the blood, with a half-life of 2-2.5 min (Beloeil et al., 2005; Scriba, 2009).

A one compartment model with linear elimination adequately described the kinetics in critically ill children. Clearance for a 10 kg-patient was 37.1 l/h and half-life was 0.9 min (Oualha et al., 2014). One other study evaluated the kinetics in septic shock and trauma patients; the clearance varied from 3 l/min in the least severely ill patients to 0.9 l/min in the most severe, while the half-life varied from 2 min to 6.8 min, respectively (Beloeil et al., 2005).

In dogs, exogenously administered norepinephrine was eliminated primarily by the liver. Additional elimination also occurs in the kidney and capillary walls, especially in lungs (Chu et al., 1999).

The toxicity of norepinephrine is generally directly related to its mechanism of action. Systemic toxicity manifests as uncontrolled hypertension (possibly associated with reflex bradycardia), headache, and peripheral ischaemia. Acute toxicity studies have been performed in mice (Pubchem, 2023b). In vitro mutagenicity tests showed that it was not genotoxic, noting negative results for in vivo micronucleus test were predicted based on epinephrine results. For the carcinogenicity endpoint, a conclusion could not be reached (Aydin and Tugcu, 2018). It is not listed by IARC.

No information about the kinetics of norepinephrine is available in horses. However, the available information in humans show that it is rapidly eliminated from the blood, even in critically ill patients

and, furthermore, it is inactive when given orally. Therefore, it can be accepted that noradrenaline/norepinephrine 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.

Vasopressin (also known as 'arginine vasopressin', 'argipressin' or 'antidiuretic hormone') promotes the renal reabsorption of solute-free water in the distal convoluted tubules and the collecting duct. It increases cyclic adenosine monophosphate (cAMP) at the tubules, which increases water permeability at the luminal surface, resulting in increased urine osmolality and decreased urine flow. It acts through at least 5 different receptors i.e. three subtypes, V1, V2, V3, the oxytocin receptor, and the purinergic P2 receptor (Plumb, 2015; Papich 2021). Vasopressin is authorised in the EU as human medicinal products.

Vasopressin's structure shares 80% homology with oxytocin, with the only difference being the third and eighth amino acids; consists of a nonapeptide with a disulphide bridge between two cysteine amino acids. Vasopressin is a pro-hormone produced primarily in the magnocellular neurons of the paraventricular and supraoptic nuclei of the hypothalamus from which migrates to the posterior pituitary (Pelletier et al., 2014).

In humans, vasopressin is known to be degraded in the gastrointestinal tract prior to being absorbed (Plumb, 2015; Drucker, 2020); thus, it is recommended to be administered either intranasally or parenterally. It distributes throughout the extracellular fluid. It is not bound to plasma proteins and is rapidly metabolised in liver and kidneys. The plasma half-life has been reported to be around 10-20 minutes in humans (Plumb, 2015).

The product information of human medicinal products states that no animal reproduction studies have been performed with vasopressin. In reproductive toxicity studies with related substances, abortions and malformations were observed. Vasopressin may cause uterus contractions and increased intra-uterine pressure during pregnancy and may reduce uterine perfusion and should therefore not be used during pregnancy unless clearly needed. No evidence is available regarding genotoxicity or carcinogenicity. Vasopressin is not classified by the IARC nor in ECHA's C&L inventory.

Residue depletion data for horses is not available.

Considering that the substance is a natural hormone, and that following oral administration is degraded by enzymes in the human gastrointestinal tract, noting the limited available evidence, it can be accepted that vasopressin 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.

4.13.3. Assessment of new substances proposed to be added to the list in the stakeholders survey

A. Considerations on the essentiality of the substance(s)

Aminocaproic acid, also known as ε-aminocaproic acid, was mentioned (once) in the survey to stakeholders and it was suggested for addition to the list as an antifibrinolytic agent for treatment of certain bleeding disorders. Scientific references were provided and are discussed below.

In horses, plasma concentrations of 5.8 µg/ml are sufficient to inhibit fibrinolysis (Fletcher et al., 2013). In a study describing its pharmacokinetics, aminocaproic acid was administered with intravenous infusion for 20 minutes in six horses and resulted in no significant change in plasma α2-antiplasmin activity at 1 or 4 hours after infusion; thirty minutes after infusion, platelet function was significantly different from that at time 0 and 1 and 4 hours after infusion (Ross et al., 2007). Aminocaproic acid is occasionally used prior to episodes of intense training in racehorses suffering from

exercise-induced pulmonary haemorrhage (EIPH) (Maxwell et al., 2023), although data are inconclusive. Buchholz et al. (2010) found that aminocaproic acid was not effective in preventing or reducing the severity of EIPH in 8 Thoroughbreds horses. Aminocaproic acid has also been used to stabilise clotting or slow bleedings in horses with haemorrhage from guttural pouches. It is suggested that aminocaproic acid may be useful only in horses with active hyperfibrinolysis (Ross et al., 2007).

Epsilon-Aminocaproic Acid (EACA) is a lysine analogue and inhibits plasminogen activator, decreases plasmin formation, and stimulates release of α2-antiplasmin from endothelial cells (Fletcher et al., 2013). It belongs to the haemostatic agents. Intravenous administration of 30 and 100mg of EACA/kg to clinically normal horses significantly modified some laboratory measures of haemostasis, consistent with its antifibrinolytic effects (Heidmann et al., 2005). It has been used for treatment of castration-associated haemorrhage, uterine artery haemorrhage, mycosis of the auditory tube diverticulum (i.e. guttural pouch), intra-abdominal haemorrhage and ongoing bleeding. It can also minimise blood loss from small vessels and seems to be useful when severe hypertension is present (Heidmann et al., 2005). It is also used by racetrack practitioners in horses suffering EIPH and is often administered as a preventative during training (Maxwell et al., 2023). However, it is known that administration of EACA to horses results in alterations in partial thromboplastin time (PTT), plasma α2-antiplasmin activity, and plasma fibrinogen concentration. Etamsylate, a haemostatic and angioprotective agent, is included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with a 'No MRL required' status for all food-producing species, including Equidae, and could serve as satisfactory alternative for which there are veterinary medicinal products authorized for equivalent indications for food-producing animals of the equine species. Aminocaproic acid is not considered to bring added clinical benefit compared to etamsylate.

Active hyperfibrinolysis, if untreated, is life-threatening and causes unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any aminocaproic acid-containing veterinary medicinal products authorised for use in equine species (neither in other animal species).

The substance aminocaproic acid is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering for the animal.

Clopidogrel was mentioned (once) in the survey to stakeholders and it was suggested for addition to the list as an antiplatelet agent to reduce platelet activity and platelet-polymorphonuclear leukocytes (PMN) aggregates in inflammatory conditions such as colitis, pleuropneumonia, retained placenta, and laminitis. The responder further stated that it is beneficial for horses at risk of endotoxaemia and laminitis secondary to obstetric, surgical, and gastrointestinal disorders. Scientific references were provided and are discussed below.

The effects of clopidogrel on equine platelet function have been investigated. Hernandez et al. (2016) reported that standard platelet inhibitors, including clopidogrel, did not affect equine herpesvirus type I (EHV-I) – induced platelet activation in two healthy adult horses ex vivo, although clopidogrel did inhibit ADP-induced aggregation in platelet-rich plasma samples obtained after that treatment session. Similarly, clopidogrel and its active metabolite inhibited ADP-induced platelet aggregation after oral administration of a single dose to 6 healthy adult horses within the first 24 hours after administration (Norris et al., 2019). Roscher et al. (2015) found that a loading dose of clopidogrel rapidly inhibited

platelet function, with maintenance doses sustaining this effect over 4 days; recover of platelet function was restored 6 days after cessation of medication. In horses with experimentally induced endotoxaemia, clopidogrel was associated with variable platelet antiaggregatory activity and attenuated some clinical signs of the condition (Watts et al., 2014).

Clopidogrel is an anticoagulant and acts like a platelet aggregation inhibitor. It is used as an antiplatelet agent for the treatment of diseases associated with platelet activation in horses such as in laminitis, thrombosis and endotoxaemia therapy (Brooks et al., 2013; Brainard et al., 2011; Watts et al., 2014). It is a prodrug that is oxidised by cytochrome P450 (CYP3A4 and CYP2C19). The active metabolite forms a covalent bond with the platelet receptor P2Y12 and irreversibly prevents ADP-associated platelet activation. An effect is measurable after two to four hours, the maximum effect is reached after three to seven days. Platelet function normalises five to seven days after discontinuation. Acetylsalicylic acid and sodium acetylsalicylate are included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with 'No MRL required' entry for all food-producing species except fin fish, including Equidae, and for acetylsalicylic acid there are veterinary medicinal products authorised for food-producing animals of the equine species for equivalent indications. Brainard et al. (2012) investigated the effect of clopidogrel and aspirin on the expression of inflammation-induced P-selectin (CD62P) in equine platelets stimulated by thrombin and on circulating PMN platelet aggregates in healthy horses. No significant changes in CD62P positive platelets or platelet-PMN aggregates were observed in horses treated with either drug suggesting both have similar clinical effect. There is no identified added clinical benefit of clopidogrel compared to acetylsalicylic acid and sodium acetylsalicylate, both of which have an MRL entry for food-producing species including *Equidae*. In addition, dalteparin is proposed to be added to the list as an anticoagulant, and heparin is included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with 'No MRL required' entry for all food-producing species.

Coagulation issues, if untreated, are life-threatening and cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any clopidogrel-containing veterinary medicinal products authorised for use in equine species (neither in other animal species).

The substance clopidogrel is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering for the animal.

Dalteparin, **enoxaparin** and **nadroparin** were mentioned (twice, twice, once, respectively) in the survey to stakeholders and suggested for addition to the list as anticoagulants, and one respondent mentioned its use for the prevention of laminitis (de la Rebière de Pouyade et al., 2009).

All three belong to the group of low molecular weight heparins (LMWHs), used as anticoagulants in horses and other species (Schwarzwald, 2002). LMWHs are derived from heparin (i.e. unfractionated heparin) through various chemical/enzymatic steps. Thus, they are essentially similar in chemical structure to heparin; differing only in the number of repeating units and the termini left and right.

Unfractionated heparin ranges in molecular weight from approximately 16,000 Da. This average molecular weight is in excess of what is appropriate for binding to the heparin-binding sites on Factor Xa and antithrombin. The above issue with unfractionated heparin led to the development of LMWHs which exhibit enhanced efficacy and safety profiles due to their optimised molecular size (e.g. enoxaparin has an average molecular weight of 4,500 Da; dalteparin 5,000 Da). This reduction in

molecular size is associated with a loss of thrombin inhibitory activity, but conversely an increase in FXa inhibition compared to unfractionated heparin (Mulloy et al., 2016).

The mechanism of action of LMWHs is like that of heparin. They are acting by inhibition of the final common pathway of the coagulation cascade (Mulloy et al., 2016). The coagulation cascade's goal is to fluid blood into a clot, thus preventing bleeding. The final common pathway is the conversion of fibrinogen into fibrin by the activity of thrombin. LMWH inhibits coagulation by activating antithrombin III. Antithrombin III binds to and inhibits factor Xa. In doing so, it prevents activation of the final common path; Xa inactivation means that prothrombin is not activated to thrombin, thereby not converting fibrinogen into fibrin for the formation of a clot.

Despite 'Heparin and its salts' being listed in Table 1 of the Annex to Commission Regulation (EU) No 37/2010, it can be accepted that LMWHs bring added clinical benefit: their reduced molecular size is associated with a loss of thrombin inhibitory activity, but conversely an increase in FXa inhibition compared to unfractionated heparin.

Dalteparin is the LMWH for which there is more available evidence in horses (Armengou et al., 2010; Whelchel et al., 2013; Rodríguez-Pozo et al., 2017) while there are no reports suggesting added benefit from one LMWH over others.

Coagulation issues, if untreated, may be life-threatening and cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any dalteparin-, enoxaparin- or nadroparin-containing veterinary medicinal products authorised for use in equine species (neither in other animal species).

The substance dalteparin is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: anticoagulant. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal. Enoxaparin and nadroparin are not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species.

Gelatinpolysuccinate (or succinylated gelatin/modified fluid gelatin) was mentioned once in the survey to stakeholders, and it was suggested for addition to the list as an alternative to hydroxyethyl-starch (hetastarch) for a fluid resuscitation regimen (in particular, given hetastarch's limited or lack of availability in many countries due to adverse issues with use in human medicine).

Gelatinpolysuccinate is a colloid solution, consisting of gelatin polypeptides derived from bovine collagen that has been used in human medicine since the 1950s (Parkins et al., 1953; Boyd et al., 2021). While it was previously considered a second-choice volume expander for critically ill patients due to a relatively high rate of anaphylactic reactions, the recent removal of the main alternative volume expander – hetastarch – from the human market in the EU has led to its increased use (Ziebart et al., 2018). Gelatinpolysuccinate use in equine patients has been reported (Boyd et al., 2021) and while its efficacy as a volume expander is well accepted, most evidence comes from experiences in human medicine or very limited experimental studies on healthy animals (Gratwick et al., 2017). To note that on 11 February 2022, EMA's pharmacovigilance committee for human medicinal products, PRAC, recommended that the marketing authorisations for hetastarch solutions for infusion should be suspended across the EU.

These products were authorised as an addition to other treatments for plasma volume replacement following acute (sudden) blood loss. Alternative products for short-term volume expansion would be

hypertonic solutions (crystalloids) such as hypertonic saline; such products are available and licensed for various animal species, including food-producing animals of the equine species. While colloids are often thought superior for long-term intravascular volume expansion, a recent large Cochrane review suggested that based on current evidence, there is probably little or no difference in outcomes between critically ill patients treated with colloids or crystalloids (Lewis et al., 2018). The smaller volume of colloids needed for a clinical effect may have advantages, however, particularly in large animals such as horses, where administrating sufficient volumes quickly enough can be challenging. Colloids are large molecules compared to crystalloid small molecules that stay longer in the intravascular space, which is an advantage for colloids for correcting hypovolemia from conditions like e.g. hypoalbuminemia.

Hypovolaemia, if untreated, is life-threatening and causes unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any gelatinpolysuccinate-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance gelatinpolysuccinate is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: to address long-term hypovolaemia resulting from conditions like e.g. low albumin. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Tranexamic acid was mentioned (once) in the survey to stakeholders and it was suggested for addition to the list. No specific indication was mentioned, and no scientific references were provided.

It is used as an antifibrinolytic drug for the treatment of various bleeding disorders such as coagulation and fibrinolysis in horses (Fletcher et al., 2013). It belongs to the group of hemostatic agents. Etamsylate, a haemostatic and angioprotective agent, is included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with a 'No MRL required' status for all food-producing species, including *Equidae*, and could serve as satisfactory alternative for which there are veterinary medicinal products authorized for equivalent indications for food-producing animals of the equine species. Tranexamic acid is not considered to bring added clinical benefit compared to etamsylate.

Active hyperfibrinolysis, if untreated, is life-threatening and causes unacceptable suffering for the animal; it does not pose a risk for public health.

A search in the veterinary medicines database does not retrieve any tranexamic acid-containing veterinary medicinal products authorised for use in equine species (neither in other animal species).

The substance tranexamic acid is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering for the animal.

Knowledge regarding hypotension or respiratory medicines during anaesthesia in horses for this assessment was derived from review articles, retrospective studies and clinical trials in horses.

B. Considerations regarding consumer safety

Dalteparin is a low molecular weight heparin (LMWH) with a high affinity binding site essential to the plasma antithrombin (ATIII), thus enhancing ATIII activity inhibiting the formation of both factor Xa and thrombin.

In dogs, dalteparin is completely absorbed after subcutaneous injection. It has a volume of distribution of 50-70 mL/kg and a half-life of about 2 hours (shorter than that reported in humans). In cats, after subcutaneous administration dalteparin is completely absorbed with a half-life of about 2 hours too (Mischke et al., 2012). Cats seem to have shorter duration of (anti-Xa) activity associated with LMWHs than humans do (Alwood et al., 2007). In horses, dalteparin pharmacokinetics is similar to that reported in humans, with the main difference being the (higher) frequency of administration needed to maintain the anti-factor Xa activity; adequate effects observed dosing twice daily (Whelchel et al., 2013).

In humans, after subcutaneous injection, dalteparin is absorbed rapidly with a bioavailability of about 87%; peak plasma levels occur in about 4 hours. Anti-factor Xa activity persists for up to 24 hours and doses are usually given once a day (rarely twice). Dalteparin is excreted via the kidneys in the urine; elimination half-life is about 3-5 hours. Half-life may be prolonged in patients with renal dysfunction (Plumb, 2015). Information in human medicinal products reports no evidence of teratogenicity or embryo-foetal toxicity in reproductive and developmental toxicity studies in pregnant rats and rabbits receiving intravenous dalteparin sodium doses up to 2,400 IU/kg in rats, and 4,800 IU/kg in rabbits, during organogenesis. These exposures were 2 to 4 times above the human therapeutic dose of 1000 IU/kg dalteparin. Limited published data on pregnant women and lactation is available. Information in human medicinal products reports dalteparin may be present in human milk in small amounts. While oral absorption in breastfeeding infants is expected to be low, any clinical implication remains unknown.

In horses, dalteparin pharmacokinetics is similar to humans. In a pharmacodynamic study where subcutaneous 50 IU/kg doses were administered twice daily, an adequate anti-factor Xa activity is reported; however, there was variability in anti-factor Xa activity between horses, which suggests the need for more studies being conducted (Whelchel et al., 2013; Plumb, 2015).

While data from depletion studies in horses is not available, available evidence suggests a similar pharmacokinetic profile in horses to that reported for humans, where peak plasma levels occur rapidly, in about 4 hours, and the anti-factor Xa activity persists for up to 24 hours with a short elimination half-life of about 3-5 hours. Considering this, it can be accepted that dalteparin 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.

Gelatinpolysuccinate is used in human medicine as a plasma volume expander similarly to the dextrans.

Little pharmacokinetic data is available. It is difficult to determine the gelatin derivatives in the blood circulation system. After infusion in humans, it rapidly distributes in the intravascular compartment. Seventy five percent of the dose is excreted in the urine in 24 hours. The half-life is about 4 hours (Scriba, 2009). From one study where hydroxyproline was used as a specific marker for the determination of gelatin plasma substitute in blood, the half-life of succinylated gelatin was calculated as 6.7 ± 0.2 h (Liu et al., 2011). According to information relating to human medicinal products, most of the infused modified fluid gelatin is excreted via the kidneys. Only a minor amount is excreted in faeces and not more than about 1% is metabolised.

No preclinical safety concerns have been identified.

Considering the characteristics of gelatinpolysuccinate, the pharmacokinetic properties reported, its low toxicity and the clinical use, it can be accepted that it 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.

4.13.4. Conclusion

Based on the above assessment and justifications, the following recommendations are proposed:

1. The following active substances, currently in Regulation (EU) 1950/2006 as amended by Regulation (EU) 122/2013, are proposed to be retained in the list, either without modification or with an amendment of the current entry as shown below:

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Allopurinol	neonatal ischaemia reperfusion injury	vitamin E	different mode of action in inhibiting the formation of reactive oxygen species (ROS) than vitamin E
Dobutamine	to manage hypotension under general anaesthesia	ephedrine	first-line medication for the treatment of hypotension in adult equines under general anaesthesia
Dopamine	as part of a treatment protocol for only acute kidney injury/renal failure	none identified	low doses have been shown to cause renal vasodilation, increased renal blood flow, and increased urine production without systemic cardiovascular effects in conscious healthy horses
Ephedrine	to treat hypotension under general anaesthesia	dobutamine	used to treat hypotension in adult equines under general anaesthesia where dobutamine is ineffective. Different mode of action to dobutamine with a more direct effect on cardiac contractility
Glycopyrrolate	to treat and prevent bradycardia	atropine	minimal central effect; suitable in conscious horses, before and after anaesthesia
Noradrenaline/ norepinephrine	for treatment of early septic shock; for support of cardiovascular function in critically ill foals	dobutamine, dopamine	in compromised (sick) foals it is generally the only catecholamine effective in treatment of hypotension
Vasopressin	treatment of circulatory collapse in foals and adult horses	epinephrine, dopamine, dobutamine	alternative in cases where standard catecholamine therapies like dopamine, dobutamine, epinephrine are ineffective or require potentiation to restore vascular tone in refractory vasodilatory shock states

2. The following active substances, currently in Regulation (EU) 1950/2006 as amended by Regulation (EU) 122/2013, are proposed to be removed from the list: hydroxyethyl-starch, pentoxifylline.

3. The following active substances, suggested for addition to the list in the survey to stakeholders, are proposed to be added to the list and the entry is proposed as shown below:

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Dalteparin	anticoagulant	heparin	reduction in molecular size is associated with a loss of thrombin inhibitory activity, but conversely an increase in FXa inhibition compared to unfractionated heparin
Gelatinpolysuccinate	to address long-term hypovolaemia resulting from conditions like e.g. low albumin	crystalloids	colloids are larger molecules compared to crystalloids (smaller molecules that stay longer in the intravascular space), which is an advantage for correcting hypovolemia from e.g. hypoalbuminemia

4. The following active substances, suggested for addition to the list in the survey to stakeholders, are not proposed for inclusion: aminocaproic acid, clopidogrel, enoxaparin, nadroparin, tranexamic acid.

4.14. Substances for tumours

4.14.1. Overview

	Active substance (ATCvet code)
Substances in Regulation (EU) 1950/2006 **proposed to be retained**, with or without amendment of the current entry	None
Substances in Regulation (EU) 1950/2006 **proposed to be removed**	None
Substances from stakeholders' survey **proposed for inclusion**	Imiquimod (D06BB10)
Substances from stakeholders' survey **not proposed for inclusion**	5-Fluorouracil (L01BC02); Cisplatin (L01XA01); Mitomycin (QL01DC03); Sarcoid Cream (no ATCvet code identified);

4.14.2. Review of the existing entries in Regulation (EU) 1950/2006, as amended by Regulation (EU) 122/2013, considering the survey results

No substance is currently listed in Regulation (EU) 1950/2006, as amended by Regulation (EU) 122/2013.

4.14.3. Assessment of new substances proposed to be added to the list in the stakeholder's survey

A. Considerations on the essentiality of the substance(s)

5-Fluorouracil (5-FU), **cisplatin** and **imiquimod** were mentioned (once each) in the survey to stakeholders and suggested for addition to the list. However, for both cisplatin and imiquimod there was neither a specific indication nor references provided; 5-FU was suggested for treatment of neoplastic lesions such as sarcoids and squamous cell carcinomas. The responder proposing 5-FU stated that intra-tumour injections of 5-fluorouracil demonstrated a 61.5% long-term resolution rate for equine sarcoids, with smaller tumour size and lack of prior treatment significantly correlating with

higher success rates, suggesting its favourable efficacy compared to other modalities, albeit with poorer outcomes for larger or previously treated sarcoids (Stewart et al., 2006). The responder stated that imiquimod could be used as alternative.

Imiquimod is a synthetic imidazoquinoline amine-based immune response modifier and belongs to a class of drugs known as toll-like receptor (TLR) agonists. Specifically, imiquimod acts as a TLR7 agonist. TLRs are a class of proteins that play a crucial role in the innate immune system by recognizing molecules that are common to pathogens. For example, TLRs have an important role in T cell activation.Imiquimod is primarily used for the treatment of certain skin conditions. It works by stimulating the local immune system response to produce interferon and other cytokines, which help the body's immune cells to recognize and destroy abnormal or infected cells. It has particularly been used to target and eliminate abnormal cells within the skin, for infectious and neoplastic conditions.

5-Fluorouracil and cisplatin belong to different chemical groups. 5-Fluorouracil is a chemotherapeutic drug that belongs to the class of drugs known as antimetabolites. It is a fluorinated analogue of the naturally occurring pyrimidine base uracil. Once taken up by a cell, 5-FU undergoes a series of biochemical transformations to become active. The active metabolites of 5-FU interfere with the normal functioning of thymidylate synthase, an enzyme involved in the synthesis of thymidine, a building block of DNA. By inhibiting thymidylate synthase, 5-FU reduces the production of thymidine, leading to an imbalance in nucleotide pools and ultimately disrupting DNA and RNA synthesis. Cisplatin is a platinum containing compound widely used as a chemotherapeutic drug. It exerts its anti-cancer effects though binding to DNA and interfering with the cell's ability to divide and replicate. The drug forms covalent bonds with purine bases (guanine and adenine) within DNA, leading to the formation of intra-strand and inter-strand crosslinks. These crosslinks disrupt the normal structure of DNA and hinder its replication and transcription processes. The cellular responses to this DNA damage can ultimately lead to cell cycle arrest and programmed cell death (apoptosis).

The expert group preparing this scientific advice has made the assumption that these substances (stated above) have been proposed for the treatment of sarcoids. Indeed, there is scientific literature available reporting the use of imiquimod, 5-FU, and cisplatin for the treatment of sarcoids.

Sarcoids are the most common cutaneous masses of horses, generally classified as being benign (i.e. rarely metastatic) that adversely impacts equine welfare due to their locally invasive, aggressive nature, or due to secondary infection or ulceration (Offer et al., 2024). Spontaneous regression without treatment has been seen in up to 48% of cases, particularly with young horses (Berruex et al., 2016). Equine sarcoids are strongly associated with impaired immune responses, as well as bovine papillomavirus (BPV) type 1 and less commonly type 2 (Ogłuszka et al., 2021; Monday et al., 2021; Jindra et al., 2023; Karalus et al., 2024), which requires different pharmacological approach. As described by Ogłuszka et al. (2021) equine sarcoids are characterized by a loss of tumour suppressor activity and changes allowing abnormal formation of the affected tissue, as well as immune defence abnormalities that weaken the host's immune response. While the aetiology is not fully understood, the most current research suggests that equine sarcoids likely result from a complex interaction between viral infection (particularly BPV-1 and BPV-2), genetic predisposition and host immune system function (Carr, 2016; Ogłuszka et al., 2021; Monday et al., 2021; Jindra et al., 2023; Karalus et al., 2024).

Imiquimod has been explored for various applications in veterinary medicine, primarily in the treatment of certain skin conditions and tumours in animals. In horses, the use of imiquimod for the treatment of aural plaques and sarcoids has been described (Nogueira et al., 2006; Torres et al., 2010; Haspeslagh et al., 2016; Petterson et al., 2020). Considering it's antiviral and antitumour activities, it is reasonable that it may have efficacy against equine sarcoids. While very high resolution of sarcoids rates of up to 84.4% were reported by Petterson et al. (2020) with topical imiquimod (5%) treatment

it would appear that case selection is important with small fibroblastic tumours more likely to respond favourably. Also, small fibroblastic sarcoids demonstrate a high rate of spontaneous regression without treatment (Berruex et al., 2016). A recent systemic review of available sarcoid treatments concluded that in terms of topical treatments the greatest evidence of efficacy exists for imiquimod or a *S. canadensis* formulation (Offer et al., 2024).

Use of 5-FU and cisplatin for the treatment of equine sarcoids has been described in the literature with notable variability of efficacy reported (Knottenbelt, 2019; Hollis, 2023; Offer et al., 2024). Since the introduction of these drugs knowledge of the pathogenesis of sarcoids has improved, indicating this pharmacological approach is not clinically appropriate. In a recent systematic review of the evidence available regarding efficacy of sarcoid treatments, the authors concluded that there was insufficient evidence available to routinely recommend one sarcoid treatment over another (Offer et al., 2024). If fact, studies available are not sufficiently powered, randomised, or placebo-controlled in order to allow for more definitive conclusions of the added clinical benefit of different pharmacological treatment strategies. Surgical excision remains the treatment of choice (Hollis, 2023). Noting a wide variation in the doses and treatment protocols, 5-Fluorouracil reported a success rate of 26.7% when used alone, or up to 77% when combined with other substances or with surgical excision (Hollis, 2023); it has also been used as an intralesional injection, alone or in combination with surgical excision (Hollis, 2023) with one case series reporting a success rate of 61.5% (Stewart et al., 2006). The use of cisplatin is also described as an intralesional injection, alone or in combination with other drugs or surgical excision and has been used compounded into an oil-based formulation or formulated into beads that are implanted intralesionally (Hollis, 2023). In electrochemotherapy protocols success rates ranging from 33% to over 90% are reported (Hollis, 2023; Offer et al., 2024). However, it should be noted that the higher success rates were associated with combination treatment protocols or electrochemotherapy.

Considering the complex aetiology of these masses, it is the expert groups opinion that the use of non-targeted, toxic chemotherapeutic drugs such as 5-FU and cisplatin cannot currently be considered optimum treatments, whereas treatments that can improve clinical signs with less negative side effects such as surgical excision, and/or therapeutics with antiviral and immunomodulating effects would be more appropriate. Imiquimod is proposed as treatment.

Sarcoids, if untreated, are not life-threatening but may cause unacceptable suffering of the animal depending on body location.

A search in the veterinary medicines database does not retrieve any imiquimod-, 5-FU- or cisplatin-containing veterinary medicinal product authorised for use in equine or other species (neither in other animal species).

The substance imiquimod is proposed to be qualified as essential because no satisfactory alternative pharmaceutical treatments are authorised for food-producing animals of the equine species for the following indication: treatment of sarcoids.

In the opinion of the expert group, neither 5-FU nor cisplatin add clinical benefit compared to imiquimod, nor are considered essential. Furthermore, use of non-targeted, toxic chemotherapeutic drugs cannot currently be considered optimum treatment for sarcoids, and there is currently insufficient evidence as to their efficacy for the treatment of equine species. Imiquimod (proposed substance) may be an appropriate therapeutic choice, also in combination with surgical debridement. The substances 5-fluorouracil and cisplatin are not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indication in animals of the equine species; these do not bring added clinical benefit compared to other treatment options. It is considered that the

alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Mitomycin was mentioned three times in the survey to stakeholders and it was suggested for addition to the list for treatment of tumours when there is not a surgical option, and neoplastic lesions such as sarcoids and squamous cell carcinomas (SCS). A reference (Rayner and Van Zyl, 2006) was provided.

Mitomycin is classified as an antineoplastic antibiotic, specifically belonging to the class of drugs known as alkylating agents. Alkylating agents work by attaching alkyl groups to DNA, which leads to damage to the DNA strands and ultimately cell death. Mitomycin specifically functions as a DNA crosslinking agent, interfering with DNA replication in rapidly dividing cells, such as cancer cells. As a result, it is used as a chemotherapy drug to treat various types of cancer.

Mitomycin has been reported as a treatment option for certain neoplasias in horses, specifically ocular squamous cell carcinoma and sarcoids (Rayner and van Zyl, 2006; Malalana et al., 2010; Clode et al., 2012; Haspeslagh et al., 2016; Hollis, 2023). The clinical efficacy of mitomycin as an antineoplastic agent has been demonstrated in horses and dogs with ocular surface SCC, both with and without coinciding surgical removal (Clode et al., 2012). In horses with corneal/conjunctival SCC, mitomycin administered topically as a 0.4 mg/mL solution successfully controlled disease in 60% to 80% of eyes utilizing either a single intraoperative (Rayner and van Zyl, 2006) or multiple postoperative (Malalana et al., 2010) dosing regimen. The results from Clode et al. (2012) further support its use in combination with CO2 laser ablation of lesions.

There are infrequent mentions in the literature of intralesional mitomycin as a treatment for equine sarcoids (Haspeslagh et al., 2016; Hollis, 2023) and there is currently limited evidence as to its efficacy for this indication (Hollis, 2023). However, evidence supports its use for the treatment of squamous cell carcinoma, which is the most common neoplasm of the equine eye and adnexa, frequently involving the cornea or corneolimbal junction, the third eyelid, and/or the eyelids (Clode et al., 2012).

Squamous cell carcinoma, if untreated, could be life-threatening and causes unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any mitomycin-containing veterinary medicinal product authorised for use in equine or other species (neither in other animal species).

The substance mitomycin is proposed to be qualified as essential because no satisfactory alternative treatments are authorised for food-producing animals of the equine species for the following indication: treatment of ocular squamous cell carcinoma.

"Sarcoid Cream" was mentioned once in the survey to stakeholders and it was suggested for addition to the list. The responder asserted that no superior alternative exists for addressing fibroblastic sarcoids on the lower limbs. No specific references were provided.

The expert group preparing this scientific advice has made the assumption that "sarcoid cream" corresponds to the topical AW4/AW5 cream commonly used in the UK. These AW formulations are compounded chemotherapy-based creams containing fluorouracil, thiouracil, heavy metal salts, and steroid (Knottenbelt, 2019). While there are some positive success rates – up to 74% nonrecurrence rates (Knottenbelt, 2019) – reported with the use of these products, the overall evidence is low and there is a lack of peer reviewed publications (Hollis, 2023). As details of exact ingredients and formulation for these creams are not available, and indeed as they are formulated on a case-by-case basis and therefore not a consistent product, the expert group preparing this scientific advice considers it unfeasible to conduct a proper review assessment of these compounds.

Sarcoids are the most common cutaneous tumour of horses. While generally classified as benign as they are rarely metastatic, they frequently can adversely impact equine welfare due to their locally invasive, aggressive nature, and secondary infection or ulceration (Offer et al., 2024).

Sarcoids, if untreated, are not life-threatening but may cause unacceptable suffering for the animal (depending on body location); they do not pose a risk for public health.

A search in the veterinary medicines database does not retrieve any veterinary medicinal product authorised for use in equine or other species (neither in other animal species).

In the opinion of the expert group, "sarcoid cream" cannot be assessed against the criteria defined in EC's mandate as regards to substances that can be considered essential for the treatment of equine species because it is not possible to accurately determine its composition.

Knowledge regarding tumours in horses for this assessment was derived from textbooks and peer-reviewed articles.

B. Considerations regarding consumer safety

Imiquimod is an immune response modifier (immunomodulator). It is marketed in the EU as human medicinal products.

Information in human medicinal products suggests that, in animal models, imiquimod is effective against viral infections (though has no direct antiviral activity) and acts as an antitumour agent, principally by induction of alpha interferon and other cytokines; such induction has been demonstrated both in pharmacokinetic studies and in clinical studies. Saturable binding studies suggest that a membrane receptor exists on responding immune cells (Plumb, 2015).

In dogs and cats, imiquimod has shown potential benefit in the treatment of feline herpes virus dermatitis, actinic keratosis, squamous cell carcinoma and Bowen's disease, papilloma virus lesions, and localized solar dermatitis or solar carcinoma in situ (Plumb, 2015). It should be noted that many of these indications are based upon anecdotal evidence, as there are very few studies published from veterinary use. In horses, imiquimod has been used with success in treating sarcoids. Studies and results are rather limited and there is still ongoing research (Pettersson et al., 2020; Offer et al., 2024). Doses and treatment regimens vary depending on the disease treated and tolerance to the drug; current recommended doses of a thin topical layer vary from once daily to 2-3 times weekly or every-other-week; it is recommended to adjust treatment duration and frequency depending on patient response and adverse reactions (Plumb, 2015).

Data in human medicinal products indicates that less than 0.9% of a topically applied single dose of radiolabelled imiquimod was absorbed through the skin in human subjects. The small amount of drug which was absorbed into the systemic circulation was promptly excreted by both urinary and faecal routes at a mean ratio of approximately 3 to 1. No quantifiable levels (>5 ng/ml) of drug were detected in serum after single or multiple topical doses. Systemic exposure (percutaneous penetration) was calculated from recovery of carbon-14 from [14C] imiquimod in urine and faeces. Minimal systemic absorption of imiquimod 5% cream across the skin of 58 patients with actinic keratosis was observed with 3 times per week dosing for 16 weeks. The extent of percutaneous absorption did not change significantly between the first and last doses of this study. Peak serum drug concentrations at the end of week 16 were observed between 9 and 12 hours and were 0.1, 0.2, and 1.6 ng/mL for the applications to face (12.5 mg, 1 single-use sachet), scalp (25 mg, 2 sachets) and hands/arms (75 mg, 6 sachets), respectively. The application surface area was not controlled in the scalp and hands/ arms

groups. Dose proportionality was not observed. An apparent half-life was calculated that was approximately 10 times greater than the 2 hours half-life seen following subcutaneous dosing in a previous study, suggesting prolonged retention of drug in the skin. Urinary recovery was less than 0.6% of the applied dose at week 16 in these patients.

Local skin reactions are common with imiquimod therapy and include application site reactions like erythema, burning, tenderness, pain, irritation, oozing, exudate, and erosion (Syed, 2001; Plumb, 2015). The product information of human medicinal products includes user safety warnings to avoid direct contact with imiquimod despite the low probability of systemic absorption of the drug and include advise to e.g. wear gloves when handling or applying an imiquimod-containing cream, and to avoid contact with eyes or mucous membranes. However, it is noted that oral mucosal papilloma has been treated dogs without significant problems (Plumb, 2015).

No clinical data on exposure to pregnancies is available. Information in human medicinal products suggest that animal studies did not indicate direct or indirect harmful effects with respect to pregnancy, embryonal/foetal development, parturition, or postnatal development. No quantifiable levels (> 5 ng/ml) of imiquimod are detected in the serum after single or multiple topical doses. Similarly, non-clinical data suggest no specific hazard for humans based on conventional safety (pharmacology) studies, mutagenicity or teratogenicity. Information in human medicinal products states that in a four-month rat dermal toxicity study, significantly decreased body weight and increased spleen weight were observed at 0.5 and 2.5 mg/kg; similar effects were not seen in a four-month mouse dermal study; local dermal irritation was observed. A two-year mouse carcinogenicity study by dermal administration (three days a week) did not induce tumours at the application site. Incidences of hepatocellular tumours among treated animals were greater than those of controls; however, the mechanism for this is unknown. Imiquimod has low systemic absorption from human skin, and is not mutagenic, so any risk to humans from systemic exposure is deemed likely to be low. Furthermore, tumours were not seen at any site in a 2-year oral carcinogenicity study in rats. An imiquimod-containing cream was evaluated in a photocarcinogenicity bioassay in albino hairless mice exposed to simulated solar ultraviolet radiation (UVR). Animals were administered imiquimod cream three times per week and were irradiated 5 days per week for 40 weeks. Mice were maintained for an additional 12 weeks for a total of 52 weeks. Tumours occurred earlier and in greater number in the group of mice administered the vehicle cream in comparison with the low UVR control group. The significance for man is unknown. Topical administration of imiquimod cream resulted in no tumour enhancement at any dose, in comparison with the vehicle cream group. Imiquimod is not listed by the IARC.

Considering the proposed use fort the substance (topically applied to in horses as an immunomodulator) and that the low absorption reported in humans, and considering the safety profile of imiquimod in humans as described in human medicinal products (a similar profile is expected for horses), it can be accepted that imiquimod 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.

Mitomycin is an antineoplastic antibiotic used in eye surgeries treating glaucoma for reduction of scar formation (DocCheck Flexikon, 2024) and used for treating cancer, i.e. cytostatic agent, chemotherapy drug against (advanced) carcinomas. It is an alkylating agent that inhibits DNA synthesis (also RNA and protein synthesis at higher doses). The complementary strands of the DNA double helix are cross-linked by mitomycin. It inhibits B cells, T cells, macrophage proliferation and impairs antigen presentation, secretion of interferon gamma, TNFa, and IL-2 (Drugbank, 2024; Knox et al., 2024). Its antibiotic effects are too unspecific to use mitomycin as an antibiotic. Mitomycin can be regarded as a prodrug. The active metabolites are formed intracellularly, i.e. after entering the respective cell

(DocCheck Flexikon, 2024). The substance is water soluble (8430 mg/L) and has a low lipophilicity (logP: -0.40) (Drugbank, 2024; Knox et al., 2024). The common administration path seems to be intravenous.

Pharmacokinetics is known in humans. After single and repeated intravenous administration in 12 patients, two phases were observed in plasma concentrations. Mitomycin is widely distributed, i.e. for seven patients the volume of distribution was 39.0 + 22.0 L/m² (Schilcher et al., 1984). While mitomycin does not go to the spleen, skin and brain, high concentrations are measured in kidney, liver, heart, lung, and muscles. The metabolism is hepatic. Elimination is mainly biliary, partly renal (at maximum 15% of dose; den Hartigh et al., 1983). Mitomycin C undergoes enterohepatic circulation (den Hartigh et al., 1983; DocCheck Flexikon, 2024). In the study by Schilcher et al. (1984) the total-body clearance was 33.9 + 18.7 L/m² per hour (mean±SD) and the terminal half-life was short (45.2 + 19.0 minutes). The elimination half-life was unaffected by dose level, infusion time, and repeated doses (Schilcher et al., 1984). In 28 patients, a different type (push) of intravenous administration with mitomycin 10-20 and 5-10 mg/m² was conducted. After intravenous push injection the beta elimination half-life was 50 (30-70) minutes; the volume of distribution is reported to be 22 L/m² (den Hartigh et al., 1983). After parenteral administration the half-lives are highly variable due to differences in liver activity but (on the whole) were also very low (4-8 min to 30-70 min) and after three hours no substance was detectable in serum. Mitomycin can go into the milk but levels are expected to be very low due to short elimination half-life (DocCheck Flexikon, 2024).

In two dogs (preliminary study) the volume of distribution and the total-body clearance were observed to be 0.48 L/kg and 0.39 L/kg per hour, respectively. The alpha- and beta-half-life values were 3.5 and 50.9 min (Schilcher et al., 1984).

Chronic toxicity of mitomycin was examined. The substance is known as a carcinogenic, cytotoxic/mutagenic and reprotoxic agent (CMR substance) (DocCheck Flexikon, 2024). A main metabolite due to reduction, the 2,7-diaminomitosene, is also an alkylating agent that inhibits DNA synthesis (Bose et al., 2016). According to the classification provided by companies to ECHA in CLP notifications this substance is fatal if swallowed and is suspected of causing cancer (ECHA, 2024).

According to the IARC evaluation from 1976, mitomycin was carcinogenic in rodents. In mice, following 35 s.c. injections 0.2 µg mitomycin twice weekly and observed for further 31 weeks, local sarcomas were developed in all animals within 39-54 weeks. In rats, following either intraperitoneal administration of 0.038 or 0.15 mg/kg bw three times weekly for 6 months followed by observation for a further 12 months, or following intravenous injection of 0.52 mg/kg bw mitomycin C within two weeks (total dose, 2.6 mg/kg bw) and observed for their lifespan, peritoneal sarcomas developed in 27/29 males and 30/31 females; out of the 79 rats surviving at the appearance of the first tumour, 27 (34%) developed malignant tumours, respectively. No human data was available (IARC, 1976). Due to this and other evidence, mitomycin was classified as a possible human carcinogen group 2b, an agent for which there is limited evidence of carcinogenicity in experimental animals. However, there was additional evidence which related apart from its genotoxicity probably to the fact that the agent itself reacts covalently with DNA so that on the whole the substance was not classified in group 3 (evidence inadequate/limited) (IARC, 1987). The US EPA (Environmental Protection Agency) Guidelines for Carcinogen Risk Assessment led to a similar assessment, classifying mitomycin as a probable human carcinogen in Group B2 using the weight-of-evidence approach (EPA, 2002).

There is no new evidence on carcinogenicity in laboratory animals publicly available since 2002. However, mitomycin is used as a positive control e.g. investigating the protective effect of aspirin against carcinogenicity induced by mitomycin C in Drosophila melanogaster (Oliveira et al., 2018).

Mitomycin is a direct-acting clastogen (induces chromosome aberrations) and was tested positive for mutagenicity in several test systems and species including bacteria and mammalian cells/species. According to IARC (1976), *"mitomycin C induced reverse mutations in Salmonella typhimurium (McCann et al., 1975). It induced mitotic crossing over in the yeast Saccharomyces ceremlsiae, in the smut fungus Ustvllago maydis (Holliday; 1964) and in the soyabean Glycine max. L. (Vig and Paddock, 1968) and induced mitotic as well as meiotic crossing over in Drosophila melanogaster (Schewe at al., 1971 a,b). It induced chromosomal aberrations in Drosophila oocytes (Walker and Williamson, 1975) and dominant and recessive mutations in the wasp Habrobracon (Smith, 1969). Mitomycin C of unspecified origin and purity injected into male (101 x C3H)F_1 hybrid mice as a single dose of 5.25 mg/kg bw induced specific locus mutations in spermatogonia (Ehling, 1973). It induced chromosomal breaks and rearrangements in cultures of human peripheral leucocytes (Cohen and Shaw, 1964)"*. Study references can be also found in the mitomycin annotation record of the PubChem database (PubChem, 2002).

In later studies, mitomoycin C led to sister chromatid exchanges in lymphocytes of human cancer patients. Mitomycin has been shown to cause synaptonemal complex structural damage in mice (genotoxic at meiosis) (Allen et al., 1988). The substance induced cytogenetic damage (micronuclei) in mouse foetal blood, demonstrated as a significant and dose-dependent increase in the incidence of micronucleated foetal blood cells (King and Wild, 1979). Mitomycin induced micronuclei in bone marrow as well as in hepatocytes using rats (Parton and Garriott, 1997).

In a NTP cytogenetic study of mitomycin C in rodents, male mice were tested positive for chromosome aberrations test and in vivo micronucleus study in bone marrow (NTP, 2024; Witt et al., 2000).

In several current studies, mitomycin was used as a positive control, e.g. in AMES assays or cell chromosome aberration tests (Luo et al., 2014), in the development of repeated-dose liver micronucleus assays (Shimada et al., 2015; Takasawa et al., 2010; Igarashi et al., 2010; Szuzuki et al., 2009) or in genotoxicity testing of Ni and NiO nanoparticles in human bronchial epithelial BEAS-2B cells (Di Bucchianico et al., 2018). In the latter test system mitomycin leaded to chromosomal damage and rearrangements, with highly significant increase of micronuclei in binucleated as well as in mononucleated cells, of nucleoplasmic bridges and of nuclear buds.

A company's test submission is summarised in the annotation record for one of the data sources of the PubChem database (see 3.4 TSCA Test Submissions (Complete)): *"Mitomycin C (CAS # 50-07-7) was evaluated for its potential to induce chromosome aberrations in cultured Chinese hamster ovary cells with and without metabolic activation. Testing was conducted using concentrations of test substance at 125, 63, 31, 16, 7.8, and 3.9 ug/ml (without activation) and 500, 450, 400, 350, 300, 250, 200, 100, and 50 ug/ml (with activation). There were statistically significant increases in the number of aberrants cells at 125 ug/ml (10-17%) without activation and at 75 ug/ml (10-11%) and 100 ug/ml (38-49%) with activation."* (PubChem, 2002).

Mitomycin was observed to be teratogenic in mice in a developmental toxicity study with some (reporting) limitations. The pregnant females were mated at age 10 to 13 weeks. Intraperitoneal injections were administered once between 7.5 and 13.5 days of gestation, at doses of 5.0, 7.5 or 10.0 mg mitomycin C/kg bw to 26 animals. Animals were sacrificed on day 18.5 of gestation and dams and live foetuses were examined. During treatment, 0, 4, and 6 dams died in the 5, 7.5, and 10 mg/kg bw treatment groups, respectively. The mortality, growth suppressing effects and gross external anomalies observed in the foetuses were dose dependent (Tanimura, 1968).

No depletion studies were found for any animal species. Only tissue levels of mitomycin C in female healthy Sprague-Dawley rats were described receiving a single intravenous dose of 2 mg of mitomycin

C per kg/bw. The half-life was highest in liver tissue (33 min) and lowest in lung tissue and plasma (14.9 and 14.4 min, respectively). Samples for this calculation had only been collected until 90 min post application (Malviya et al., 1986), which does not take into account the biphasic nature of elimination of the substance in humans (Schilcher et al., 1984).

Considering that there is no information available on pharmacokinetics or residue depletion of mitomycin in horses and the substance is known to be a possible human carcinogen group 2b by IARC, an agent for which there is evidence of carcinogenicity in only one experimental animals species but additional evidence is available, and to be mutagenic and teratogenic, it can be concluded that mitomycin will pose an unacceptable risk for consumers when used in food-producing animals of the equine species and a six-month withdrawal period is respected.

4.14.4. Conclusion

Based on the above assessment and justifications, the following recommendations are proposed:

1. The following active substance, suggested for addition to the list in the survey to stakeholders, is proposed to be added to the list with an entry as shown below:

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Imiquimod	treatment of sarcoids	none identified	current research suggests that equine sarcoids likely result from a complex interaction including host immune system disfunction

2. The following active substances, suggested for addition to the list in the survey to stakeholders, are not proposed for inclusion: 5-fluorouracil, cisplatin, mitomycin, 'sarcoid cream'.

4.15. Z. Miscellaneous

4.15.1. Overview

	Active substance (ATCvet code)
Substances in Regulation (EU) 1950/2006 **proposed to be retained**, with or without amendment of the current entry	Domperidone (QA03FA03, noting the proposed use is not adequately covered by any ATCvet code for this substance)
Substances in Regulation (EU) 1950/2006 **proposed to be removed**	Cyproheptadine (QR06AX02); Imipramine (QN06AA02, noting the proposed use is not adequately covered by any ATCvet code for this substance)
Substances from stakeholders' survey **proposed for inclusion**	Cetirizine (QR06AE07, QS01GX12); Sulpiride (QN05AL01)
Substances from stakeholders' survey **not proposed for inclusion**	CBD (no ATCvet code identified); Fipronil (QP53AX15); Hydroxyzine (QN05BB01)

4.15.2. Review of the existing entries in Regulation (EU) 1950/2006, as amended by Regulation (EU) 122/2013, considering the survey results

A. Considerations on the essentiality of the substance(s)

Cyproheptadine is a potent competitive antagonist of both serotonin and histamine receptors. It is assumed that it exerts antihistamine and antiserotonin effects by competing with free histamine and serotonin for binding at their respective receptors resulting in reduction of central nerve conduction (Roberts, 2019).

Cyproheptadine is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for treatment of the headshaking syndrome. No alternatives are identified in the current list. Specific advantages captured in the current list is that horses exhibiting signs of photic headshaking respond favourably to treatment with the antihistaminic drug cyproheptadine. In addition to antihistaminic action, cyproheptadine has anticholinergic action and is a 5-hydroxytryptamine (serotonin) antagonist. Relief from the behaviour is usually seen within 24 hours of beginning cyproheptadine therapy and often resumes within 24 hours of discontinuing therapy. Other antihistamines are not considered effective at eliminating headshaking.

There are various treatments and management procedures described for the treatment of the headshaking syndrome (Roberts, 2019). Percutaneous electrical nerve stimulation (PENS) therapy and surgical platinum coil ablation of the superficial sensory branches of the trigeminal nerves are effective treatment options. Diazepam, which is proposed to be retained in the list of essential substances, could be considered as an alternative for the same indication. For diazepam there are veterinary medicinal products authorised for use in species other than equines (i.e. dog and cat). Since the etiology of head shaking syndrome is not known and treatment with cyproheptadine does not result in resolving the condition (Roberts, 2019; Newton et al., 2000), it is not considered to bring added clinical benefit compared to other treatment options. As an antihistamine in horses, chlorphenamine is included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with an entry covering for *Equidae* and cetirizine, which is considered a more effective alternative, is proposed for addition to the list (see below).

Histamine-mediated conditions, if untreated, are potentially life-threatening and cause unacceptable suffering of the animal.

Cyproheptadine was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any cyproheptadine-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance cyproheptadine is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indication in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Domperidone is a benzimidazole derivative and dopamine receptor antagonist with prokinetic properties increasing gastrointestinal peristalsis (Kilbinger and Weihrauch, 1982). It also affects thermoregulation, prolactin release, and the chemo-effector trigger zone (Boothe, 2001).

Domperidone is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) for treatment of agalactia in mares. No alternatives are identified in the current list and the specific

advantage captured is that domperidone is a dopamine antagonist and up-regulates prolactin production. Oxytocin is not a suitable alternative because it produces milk letdown as opposed to increasing milk production, which is the aim of domperidone therapy. Additionally, oxytocin is likely to cause abdominal pain if used in large doses.

According to Morresey (2012) domperidone is the most used lactogenic agent due to its ability to stimulate prolactin secretion in situations of dopaminergic inhibition such as with 'fescue toxicity'. It is extensively used in the eastern USA in mares grazing on hayfields with fescue toxicity, infected with *Epicloe coeanophialum*. Although fescue grasses occur in European horse pastures and hayfields, it remains unclear whether fescue toxicity constitutes a clinical disease as it does in the USA. Expertise within the expert group preparing this scientific advice confirmed that domperidone would be widely used for the treatment of agalactia/dysgalactia in mares irrespective of the occurrence of fescue toxicity in the EU. Sulpiride is an alternative recommended for addition to the list.

Agalactia/dysgalactia, if untreated, may cause unacceptable suffering of the animal.

Domperidone was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any domperidone-containing veterinary medicinal product authorised for use in equine species. One veterinary medicinal product authorised for dogs for a different indication (prophylaxis of Leishmaniosis) is authorised in the EU.

The substance domperidone is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: treatment of agalactia/dysgalactia in mares. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Imipramine is a dibenzoazepine-derivative prototypical tricyclic antidepressant structurally similar to phenothiazines and is a potent inhibitor of serotonin and norepinephrine reuptake (Gillman, 2007). It has also been described to lower the ejaculatory threshold in stallions (McDonnell, 1999).

Imipramine is listed in Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) to pharmacologically induce ejaculation in stallions with ejaculatory dysfunction. No alternatives are identified in the current list, and the specific advantage captured for the substance is that there are no alternatives available.

Diazepam, gonadotropin-releasing hormone, xylazine, or prostaglandin F2α have also been described to enhance ejaculatory function in stallions (McDonnel et al., 1985; Sieme et al., 2004). Regarding these alternatives, it is noted that diazepam is proposed to be retained in the list of essential substances; gonadotropin-releasing hormone and xylazine are listed in Table 1 of the Annex to Commission Regulation (EU) No 37/2010, with a 'No MRL required' entry for all food producing species and *Equidae,* respectively; for prostaglandin F2α a veterinary medicinal product is authorized for use in mares. Expertise within the expert group preparing this scientific advice confirmed the effectiveness of imipramine for a specific and narrow indication, i.e. to pharmacologically induce ejaculation in stallions with ejaculatory dysfunction, and therefore proposes qualifying the substance as essential.

Ejaculatory dysfunction, if untreated, may cause unacceptable suffering of the animal.

Imipramine was not mentioned in the survey to stakeholders.

A search in the veterinary medicines database does not retrieve any veterinary medicinal product authorised for use in equine species. In the EU, there is one veterinary medicinal product authorised

for dogs containing imipramine as hydrochloride for the treatment of frequent urination due to excitement in young dogs.

The substance imipramine is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: for the pharmacological induction of ejaculation in stallions with ejaculatory dysfunction. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Knowledge regarding miscellaneous substances in horses for this assessment was derived from peer-reviewed articles.

B. Considerations regarding consumer safety

Domperidone is a dopamine antagonist used in humans, specially, as an antiemetic for the short-term treatment of nausea and vomiting of various aetiologies. It speeds gastrointestinal peristalsis and causes prolactin release (Scriba, 2009). It is used for the treatment of agalactia in mares. It is a lipophilic agent, classified under class II according to BCS, with limited solubility and high permeability.

In humans the absorption is rapid, but the systemic bioavailability is only about 15% in fasting subjects given an oral dose, which is increased when given after food consumption. The poor aqueous solubility, first pass effect and extensive hepatic metabolism are probably the main possible reasons for its low bioavailability (Scriba, 2009; Helmy and Bedaiwy, 2014; Ye et al., 2022). In humans, the apparent volume of distribution is 5.7 L/kg after intravenous administration (Brogden et al., 1982). It is more than 90% bound to plasma proteins and has a terminal elimination half-life of about 7.5 hours in healthy subjects and prolonged to up to 20.8 hours in patients with severe renal dysfunction. It undergoes extensive hepatic metabolism. About 30% of an oral dose is excreted in urine within 24 hours, almost entirely as metabolites; the remainder is excreted in faeces over several days. It does not readily cross the blood-brain barrier (Brogden et al., 1982; Scriba, 2009).

No differences in the rate and extent of absorption were observed under fasting state between 20 mg domperidone suspension and tablet dosage. The area under the curve (AUC_{0-t}) was 249.5 and 202.8 ng h/ml and the elimination half-life was 8.1 and 7.9 hours for suspension and tablets, respectively (Helmy and Bedaiwy, 2014). Ye et al. (2022) compared the pharmacokinetics and bioequivalence of two 10-mg domperidone tablets in fasting and fed states and concluded that a high-fat meal slowed down the absorption and elimination of domperidone and increased domperidone exposure. In this study, AUC_{0-t} was reported to be 77.6 and 50.7 ng h/ml, maximum blood concentration (C_{max}) 15.3 and 18 ng/ml and the terminal-phase elimination half-life 12.5 and 14 hours, for the fasting and fed test, respectively. Other study reported elimination half-life of 8.72 hours in fed adults and 7.15 hours in fasting adults. Clearance was reported as 0.12 and 0.20 l/h in fed and fasting adults, respectively (Wang et al., 2020).

The product information of human medicinal products describes a pharmacokinetic study in horses which showed an average terminal elimination half-life of 6 and 5 hours for oral and intravenous administration of domperidone. Average AUC from time 0 to 24 hours was 26.2 (+15.3 SD) and 182 (+156 SD) ng hr/mL, for 1.1 and 5.5 mg/kg oral dose groups, respectively, and 1387 (+1911 SD) ng hr/mL, for 0.2 mg/kg intravenous dose. The average clearance in the 0.2 mg/kg intravenous dose group was 143 ml/hr/kg. Oral bioavailability was 1.2% for the 1.1 mg/kg dose and 1.4% for the 5.5 mg/kg dose. In Beagle dogs, half-life of 2.45 hours was described, and the time-courses of the drug

plasma levels were similar for single and repeated doses, indicating that chronic administration of domperidone did not alter its pharmacokinetics (Heykants et al., 1981).

Excretion and metabolism were studied in rats, dogs and man after oral and intravenous administration of the 14C-labeled domperidone. Excretion of the radioactivity was almost complete within four days. Unchanged domperidone accounted in urine was 0.3 and 0.4% in dogs and in man, respectively, while in faeces it was 9 and 7% (Meuldermans et al., 1981). Tissue distribution was also studied in rats showing large amounts of radioactivity in the stomach and in the bilious contents of the intestine. High activity was detected in the liver, kidney, lung and some glandular tissues. The half-life reported was 8-10 hours (Michiels et al., 1981).

The safety of domperidone in humans was assessed by EMA in 2014. A review of the evidence confirmed a small increased risk of serious cardiac adverse drug reactions related to the use of domperidone. A higher risk was observed in patients older than 60 years, adults taking oral doses of more than 30 mg and those taking QT-prolonging medicines or CYP3A4 inhibitors. It was concluded that domperidone should be used at the lowest effective dose for the shortest possible duration (EMA/465179/2014). Plasma-prolactin concentrations may be increased, which may lead to galactorrhoea or gynaecomastia (Scriba, 2009). Keating and Rees (1991) reported a case of gynaecomastia after a long treatment with 20 mg domperidone 3 times a day. Cann et al. (1983) reported that 7 out of 28 women developed mastalgia and galactorrhoea after receiving 20 mg four times a day and concluded that the high incidence of these effects is likely to be a problem only if the drug is to be used for persistent symptoms in an essentially benign condition such as the irritable bowel syndrome.

Domperidone has been concluded not to induce chromosome aberrations and/or gene mutations in studies in vivo and in vitro (Vanparys et al., 1982, 1985). The product information of human medicinal products mentions that domperidone did not show mutagenic potential in Ames test or in vitro models either.

In spite of the safety concerns in humans recently assessed, there is pharmacokinetic information available in humans, in the horse and other animal species that suggests a rapid elimination and an extensive metabolism. Furthermore, serious cardiac adverse reactions have been related to high doses, treatment duration and other medicinal products taken simultaneously. Therefore, it can be accepted that domperidone 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.

Imipramine is highly lipid-soluble with a predicted logP value of 4.28 which is important to reach effective concentrations in the central nervous system, but it also demonstrates solubility in water, measured at 18.2 mg/l at 24°C (Drugbank, 2024).

Pharmacokinetics in horses was studied in five narcoleptic adult animals administered imipramine solution of 25 mg/ml intravenously, achieving a single dose of 2 mg/kg bw. Blood was collected up to 24 hours and the serum was analysed (Peck et al., 2001). The mean volume of distribution at steady state was 584 ml/kg, which approximates the total body water. The mean residence time and total body clearance were at average 1.8 hours and 522 ml/kg/h, respectively. Desipramine is the major metabolite in human but was not detected in any of the horses (Peck et al., 2001). With the following equation the half-life is calculated, using the above reported values, to be approximately 21 hours in horses: Half-life (hours) = 0.693 x (Volume of distribution (l) / Clearance (l/hr)) (Mansoor and Mahabadi, 2023).

In a pharmacokinetic study in an unspecified number of cattle, an (over-) dose of 2 mg/kg bw was administrated intravenously and revealed a high volume of distribution at steady state of 4.2 ± 0.9

l/kg bw. Imipramine had a short plasma terminal elimination half-life of 140 ± 15 minutes and the total body clearance was rapid with 22.7 ± 7 ml/min/kg (Cordel et al., 2001).

The oral absorption in humans is reported to be rapid and high (>95%) (Sallee and Pollock, 1990; Gillman, 2007; Drugbank, 2024), and not to be affected by food. However, bioavailability is highly variable, i.e. 29-77%. After oral administration the peak plasma concentration is achieved after 2-6 hours (Sallee and Pollock, 1990; Drugbank, 2024). At therapeutic plasma concentrations in humans all tricyclic antidepressants bind 90-95% to plasma albumin, and also bind to extravascular tissues, so that large distribution volumes occur in a range 10-50 l/kg (Gillman, 2007). For imipramine, the volumes of distribution for both the parent compound and metabolite were higher than in horse and cattle, with 10±20 l/kg for imipramine and 10±50 l/kg for desipramine, also suggesting an extensive peripheral distribution due to high lipid solubility and an apparently low plasma binding (Sallee and Pollock, 1990; Besret et al., 1996; Cordel et al., 2001).

In humans, imipramine is rapidly metabolised to desipramine, which is an active metabolite (Drugbank, 2024). However, the speed of the metabolism and thus the systemic availability of imipramine is highly variable, with up to a four-fold difference among individuals. The plasma elimination in humans was slower compared to that of cattle and rat (Sallee and Pollock, 1990; Besret et al., 1996; Cordel et al., 2001) and is reported to be 19 hours (PharmaWiki, 2024). The elimination half-lives of imipramine in rat from brain tissue were similar to those reported for serum, approximately 204 minutes or 3.4 hours (Besret et al., 1996). Desipramine was the major metabolite detected in brain in a study in rats. Imipramine and desipramine undergo further hydroxylation (Sequeira and Strobel, 1995). Less than 5% of unchanged imipramine is excreted in urine. (Drugbank, 2024).

No residue depletion data were found for imipramine administrated in horses or in closely related species of the *Equidae* family. The preferred tissue binding for tricyclic antidepressants are myocardial, hepatic, pulmonary and brain tissue (Johnson, 1990; Besret et al., 1996; Cordel et al., 2001). In a rat study with administration of imipramine of 20 mg/kg intraperitoneally twice a day for 14 days the concentrations of imipramine and desipramine were much higher in brain tissue than in serum (Besret et al., 1996).

The most important side effects of tricyclic antidepressants by increasing the dose or by overdosing are cardiovascular symptoms (Brown, 1976) and neurological symptoms. Imipramine has antihistaminic effects in the central and autonomic nervous system and can cause mainly cardiac dysrhythmia (Fayez and Gupta, 2023).

According to the notifications provided by companies to ECHA in REACH registrations no hazards have been classified due to data lacking. No toxicological data is available in the chemical dossier (ECHA, 2024b). Imipramine is not classified for carcinogenic hazards by IARC (IARC, 2024). Carcinogenicity of imipramine is predicted to be negative with a probability of 0.9329 (Drugbank, 2024). Publicly available studies on carcinogenicity of imipramine have not been found. The predicted mutagenicity is reported to be negative in the Ames test with a probability of 0.9132 (Drugbank, 2024). However, several studies on the genotoxicity of imipramine are available with both positive and negative results in vitro and in vivo. Imipramine was tested in *Salmonella typhimurium* (TA 1535, TA 1537, TA 100 and TA 98) with and without metabolic activation with negative results up to 5 mg/plate (Balbi et al., 1980). In leukocyte cultures from seven healthy blood donors, no increase in chromosome breaks was found for the tested concentrations up to 62.5 µg/ml and durations of exposure of 4 to 68 hours (Fu and Jarvik, 1977). Cytogenetic effects in human lymphocytes cultures were studied with three concentrations. A small but significant increase in chromosome damage was observed at the highest concentration of 5,000 ng/ml, which is reported to be above the plasma level after therapeutic doses of

imipramine in humans. The sister chromatid exchange frequency was slightly but significantly increased only at the concentration higher than the therapeutic plasma level. The mitotic index was not affected (Saxena and Ahuja, 1988). Both imipramine and desipramine were reported to induce micronuclei in the bone marrow of mice following oral exposure via drinking water with up to 9.9 mg/kg corresponding to doses up to 1.6 mg/kg bw per day, calculated based on default water consumption and body weight of 5 ml and 0,03 kg, respectively, in a 4-week assay (Madrigal-Bujaidar et al., 2008). Chromosomal aberrations were observed in mouse bone marrow cells following intraperitoneal injection of imipramine and desipramine in all three dosage groups (up to 60 mg/kg bw) without a clear dose response for imipramine and with desipramine inducing the stronger effects (Madrigal-Bujaidar et al., 2010). Imipramine did not induce chromosomal aberrations in rat bone-marrow cells following intraperitoneal exposure to up to 100 mg imipramine/kg bw (Bishun et al., 1974). Imipramine and desipramine induced sister-chromatid exchanges in mouse bone marrow cells after intraperitoneal administration (Paniagua-Pérez et al., 2002). A comet assay was conducted using the blood of 35 children treated with imipramine for primary nocturnal enuresis, and of 20 untreated controls. A significant DNA damaging effect of imipramine in human lymphocytes was observed. However, further research was considered necessary, as psychological stress among the children was also considered to be a potential contributing factor to this outcome (Dündaröz et al., 2002). Overall, there is evidence of genotoxicity of imipramine in mice after oral exposure to rather low doses and after intraperitoneal exposure, an exposure route less relevant for consumer risk assessment.

In a mating study on imipramine-treated rabbits, one of 53 young rabbits in the imipramine group and one of 59 in the imipramine-N-oxide-group were abnormal while no abnormalities were observed in the 25 animals control group. Fertility was not affected due to imipramine and imipramine-N-oxide administration of 15 mg and 25 mg/kg daily, respectively (Larsen, 1963). A developmental toxicity test with imipramine administered subcutaneously at 0, 5, or 10 mg/kg/day to pregnant rats on gestation days 8-20 induced effects regarding several parameters which suggested that alterations in postnatal heart and brain development can occur when mothers are treated with imipramine (Harmon et al., 1986).

Considering the genotoxic effects of imipramine in somatic cells in vivo, despite its fast total body clearance in horses, it can be concluded that imipramine will potentially pose an unacceptable risk for consumers when used in food-producing animals of the equine species and a six-month withdrawal period is respected.

4.15.3. Assessment of new substances proposed to be added to the list in the stakeholders survey

A. Considerations on the essentiality of the substance(s)

Cannabidiol (CBD) was mentioned (twice) in the survey to stakeholders and it was suggested for addition to the list. The survey responders indicated it is designated as a nutritional supplement that has anti-anxiety, analgesic, and anticonvulsant properties, and that may offer an alternative to other commonly used analgesic drugs; also proposed for pain relief and welfare on (zolder) horses. No scientific evidence was provided in support these statements.

Scientific evidence supporting their use in animals is currently limited (De Briyne, et al., 2021). In horses, similarly to other animals, the vast majority of reports available studied animal feed supplements (Turner et al., 2022; Williams et al., 2022). Animal feed supplements do not fall under Regulation (EU) 2019/6. Although cannabinoids such as CBD may have potential for therapeutic promise as analgesics, sufficient alternatives are currently included in Table 1 of the Annex to

Commission Regulation (EU) No 37/2010 or proposed to be retained in the list of essential substances (see section 4.2 of this scientific advice).

A search in the veterinary medicines database does not retrieve any cannabidiol-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance cannabidiol is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indication in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Cetirizine was mentioned (four times) in the survey to stakeholders and it was suggested for addition to the list as a safe antihistamine drug for horses that has proven to reduce histamine-induced cutaneous wheal formations (Olsen et al., 2008).

Cetirizine is a potent, second-generation histamine-1 (H1) receptor inverse agonist that interferes with the activity of the endogenous ligand, histamine, thereby lessening histamine-mediated inflammatory responses. Antihistamines have traditionally been used for treatment of allergies in horses, whereas glucocorticoids are the drugs of choice and there are a number of alternatives listed in Table 1 of the Annex to Commission Regulation (EU) No 37/2010, e.g. dexamethasone. Long term use of glucocorticoids in horses can lead to potential adverse effects, so the addition of antihistamines can reduce both the dose and length of glucocorticoid treatment.

As an antihistamine in horses, chlorphenamine is included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 with an entry covering for *Equidae*; its use in horses is well established (Kuroda et al., 2013). However, it has been proven that second-generation histamine-1 (H1) receptor inverse agonists are alternatives with fewer CNS (sedative) side effects available that may be preferable (Olsen et al., 2008; Knych et al., 2019).

Histamine-mediated conditions, if untreated, may cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any cetirizine-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance cetirizine is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: for the treatment of conditions where an antihistamine is deemed necessary. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Fipronil was mentioned (once) in the survey to stakeholders and it was suggested for addition to the list for treatment of lice in horses. The responder stated that fipronil had shown to be effective in these cases (Da Silva et al., 2013; Kondratjeva et al., 2022), being easily applicable in horses and with less environmental risks compared to other treatment options, as it remains mostly on the horse and is not easily washed off.

Fipronil is a broad-spectrum insecticide belonging to the phenylpyrazole chemical family. Fipronil disrupts the insect central nervous system by blocking the ligand-gated ion channel of the GABAA receptor and glutamate-gated chloride (GluCl) channels. This causes hyperexcitation of contaminated insects' nerves and muscles. Fipronil's specificity towards insects is believed to be due to its greater binding affinity for the GABAA receptors of insects than to those of mammals, and for its action on

GluCl channels, which do not exist in mammals. Fipronil is widely used in companion animals such as dogs and cats for the treatment of flea, tick and lice infestations. There are a few reports in the literature of the successful use of fipronil for the treatment of lice in horses (Hugnet et al., 1999; Da Silva et al., 2013). While there are substances in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 that can be used for the treatment of lice (e.g. ivermectin, which is authorised for the same indication in food-producing animals of the equine species) fipronil brings added clinical benefit compared to these.

Lice infestation, if untreated, may cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any fipronil-containing veterinary medicinal product authorised for use in equine species. There are veterinary medicinal products authorised for use in species other than the equine (i.e. dog and cat).

The substance fipronil is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: treatment of lice in horses. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Hydroxyzine was mentioned (once) in the survey to stakeholders. No specific indication was mentioned, and no scientific references were provided.

It is a first-generation histamine-1 (H1) receptor inverse agonist that interferes with the activity of the endogenous ligand, histamine, thereby lessening histamine-mediated inflammatory responses. Antihistamines have traditionally been used for treatment of allergies in horses, whereas glucocorticoids are the drugs of choice and there are a number of alternatives listed in Table 1 of the Annex to Commission Regulation (EU) No 37/2010, e.g. dexamethasone. While hydroxyzine has been widely used in horses for the treatment of allergic reactions (Olsén et al., 2008; Petersen and Schott, 2009), it has been suggested that second-generation histamine-1 (H1) receptor inverse agonists are alternatives with fewer CNS (sedative) side effects available that may be preferable (Knych et al., 2019). Cetirizine (a second-generation H1 receptor inverse agonist) is recommended for addition to the list.

Histamine-mediated conditions, if untreated, may cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any hydroxyzine-containing veterinary medicinal product authorised for use in equine species There are veterinary medicinal products authorised for use in species other than the equine (i.e. dog and cat).

The substance hydroxyzine is not proposed to be qualified as essential, nor as bringing added clinical benefit for the above-mentioned indications in animals of the equine species; satisfactory alternative treatments are authorised for food-producing animals of the equine species, and it does not bring added clinical benefit compared to other treatment options. It is considered that the alternatives do yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Sulpiride was mentioned (five times) in the survey to stakeholders and it was suggested for addition to the list for stimulating lactation in adoptive mares and milk production in non-cycling mares, as an alternative to domperidone, and in some cases to improve colostrum quality. The proposed indications were supported by published peer-reviewed references (Chavatte-Palmer et al., 2002; Guillaume et al.,2003; Panzani et al., 2011; Valencia et al., 2018).

Sulpiride is a selective D2 dopamine receptor antagonist. In human medicine it is used for treatment of acute and chronic schizophrenia and in Meniere's disease. Its side effects relate to its endocrine-disruptive effects and include hyperprolactinemia, gynecomastia, menstrual disorders, hyperglycemia, and weight gain. It is due to that side effects that is used as a lactation stimulant in adoptive and non-cycling mares, also in suckling foals. As a dopamine receptor antagonist, it is used together with domperidone for treatment of fescue toxicity (Blodgett, 2001). The efficacious use of sulpiride in horses treatment of agalactia/dysgalactia in mares (Chavatte-Palmer et al., 2002; Guillaume et al.,2003; Valencia et al., 2018) or to advance ovulation (Panzani et al., 2011) is noted from the references provided. Redmond et al. (1994) had already assessed the efficacy of domperidone and sulpiride for fescue toxicosis in 16 gravid mares and showed that treated mares had higher serum progesterone and lower estradiol-17β concentrations than did control mare, and significantly higher mammary gland development. Although it is noted that domperidone is proposed to be kept on the list of essential substances, no reference is found claiming a better clinical profile of one substance over the other.

Agalactia/dysgalactia, if untreated, may cause unacceptable suffering of the animal.

A search in the veterinary medicines database does not retrieve any sulpiride-containing veterinary medicinal product authorised for use in equine species (neither in other animal species).

The substance sulpiride is proposed to be qualified as bringing added clinical benefit compared to other treatment options for food-producing animals of the equine species for the following indication: treatment of agalactia/dysgalactia in mares. It is considered that the alternatives do not yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Knowledge regarding miscellaneous substances in horses for this assessment was derived from textbooks and review articles.

B. Considerations regarding consumer safety

Cetirizine is a H(1) receptor blocking antihistamine agent. In the EU is marketed as a human medicinal product presented as crystal powder freely soluble in water.

After oral administration to humans, cetirizine peak concentration occurs in about one hour; it is highly (93%) bound to plasma proteins, and brain levels are approximately 10% of those found in plasma (Plumb, 2002). Approximately 80% is excreted in urine, primarily as unchanged drug. Terminal elimination half-life is 8 hours; antihistaminic effect generally persists for 24 hours after the dose (Plumb, 2002).

In horses, after three consecutive oral administrations of cetirizine (0.2 mg/kg bw) every 12h, the plasma concentration was 16±4 ng/mL (mean±SD); after four additional administrations of 0.4 mg/kg bw every 12 h, the plasma concentration was 48±15 ng/mL (Olsén et al., 2008). The terminal elimination half-life is reported to be around 6 hours.

In pregnant mice, rats, and rabbits, dosages of approximately 40X, 180X, and 220X human doses (respectively) caused no teratogenic effects. Reported minimum lethal oral doses for mice and rats are 237 mg/kg (95X human adult dose on a mg/m2 basis) and 562 mg/kg (460X human adult dose on a mg/m2 basis), respectively. Cetirizine may cross into the CNS in overdose situations and cause neurologic signs (Papich, 2021).

In humans, the FDA categorizes cetirizine as category B for use during pregnancy (i.e. animal studies have not demonstrated risk to the foetus, but there are no adequate studies in pregnant women). Cetirizine is not considered carcinogenic to humans by the IARC.

Residue depletion data for horses are not available.

Considering the characteristics of the substance and its short terminal half-life in horses, it can be accepted that the cetirizine 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.

Fipronil is a phenylpyrazole used as a contact broad-spectrum insecticide in veterinary medicinal products for non-food producing animals, recently tested when orally administered (Dos Santos et al., 2022). It is often combined with other active substances for improving the effect against the various stages of the target organism. Fipronil is also approved as an active substance in biocidal products (ECHA, 2011) but was not renewed as an active substance for plant protection products (Regulation (EU) 2019/330)[52] possibly due to the former restriction in use based on bee toxicity (European Commission, 2013) with expiration of approval in 2017 (European Commission, 2024). Inappropriate management in poultry farming has led to high contamination in eggs and chicken meat on farms in Belgium and Netherlands in 2017, subsequently affecting numerous other countries (Corrias et al., 2021). Fipronil has a long half-life in the environment (Corrias et al., 2021).

Its water and organic solvent solubility is low, high in some polar organic solvents such as acetone (545.9 g/l at 20°C) and methanol (137.5 g/l at 20°C). Partition coefficient n-octanol/water for the purified material is 3.5 – 4.0 at 20°C (ECHA, 2011).

No pharmacokinetic data were found for fipronil administrated in horses or in closely related species of the *Equidae* family. According to the fipronil assessment report under the biocidal product legislation, after oral administration, absorption is estimated to be approximately 90%. Dermal absorption was estimated to be around 11% based on available information for fipronil biocidal product formulations. Additionally, the assessment report provides information that fipronil is widely distributed with a preference for fatty tissues and is extensively metabolised. Fipronil is slowly eliminated via both bile (metabolites) and faeces and urine (parent residue). The slow elimination appears to be partly due to the distribution of fipronil into fatty compartments and to the extent of gastrointestinal reabsorption of biliary excreted fipronil metabolites (ECHA, 2011).

Several metabolism pathways are suggested, and one results in fipronil sulfone which is a lipophilic and persistent compound known to be also a neurotoxicant and an endocrine disruptor (Cravedi et al., 2013). Fipronil undergoes bioactivation as the predominant metabolism pathway by hepatic cytochrome P450 leads to at least one toxicological relevant metabolite, the fipronil sulfone (Das et al., 2006; Romero et al., 2016). Fipronil sulfone seems to be also formed in humans based on a case study (Mohamed et al., 2004).

Not fipronil nor fipronil sulfone are easily eliminated from the body; fipronil sulfone taking longer. In an oral study in 24 dogs the terminal half-lives were at maximum in the highest dose group of 6 mg/kg bw with the average of 338.82 ± 167.81 hours for fipronil and of 394.31 ± 185.30 hours for fipronil sulfone, i.e. an average of 16.4 days for fipronil sulfone to be eliminated at maximum (Dos Santos et al., 2022). Rats were administrated fipronil intravenously 1 mg/kg bw and orally 3, 10, or 30 mg/kg bw resulting in average half-lives of 2.66 ± 0.56, 12.16 ± 5.21, 9.16 ± 11.28 and 4.44 ±

[52] Commission delegated Regulation (EU) No 2019/330 of 11 December 2018 amending Annexes I and V to Commission Regulation (EU) No 649/2012 of the European Parliament and of the Council concerning the export and import of hazardous chemicals.

1.19 hours, respectively. Fipronil sulfone stayed longer in the body; half-lives were not given (Chang and Tsai, 2020). Thus, terminal half-life seems to be species dependent.

Fipronil and fipronil sulfone can be found in chicken eggs. In rats, they cross the blood placental barriers and enter the foetus (Chang and Tsai, 2020).

No residue depletion data were found for fipronil administrated in horses or in closely related species of the *Equidae* family. In rats, radiolabelled fipronil was administered orally at a single dose of 10 mg 14C-fipronil/kg bw. After 72 hours the highest levels of radioactivity could be found in adipose tissue and adrenals. The main part of the radioactivity present in investigated tissues (adipose tissue, adrenals, liver, kidney, testes) was due to fipronil-sulfone, accounting for over 90% (Cravedi et al., 2013).

Regarding toxicology, the existing harmonised chemical classification according to CLP, identifies fipronil as acutely toxic by inhalation, in contact with the skin and by ingestion but fipronil is not locally toxic. Fipronil exhibits systemic toxicity upon repeated administration by causing damage to the central nervous system (ECHA, 2024a; ECHA, 2011). The mechanism in humans and other species and at the same time the mode of action for using it as an insecticide is fipronil's action on the nervous system by blocking the gamma-aminobutyric acid (GABA)-regulated chloride channels with a higher affinity in insects (ECHA, 2011).

Repeated dosing in rats also induced hepatic effects and hypertrophy in thyroid after 28 days and 90 days with a NOAEL of 0.35 mg/kg bw/day. In a 1-year study in dogs, neurological effects were induced by fipronil (ECHA, 2011). Under the biocidal products legislation a long-term reference value of 0.0002 mg/kg bw/day was derived on the basis of the NOAEL of 0.019 mg/kg bw/day from a combined chronic toxicity/carcinogenicity study in rats due to neurological signs and functional and morphological changes in the liver, thyroid and kidney (ECHA, 2011). Also, an ADI of 0.0002 mg/kg bw/day under the pesticides legislation exists (European Commission, 2024).

According to the 2011 assessment report for inclusion of fipronil in the list of approved active substances under Directive 98/8/EC for placing biocidal products on the market (ECHA, 2011), fipronil does not appear to be mutagenic based on in vitro and in vivo industry owned tests conducted between 1988-2005. The assessed in vitro tests comprised negative results in Ames tests, in a gene mutation assay in Chinese hamster cells (V79) and in a cytogenetic study in human lymphocytes. The assessed in vivo test results were all negative, including two micronucleus tests in mouse and an unscheduled DNA synthesis (UDS) assay in primary hepatocytes from treated rats. However, recent findings from published in vivo studies suggest that fipronil may be genotoxic in mammals, contradicting the negative test results and thus, the suspicion of genotoxicity can no longer be ruled out. Also, fipronil causes oxidative stress-induced DNA damage and apoptosis in spermatozoa (Khan et al., 2015; Badgujar et al., 2017; DPR, 2022). Due to limitations in the test design across all studies, neither a definitive conclusion that fipronil is negative for genotoxicity nor a clear establishment of its genotoxic potential could be reached. Thus, new tests are required (DPR, 2022).

Fipronil was not carcinogenic in mice and in rats in combined chronic toxicity/carcinogenicity studies and in an 89/91 weeks-study in rats. In rats the observed thyroid follicular cell adenomas and carcinomas were not relevant for humans and in mice no evidence of carcinogenicity was observed (ECHA, 2011).

Fipronil was not regarded as a reproduction toxicant or teratogenic substance under the biocidal products legislation based on an oral two-generation reproduction study conducted in rats, with observed slightly reduced mating performance and litter size in the presence of significant parental toxicity and two oral teratogenicity studies in rats and rabbits without evidence of developmental

toxicity even at maternally toxic dose levels (ECHA, 2011). However, fipronil may cause male reproductive toxicity through DNA damage and apoptosis in spermatozoa (Khan et al., 2015).

Considering the pharmacokinetics and metabolite neuro-toxicology of fipronil leading possibly to toxic residues in fat tissue, as indicated by the very low ADI, and the possible genotoxicity of fipronil and fipronil metabolite, it can be concluded that fipronil will pose an unacceptable risk for consumers when used in food-producing animals of the equine species and a six-month withdrawal period is respected.

Sulpiride is a substituted benzamide antipsychotic selective antagonist of central dopamine receptors. It is used in humans orally or parenterally.

In humans, sulpiride is slowly absorbed from the gastrointestinal tract. Bioavailability is low and subject to interindividual variation. It is rapidly distributed to tissues but passage across the blood-brain barrier is poor because of its low lipid solubility. It is about 40% bound to plasma proteins and is reported to have a plasma half-life of about 8-9 hours. It is excreted in the urine and faeces, mainly as unchanged drug. It is known to induce prolactin elevation in both serum and cerebrospinal fluid (CSF) (Sweetman, 2009; Mauri et al., 1996). Other authors have described oral bioavailability estimates approximating 35%. It does not appear to have an extensive first-pass metabolism, nor is it extensively protein-bound. No identified active metabolites have been described (Caley et al., 1995).

After intravenous administration of 100 mg sulpiride, the data obtained were consistent with a two-compartment model. The apparent elimination half-life was 6.47 ± 1.00 hours and the mean volume of distribution at steady state was 0.94 ± 0.23 l/Kg. Renal clearance (119.5 ± 28.2 ml/min) was very close to total clearance (127.8 ± 26.2 ml/min). In urine, the mean unchanged drug was 90.0 ± 9.68% of the administered dose (Brès and Bressolle, 1991). In other study, the serum half-life of the terminal slope following 100 mg by intravenous administration averaged 5.3 hours and 70± 9 % of the dose was recovered unchanged in urine within 36 hours. The mean renal clearance was 310 ± 91 ml/min. In the same study, after oral administration, sulpiride was absorbed slowly, with peak concentrations appearing between 3 and 6 hours after oral administration. The recovery of unchanged drug in urine following oral administration was 15 ± 5% of the dose. The bioavailability determined from combined plasma and urine data was 27 ± 9% (Wiesel et al., 1980).

Some pharmacokinetic data is available from horses treated with 1 mg/kg via intravenous, intramuscular and oral routes. After intravenous administration, sulpiride was detectable up to 24 hours and had dropped below the limit of detection (LOD) at 34 hours. Distribution in peripheral tissues was rapid, with a transfer rate constant from tissue to plasma that was double the elimination rate constant. Main clearance values were 58.2 ± 14.5, 71.7 ± 57.8 and 165 ± 66 ml/h/kg, with volumes of distribution of 581 ± 230, 952 ± 706 and 398 ± 224 ml/kg, by intravenous, intramuscular and oral routes, respectively. Elimination half-lives reported were 7.0 ± 1.8, 9.2 ± 1.8 and 6.9 ± 1.5 hours, for the intravenous, intramuscular and oral routes, respectively. Surprisingly, the intramuscular absolute bioavailability was 118%, due to an increase in the plasma concentration in the terminal phase, increasing the AUC value. The absolute oral bioavailability described was low (20.4%) (Giorgi, et al., 2013).

Levosulpiride is the levorotatory enantiomer of sulpiride. No adverse effects were observed after subacute and subchronic levosulpiride administration. Regarding toxicity to reproduction, it has been reported that levosulpiride does not cause any adverse effect to reproduction. However, other studies suggest that sulpiride can alter reproductive function in female offspring rats and may impact the reproductive development of male rats: the testes seem to be the main target organ at adulthood. Sulpiride induced ovarian toxicity in rats treated with 1 mg/kg or more in 2- and 4-week repeated-dose toxicity studies, and the observed ovarian histological changes were considered to be related to

adverse effects on female fertility. Levosulpiride was studied by different test methods and was not found to be genotoxic. In addition, the substance was predicted to be non-mutagenic by 3 individual QSAR models and it was not found to be carcinogen in both rats and mice (ECHA, 2024c; Ishii et al., 2009).

There are no residue depletion studies in food-producing animals. However, considering the pharmacokinetics and toxicity of sulpiride and its enantiomer, it can be accepted that sulpiride 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.

4.15.4. Conclusion

Based on the above assessment and justifications, the following recommendations are proposed:

1. The following active substance, currently in Regulation (EU) 1950/2006 as amended by Regulation (EU) 122/2013, is proposed to be retained in the list, either without modification or with an amendment of the current entry as shown below:

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Domperidone	treatment of agalactia/dysgalactia in mares	sulpiride	its ability to stimulate prolactin secretion in situations of dopaminergic inhibition

2. The following active substances, currently in Regulation (EU) 1950/2006 as amended by Regulation (EU) 122/2013, are proposed to be removed from the list: cyproheptadine, imipramine.

3. The following active substances, suggested for addition to the list in the survey to stakeholders, are proposed to be added to the list with an entry as shown below:

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Cetirizine	for the treatment of conditions where an antihistamine is deemed necessary	chlorphenamine	second-generation histamine-1 (H1) receptor inverse agonists are alternatives with fewer CNS (sedative) side effects
Sulpiride	treatment of agalactia/dysgalactia in mares	domperidone	its ability to stimulate prolactin secretion in situations of dopaminergic inhibition

4. The following active substances, suggested for addition to the list in the survey to stakeholders, are not proposed for inclusion: cannabidiol, fipronil, hydroxyzine.

5. Review of the entries in Table 2 of the Annex to Commission Regulation (EU) No 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal, as provided for in the European Commission's mandate

The request received from the European Commission on 9 February 2023 to provide scientific advice according to Article 115(5) of Regulation (EU) 2019/6, included a call for considering substances included in Table 2 of the Annex to Commission Regulation (EU) 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin, for inclusion in the list, unless there are concerns related to consumer protection.

According to Article 15 paragraph 6 of Regulation (EC) No 470/2009 of 6 May 2009, *the administration of a substance to food-producing animals shall be prohibited (…) in either of the following circumstances:*

a) *where any presence of a pharmacologically active substance or residues thereof in foods of animal origin may constitute a hazard to human health;*

b) *where no final conclusion concerning the effect on human health of residues of a substance can be drawn*.

Table 2 the Annex to Commission Regulation (EU) 37/2010 lists the so called "prohibited substances", as follows:

- *Aristolochia spp.* and preparations thereof,
- Chloramphenicol,
- Chlorpromazine,
- Colchicine,
- Dapsone,
- Dimetridazole,
- Metronidazole,
- Nitrofurans (including furazolidone),
- Ronidazole.

For all these substances, an assessment was previously conducted by the Committee for Veterinary Medicinal Products (CVMP) according to Regulation (EC) No 470/2009 of 6 May 2009, and it was concluded that an "MRL cannot be established". The outcome of these assessments is captured in the respective European Public MRL Assessment Reports (EPMARs) for each of the concerned substances. Such EPMARs can be accessed in the Agency's corporate website (Maximum residue limit assessment reports | European Medicines Agency (europa.eu)). None of these prohibited substances for food producing animals were considered for inclusion in the list of substances essential for the treatment of

Equidae in accordance with Article 4 of Regulation (EU) 1950/2006[53] (as amended by Regulation (EU) 122/2013).

It Is worth mentioning that Regulation (EU) 2019/6 provides, amongst other things, for specific rules for the administration of veterinary medicinal products to food-producing animals, including equine animals. In particular, it lays down record-keeping obligations as regards equine animals and information to be contained in the single lifetime identification document referred to in point I of Article 114(1) of Regulation (EU) 2016/429[54] and in any acts adopted on that basis thereof. Commission Delegated Regulation (EU) 2021/577[55] lays down rules concerning the content and format of the information necessary to apply Articles 112(4) and 115(5) of Regulation (EU) 2019/6 and to be contained in the single lifetime identification document.

A. Clinical considerations on the essentiality of the prohibited substances

Aristolochia spp. is a plant genus of the family *Aristolochiaceae* with ecological significance due to its large size and huge geographic distribution (Nath et al., 2022). Medicinal (homeopathic) preparations of these plants contain complex mixtures of compounds including aristolochic acids and aristolactams, as the main medicinal principles, together with the quaternate aporphin base, magnoflorine (EMEA/MRL/271/97-FINAL). While the pharmacodynamic properties of *Aristolochia* have not been investigated according to current standards, aristolochic acids are reported to stimulate immune defence mechanisms against infections and inflammations in several mammalian species, including humans.

Though the EPMAR mentions that *Aristolochia* is used parenterally or orally for several days in horses, among other food-producing species, for the regulation of sexual functions, immunostimulation, antiphlogistic effects and others, such uses could not be backed up by the current scientific literature references. Indeed, a literature search could not reveal any published study supporting the use of *Aristolochia* in equine species. It has been recognised that horses ingesting hay contaminated with *Aristolochia clematitis* were observed to develop renal failure (Grollman and Marcus, 2016).

No specific condition has been identified for which *Aristolochia* could either represent a treatment alternative needed in equine species and the substance was not identified by stakeholders in the responses provided to the survey. Thus, it is concluded that *Aristolochia spp.* does not meet the criteria defined in EC's mandate as regards of substances that can be considered essential for the treatment of equine species or provide added clinical benefit.

Chloramphenicol is a broad-spectrum antibiotic initially isolated from the bacteria *Streptomyces venezuelae* in 1948 (Ehrlich et al., 1947). Chloramphenicol inhibits protein synthesis by reversibly binding to the peptidyl transferase cavity of the 50S subunit of the bacterial 70S ribosome. This prevents the aminoacyl-tRNA from binding to the ribosome, thus terminating polypeptide chain synthesis. Chloramphenicol is effective against a wide range of aerobic and anaerobic bacteria, including Gram-positive (e.g. *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *S. pneumoniae*, alpha-haemolytic *streptococci*, *Enterococcus faecalis)* and Gram-negative

[53] Commission Regulation (EC) No 1950/2006 of 13 December 2006, as amended by Commission Regulation (EU) No 122/2013 of 12 February 2013, establishing, in accordance with Directive 2001/82/EC of the European Parliament and of the Council on the Community code relating to veterinary medicinal products, a list of substances essential for the treatment of *equidae* and of substances bringing added clinical benefit.
[54] Regulation (EU) 2016/429 of the European Parliament and of the Council of 9 March 2016 on transmissible animal diseases and amending and repealing certain acts in the area of animal health (Animal Health Law)
[55] Commission Delegated Regulation (EU) 2021/577 of 29 January 2021 supplementing Regulation (EU) 2019/6 of the European Parliament and of the Council as regards the content and format of the information necessary to apply Articles 112(4) and 115(5) and to be contained in the single lifetime identification document referred to in Article 8(4) of that Regulation

bacteria (*Bacteroides fragilis*, *Neisseria*-meningococci and gonococci, *Haemophilus* spp., *Salmonella typhi*).

Chloramphenicol-containing VMPs are marketed for dogs and cats for the treatment of superficial eye (bacterial) and skin infections. In horses, off-label uses of chloramphenicol include the systemic use for the treatment of bacterial infections as well as topical use for bacterial eye infections.

The short systemic half-life of chloramphenicol in horses (1 hour), together with its generally bacteriostatic action, makes intravenous administration impractical because frequent administrations throughout a 24-hour period would be necessary to maintain therapeutic concentrations. Tablets of the free base drug can be administered orally, or the sodium succinate formulation can be given via intramuscular injection. After absorption from injection sites, the inactive succinate ester is rapidly hydrolyzed to the active drug. The oral bioavailability of chloramphenicol in foals is 83%, but only 40% after a single administration in mares; declining to 20% after 5 doses (Brumbaugh et al., 1983; Gronwall et al., 1986). Despite reasonable oral bioavailability and good tissue distribution, systemic administration to horses adversely alters faecal consistency and gastrointestinal microflora.

Chloramphenicol was mentioned by stakeholders in the survey (three times) and proposed for inclusion in the list. According to the information provided by stakeholders, the use of the substance should be restricted to ophthalmic use for the treatment of e.g. corneal ulcers, when a bacterial agent is diagnosed. However, no specific need for chloramphenicol has been identified in equine ophthalmology. For example, multi-resistant bacterial pathogens involved with equine eye infections have not been identified, whereby alternative antimicrobials available are still effective (Foote et al., 2023). A search in the veterinary medicines database retrieves at least two veterinary medicinal products authorised for use in equine species (non-food-producing horses) containing chloramphenicol for ocular use.

In the opinion of the expert group, chloramphenicol does not meet the criteria defined in EC's mandate as regards to substances that can be considered essential for the treatment of equine species or provide added clinical benefit. For systemic uses, other safer broad-spectrum antimicrobials are available for use in horses. Also, other antimicrobial classes of topical broad-spectrum antimicrobials for eye infections are available as well as proposed for inclusion in the list.

Alternatives within the same class as chloramphenicol (Amphenicols) include florfenicol and thiamphenicol. Thiamphenicol, for which MRLs are set for all food producing species, is authorised as a cutaneous spray for horses, in the EU, for the treatment of superficial wound infections as well as hoof lesions. Florfenicol is not authorised for horses but for other food animals (pigs, cattle) with established MRLs. Florfenicol is used outside the terms of the marketing authorisation in horses, although is associated with a risk of adverse events.

Chloramphenicol is thus not considered to bring added clinical benefit compared to the existing alternative medicinal product(s) authorised for food producing animals of the equine species, because the alternatives yield equally satisfactory results in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Chlorpromazine and related phenothiazine drugs have been used in human and veterinary medications for more than 40 years, predominantly as psychotropic agents. In human medicine, chlorpromazine is a well-established psychotropic agent indicated for the treatment of schizophrenia. It also exerts sedative and antiemetic activity and has actions at all levels of the central nervous system as well as on multiple organ systems (PubChem, 2023). Scientific reports in veterinary medicine do not reveal a specific use of chlorpromazine in equine veterinary practice that could be claimed as a treatment alternative needed in this animal species.

Chlorpromazine has not been identified by stakeholders in the responses provided to the survey. In addition, it is noted that the list of essential substances established by Regulation (EU) 1950/2006 (as amended by Regulation (EU) 122/2013) includes acepromazine, for its use prior to general anaesthesia (i.e. premedication) and for mild sedation. Acepromazine constitutes a viable and safe alternative to the potential use of chlorpromazine in *Equidae*. After revision of this entry, acepromazine is proposed to be retained in the list of essential substances for *Equidae*.

In the opinion of the expert group, chlorpromazine does not meet the criteria defined in EC's mandate as regards to substances that can be considered essential for the treatment of equine species or provide added clinical benefit.

Colchicine is a plant alkaloid that has been used for decades as an anti-inflammatory agent for the treatment of various diseases including gout, familial Mediterranean fever (FMF) and pericarditis, and in more recent years for dermatological indications including chronic urticaria, cutaneous vasculitis and psoriasis (Imazio, 2015; Pascart and Richette, 2018; Robinson and Chan, 2018; Deftereos et al., 2022). It has a narrow therapeutic index, with no clear-cut distinction between nontoxic, toxic, and lethal doses, causing substantial confusion among clinicians (Finkelstein et al., 2010).

A literature search did not reveal any published use for this substance in *Equidae* and nor was the substance identified by stakeholders in the responses provided to the survey. In the opinion of the expert group, colchicine does not meet the criteria defined in EC's mandate as regards to substances that can be considered essential for the treatment of equine species or provide added clinical benefit.

Dapsone is an aniline derivative belonging to the group of synthetic sulfones. Dapsone is not in the same chemical class as sulfonamides, but its mechanism of action is similar to sulfonamides as it acts via inhibition of bacterial synthesis of dihydrofolic acid by competing with para-aminobenzoate for the active site of dihydropteroate synthase. Since it became available in the 1940s, dapsone has been the principal drug in a multidrug regimen recommended by the World Health Organization for the treatment of leprosy (Wozel, 1989). It is reported to be used as an anti-infective agent for treating malaria and, more recently, for *Pneumocystic carinii* pneumonia (now known as *Pneumocystis jiroveci*) in human patients with AIDS (Wolf et al., 2002).

There is no veterinary approved formulation of dapsone. It is potentially useful for the oral treatment of some protozoal infections in horses (e.g. cutaneous leishmaniasis); however, safer alternative therapies exist, including spontaneous regression of lesions, surgical resection, pentavalent antimony, and fluconazole (Reuss, 2013). Also, dapsone is carcinogenic and not recommended in pregnant and nursing animals (Constable et al., 2016). Toxic effects include hepatotoxicity, anaemia, thrombocytopenia, neutropenia, and gastrointestinal effects.

Foals can also acquire *Pneumocystis carinii* pneumonia, and one case report describes the use of dapsone for the treatment of this infection (Clark-Price et al., 2004). However, the treatment-of-choice for *Pneumocystis carinii* pneumonia in foals is trimethoprim sulfonamides (approved VMPs for horses).

Since no specific condition has been identified for which dapsone could represent a safe treatment alternative needed in equine species and nor was it identified by stakeholders in the responses provided to the survey, it is concluded that dapsone does not meet the criteria defined in EC's mandate as regards of substances that can be considered essential for the treatment of equine species.

Dimetridazole, **metronidazole**, and **ronidazole** are antibiotics of the nitroimidazole class. The antimicrobial activity is bactericidal to most Gram-negative and many Gram-positive anaerobic bacteria. This antimicrobial class is highly specific for their activity against anaerobes, as well as *Brachyspira* (*Serpulina*) *hyodysenteriae* and a variety of protozoa (*Tritrichomonas foetus, Giardia*

lamblia, Histomonas meleagridis). Nitroimidazoles are heterocyclic compounds based on a 5-membered nucleus similar to that of the nitrofurans. Like the nitrofurans, the nitroimidazoles were once widely used in veterinary medicine, but due to carcinogenicity, have now been banned from use in food animals in the United States, Canada, and the European Union. Nitroimidazoles have been shown to be carcinogenic in some laboratory animals (e.g. rodents) and mutagenic in some in vitro assays.

A literature search did not reveal any published use for dimetridazole or ronidazole in *Equidae* and the substances were not identified by stakeholders in the responses provided to the survey. Thus, it is concluded that dimetridazole and ronidazole do not meet the criteria defined in EC's mandate as regards of substances that can be considered essential for the treatment of equine species or provide added clinical benefit.

The use of metronidazole In *Equidae* is well established. Metronidazole is moderately lipophilic and widely distributed in tissues. It penetrates bone, abscesses, and the central nervous system. Metronidazole is used to treat anaerobic infections, especially pleuropneumonia and lung abscesses caused by penicillin-resistant *Bacteroides fragilis* as well as clostridial enterocolitis in horses (Mair and Yeo, 1987; Baverud et al., 2003). While other antibiotics approved for horses are effective against anaerobes (e.g. beta-lactams, trimethoprim-sulfonamides, tetracyclines), none have the PK/PD characteristics of metronidazole to widely distribute in tissues.

Another example of metronidazole use is for *Clostridioides difficile* associated with colitis (i.e. antimicrobial-associated diarrhoea) in horses. As in human medicine, *Clostridioides difficile* diarrhoea carries a grave prognosis without treatment (Cohen and Woods, 1999; Magdesian et al., 2002). There are no approved medicines for this condition, and thus many horses are treated with metronidazole. However, AMR to metronidazole builds quickly in *C. difficile*; for example, in surveys up to 43% of metronidazole-resistant *C. difficile* isolates from horses have been reported in certain geographic locations (Jang et al., 1997; Magdesian et al., 2002), and the incidence is likely to be higher since these old surveys.

Clinically, the most serious adverse effect from the administration of antimicrobials in horses is antimicrobial-associated colitis (AAC). This syndrome develops in temporal association with antimicrobial therapy and may be caused by changes in the proportions of the intestinal microflora or allowing the proliferation and toxin production of *Clostridioides difficile*. Drugs, such as metronidazole, with activity against anaerobes are considered more likely to cause colitis, especially with greater disturbances of the bacterial communities of the hindgut and subsequent metabolic changes that result in more severe clinical symptoms (Arnold et al., 2021).

Metronidazole was mentioned (three times) in the survey to stakeholders and proposed for inclusion in the list. As indicated by stakeholders, metronidazole constitutes an essential alternative for the treatment of some anaerobic infections (Hollis and Wilkins, 2009).

The substance, metronidazole, is proposed, from a clinical perspective, to provide added clinical benefit compared to other treatment options for food-producing animals of the equine species for the treatment of specific anaerobic infections. Metronidazole treatment improves anaerobic coverage with better pharmacodynamic/pharmacokinetic characteristics for long-acting penetration into difficult to reach body sites. The drug therefore offers advantages in terms of successfully treating the animal or avoiding unnecessary suffering of the animal.

Nitrofurans (furazolidone, furaltadone, nitrofurantoin, and nitrofurazone) are synthetic chemotherapeutic agents with a broad antimicrobial spectrum (Chamberlain, 1976), but their toxicity limits their use. Nitrofurans, introduced in the 1940s and 1950s, have been signalled as potential

candidates for the revival and reintroduction of old antimicrobials to fight against the spread of antimicrobial resistance (Le and Rakonjac, 2021).

However, the carcinogenicity of the nitrofurans led to their ban in food animals in the United States, Canada, and the European Union. Nitrofurazone, once used orally as a veterinary antimicrobial drug, causes mammary and ovarian tumours in animals. Nitrofurazone stimulates the proliferation of estrogen-dependent cells, nitrofurazone metabolites are involved in tumour initiation through oxidative DNA damage, and nitrofurazone itself enhances cell proliferation, leading to promotion and/or progression in carcinogenesis (Hiraku et al., 2004).

The nitrofurans are rapidly metabolized after administration, resulting in stable tissue-bound metabolites, which persist in muscle and liver for weeks to months. These metabolites, 3-amino-2-oxazolidinone (the metabolite of furazolidone), 1-aminohydantoin (the metabolite of nitrofurantoin), and semicarbazide (the metabolite of nitrofurazone), are the marker residues for their parent compounds in animal tissues. Nitrofuran metabolite residues in food animal products are resistant to degradation during storage or by cooking (Cooper and Kennedy, 2007).

With regards to its use in *Equidae*, a number of considerably old scientific publications provide an account of the use of this class of molecules for topical wound treatment and management of contagious equine metritis (CEM) (Davis and Abbitt, 1977; Eaglesome and Garcia, 1979). Regarding the latter, the causative agent is a gram-negative, non-motile, microaerophilic bacterium of the genus *Taylorella* (*Taylorella equigenitalis*) and the disease manifestation is characterised by shortened inter-oestrus intervals and poor pregnancy rates associated with a muco-purulent vulvar discharge. However, changes in husbandry management of reproduction have impacted the epidemiology as well as the classical manifestations affecting equines worldwide, and the condition has essentially disappeared after almost four decades of international regulation and disease surveillance (Schulman et al., 2013).

It is considered that there are satisfactory alternative treatments for CEM for food-producing animals of the equine species. Infections in carrier animals may be cleared by washing the external genitalia with disinfectants, combined with local antimicrobial treatments; systemic antibiotics might also be recommended in some animals (Timoney, 1996; Erdman et al., 2011; CFSPH, 2015).

A veterinary-approved product in the United States and Canada is available for topical wound formulations of nitrofurazone and furazolidone.

No specific condition has been identified for which nitrofurans (including furazolidone) could represent a treatment alternative needed in equine species nor it has been identified by stakeholders in the responses provided to the survey. It is thus concluded that nitrofurans (including furazolidone) do not meet the criteria defined in EC's mandate as regards of substances that can be considered essential for the treatment of equine species.

The above judgement mainly relies on published evidence from peer-reviewed scientific reports. Expert judgement has also been taken into consideration when drafting the above conclusions.

B. Considerations regarding consumer safety

Two of the substances listed in Table 2 of the Annex to Commission Regulation (EU) No. 37/2010 were proposed by stakeholders for inclusion in the list (metronidazole and chloramphenicol), whereas only the first one is deemed as providing added clinical benefit for *Equidae* following part 'A' of the

assessment. Major considerations are given towards a high-level of consumer protection that may arise from the use of metronidazole in food horses.

For chloramphenicol, for which a well-documented use in equine practice was recognised, a complementary safety assessment was undertaken despite it did not qualify as essential following part 'A' of the assessment. The consumer safety assessment is presented in the annex (please refer to section 7.3).

For **metronidazole**, the CVMP noted in its summary report (i.e. EPMAR)

- that the information provided did not address the formation and toxicological relevance of covalently bound tissue residues with the intact imidazole structure, which has been demonstrated for other nitroimidazoles,
- that no toxicological NOEL could be identified in repeat dose toxicity studies,
- that its influence on fertility had not been specifically tested although impairment of male fertility was noted in the repeated dose toxicity studies,
- that teratogenicity was not adequately tested though it had been shown to have such teratogenic potential,
- that the substance was proven to be mutagenic in mammalian cell systems and human cells in vitro and in mouse in vivo,
- that genotoxic effects are known in man following oral treatments,
- that the substance is a proven carcinogen in mice and rats and according to IARC metronidazole is considered a possible carcinogen in humans, and
- that no data on a possible tumour-promoting mechanism are available.

The Committee concluded that no ADI could be established due to the genotoxic mechanism of the carcinogenicity of metronidazole, since it was not possible to establish a threshold level to calculate an ADI.

Regarding residue depletion, there was no full residue file and the pharmacokinetic data provided in the target animals focused mainly on absorption and plasma elimination following parenteral and oral routes of administration. There was no total residue study data on metabolism in the target animals and no information available on bound residues in tissues of target animals. No information was provided on depletion of total drug-related residues, nor on the ratio of marker to total residues. The routine analytical method was deemed inadequate.

The Committee, therefore, concluded that no MRL values could be recommended (EMEA/MRL/173/96-FINAL).

As concluded in part 'A' of this assessment, metronidazole is proposed to be qualified as providing added clinical benefit compared to other treatment options for food-producing animals of the equine species; it was also proposed by stakeholders to be included in the list. Therefore, the most recent scientific evidence is discussed hereinafter. While this expert group has no mandate to question the conclusions previously reached by CVMP, as indicated in the European Commission's mandate, any concerns related to consumer protection merits further consideration.

The most recent assessment report at the WHO level is still the JECFA report from 1989 that concluded that a toxicological assessment was not possible at the time and residue depletion had not been studied but "significant health concerns were identified". Therefore, it was concluded that residues of

metronidazole in food are best prevented by not using metronidazole in food-producing animals (JECFA, 1989; FAO, 2021). Bendeskya et al. (2002) underpinned the lack of data for classifying metronidazole as a human carcinogen. However, the United States National Toxicology Program (NTP) has lately classified metronidazole as falling into the group of "reasonably anticipated to be human carcinogens" based on sufficient evidence of carcinogenicity from studies in experimental animals (NTP, 2021).

In the EU, metronidazole is regularly detected in food of animal origin under National Residue Control Plans (EFSA, 2018). Thus, the published scientific literature on metronidazole since the MRL assessment in 1997 was considered and a summary is presented hereinafter. It was checked whether any new findings may change the substance evaluation since the MRL assessment was conducted by the CVMP.

Toxicology

Metronidazole is moderately lipophilic (octanol/water partition coefficient -0.02) (Drugbank, 2023). Reduction of the nitro group, leading to imidazole fragmentation, is required in order for it to become antimicrobially active (EMEA/MRL/173/96-FINAL; Dingsdag and Hunter, 2018).

Metronidazole is part of the nitroimidazoles antimicrobial class, heterocyclic compounds based on a 5-membered nucleus, similar to that of the nitrofurans. As described above in the clinical assessment, nitrofurans are established as being carcinogenic.

No adequate repeat dose studies could be found in the literature since 1997. Maternal hepatoxicity was observed in a 28-day repeat dose study in pregnant rats at all dosage groups (AbdRabou et al., 2023). Thus, there is still no NOAEL available.

Since 1997, studies on genotoxicity have been published both in vitro and in vivo in different animal species. These studies demonstrate mutagenicity of metronidazole in bacterial test systems (Sisson et al., 2000; Buschini et al., 2009). In male rats receiving a high fat diet, DNA double strand breaks and an increased number of pre-neoplastic lesions in the liver were seen (Eguchi et al., 2022). In male mice chromosomal aberrations and micronuclei were detected in bone marrow following oral exposure to metronidazole (El-Nahas and El-Asmawy, 2004). When instilled into the vagina of rats, metronidazole caused micronucleus formation in proestrus rat vaginal mucosal cells at the highest tested dose, i.e. 100 mg/kg bw/day for five days (Ornelas-Aguirre et al., 2006). The route of administration is not relevant for the risk assessment for consumers and could only be used for the assessment of genotoxic effects at the site of first contact. Oral administration of a therapeutic dose of metronidazole for 7 days did not cause DNA damage in peripheral blood mononuclear cells of dogs; in vitro, however, metronidazole led to a significant increase in DNA damage at 100 µg/mL in both canine and feline PBMCs (Peterson et al., 2022). In three cats, a COMET assay in PBMC was positive after the oral administration of 20 mg/kg of metronidazole benzoate every 12 hours for 7 days as well as in vitro in PBMC and T-cell lymphoma cell lines (Sekis et al., 2009).

Few in vitro studies on genotoxicity were published on human lymphocytes. Two of them show negative results on sister chromatid exchange (Gomez-Arroyo et al., 2004), formation of micronuclei or DNA strand breaks (Buschini et al., 2009). Another in vitro study investigated DNA damage in human lymphocytes with and without metabolic activation and in anaerobic conditions, the results of which suggest a role of oxidative stress as the cause for the DNA damage found following metronidazole treatment (Re et al., 1997).

Following oral administration of metronidazole in the therapeutic range, no DNA strand breaks and no sister chromatid exchange were observed in human volunteers (Fahrig and Engelke, 1997; Akyol et al.,

2000). A further study found increased DNA damage at the therapeutic range after oral administration of metronidazole to human volunteers and investigated the role of its hydroxyl metabolite in vitro (Menéndez et al., 2001, 2002). Its hydroxy metabolite has been reported to also have the capacity to produce damage to macromolecules, such as the DNA. In this study, the hydroxy metabolite was able to induce the expression of P53, that has been proposed as a biomarker of carcinogenicity, so it was concluded that the carcinogenicity of this metabolite requires further evaluation.

The available studies of genotoxicity are heterogeneous, and some have shortcomings (in vitro: lack of positive controls, lack of information on cytotoxicity; in vivo: small number of animals, small number of volunteers, lack of information on confounding factors). Overall, the new data available supports the assessment and conclusion previously reached by the CVMP in the MRL report with regards to genotoxicity and/or carcinogenicity of metronidazole.

The potential of metronidazole to cause reproductive effects including teratogenicity is still not finally concluded as recently published data in laboratory animals are still not suitable to derive such evidence. The studies found in the public literature were not designed in accordance with the respective OECD Guidelines (El Nahas and El-Ashmawy, 2004; Kumari and Singh, 2013; Abd Rabou et al., 2023) and the derivation of a NO(A)EL/LO(A)EL was not possible. However, adverse impacts on fertility (El Nahas and El-Ashmawy, 2004; Kumari and Singh, 2013) and on development of the foetus (Abd Rabou et al., 2023) were observed, which support the CVMP assessment of 1997.

On the other hand, data from pregnant women in publicly available literature did not show an association between the oral administration of metronidazole during pregnancy and teratogenicity (Caro-Paton et al., 1997; Struthers, 1997; Czeizel and Rockenbauer, 1998; Sheehy et al., 2015; Nwosu and Bloom, 2021). After vaginal treatment during pregnancy an association between metronidazole and congenital hydrocephalus cannot be excluded (Kazy et al., 2005). A possible increase in spontaneous abortions following the administration of metronidazole needs further investigation (Cram et al., 2015).

Overall, the new data do not add significantly to the assessment of the CVMP in the MRL report and do not allow a final conclusion on the reproductive including developmental toxicity potential of metronidazole.

Pharmacokinetics in horses

The administration routes in horses may be oral (tablets), intravenous (injection) or rectal (crushed tablets).

The MRL assessment report does not address kinetics in horses. Thus, the literature on the pharmacokinetics of metronidazole in horses from 1986-2020 was screened (Sweeney et al., 1986; Baggot et al., 1988; Specht et al., 1992; Steinman et al., 2000; Britzi et al., 2010; Swain et al., 2014; Kuroda et al., 2020). In general, for metronidazole a very high systemic availability after oral intake and a lower absorption after rectal intake is reported (Lamp, 1999). Metronidazole has a low protein binding and a large volume of distribution, also in horses, i.e. is widely distributed in the body fluids and tissues resulting in high concentration in these (Sweeney et al., 1986; Specht et al., 1992; EMEA/MRL/173/96-FINAL; Steinman et al., 2000). However, there are no details on (possible differences in the) distribution and possible accumulation of metronidazole in different equine tissues available. It was only observed to accumulate in the peritoneal fluid in horse (Sweeney et al., 1986; Specht et al., 1992; Kuroda et al., 2020). In mice metronidazole and its metabolites pass through the placenta and are transferred into milk (EMEA/MRL/173/96-FINAL). Also, metabolism was only studied in rats, dogs, rabbits and humans so far. Metronidazole is rapidly metabolised resulting in several break-down substances. Their distribution is strongly dependent on species. A small proportion of

administered metronidazole is excreted unchanged even in horses (Specht et al., 1992; EMEA/MRL/173/96-FINAL; Lamp, 1999). In addition, there is still no new information on bound residues (with possibly intact 5-imidazole structure). Not all metabolites after reductive activation can be determined due to their short lives (Dingsdag and Hunter, 2018). Metronidazole is generally excreted mainly via urine (EMEA/MRL/173/96-FINAL).

In adult horses the plasma elimination half-life of metronidazole was determined to be approximately 5 hours for a single oral administration of 15 mg/kg bw (Kuroda et al., 2020) and 2.9 hours when 25 mg/kg bw was given intravenously (Sweeney et al., 1986); other authors reported it to be within the range 2.5 to 4.17 hours, depending on the route of administration (oral, intravenous or rectal administration) at single doses of 10, 15 or 20 mg/kg bw (Baggot et al., 1988; Specht et al., 1992; Steinman et al., 2000; Britzi et al., 2010). Baggot et al. (1988) gives an apparent half-life of six hours following oral administration of 20 mg/kg bw in adult horses. In foals, similar volume of distribution as in adult horses was determined but with terminal elimination half-life being longer in foals when metronidazole was administered by intravenous and intragastric routes at a dose of 15 mg/kg bw, i.e. mean 9.07 to 13.78 hours (Swain et al., 2014). Metabolites may have longer half-life which was shown for the primary metabolite in a human volunteer study (metronidazole 8.9 hours, 2-hydroxymetronidazole 13.1 hours) (Stancil et al., 2018).

Residue depletion

There is no residue depletion literature available in horses. Two analytical methods were published with studies presenting residue depletion data in tissues in broilers and pigs (Pan et al., 2017; Církva et al., 2019). In the study by Pan et al. (2017) it was observed that, after a single oral administration of metronidazole at a dose of 25 mg/kg bw in broilers and pigs, metronidazole concentrations were lower in fat than in other tissues, and average residue concentrations were not depleted at the same rate in the different tissues investigated. Metronidazole could still be detected in liver, kidney and muscle up to 14 days after treatment, nevertheless the main portion was eliminated, and was below the limit of detection (LOD) in fat, heart, lung, stomach, intestine after 14 days. The elimination half-lives of metronidazole and the primary metabolite were similar in both species (Pan et al., 2017). Also, after a single administration of 40 mg/kg bw in broilers, where samples were taken for up to 19 days, metronidazole and metabolites in muscle were below LOD within five days, and in serum within 12 days (Církva et al., 2019).

Evaluation for metronidazole

Overall, the positive results in genotoxicity tests cannot be ignored even though there were also negative test results. Available data indicates that the main metabolite may also be genotoxic, and there is still a lack of data regarding toxicology of the metabolites. Moreover, based on sufficient animal studies, metronidazole is regarded to be a potential human carcinogen (NTP, 2021; FAO, 2021; EMEA/MRL/173/96-FINAL; IARC, 1987). There was no recent data found to sufficiently suggest otherwise. Therefore, the CVMP's original conclusion remains valid and metronidazole must be considered as genotoxic carcinogen for which no threshold can be determined. As no (mechanistic) data are available demonstrating that the mechanism underlying the observed genotoxicity is not relevant for consumers, metronidazole should not enter the food chain in line with Commission Regulation (EU) 2018/782[56] section II.6.3.6.7.

There is no robust information about the behaviour of metronidazole and its metabolites within the body or bound to tissues, especially not in horses. Due to the wide distribution of metronidazole in

[56] Commission Regulation (EU) 2018/782 of 29 May 2018 establishing the methodological principles for the risk assessment and risk management recommendations referred to in Regulation (EC) No 470/2009

tissues, plasma pharmacokinetics cannot be regarded as reflective of tissue concentrations. The assumption of a rapid residue depletion of metronidazole in horses is associated with a high degree of uncertainty. Residue depletion knowledge of metronidazole in horses is lacking and the scarce available data for other species can only be considered as supporting information. Moreover, there is still no agreement on which biomarkers (parent compound and/or metabolites) to study in such testing.

Therefore, since there is still a concern regarding toxicity (including the carcinogenicity potential) for humans for metronidazole (Group 2B IARC), and in the absence of tissue depletion studies in relevant tissues of the horse it is not possible to conclude that a six-months withdrawal period can be considered safe for consumers.

5.1.1. Conclusion

Based on the above assessment and justifications, the following recommendations are proposed:

As provided for in the European Commission's request received on 9 February 2023, consideration has been given to substances included in Table 2 of the Annex to Commission Regulation (EU) 37/2010. The European Commission's request indicated these substances *should be considered unless there are concerns related to consumer protection*.

The expert group preparing the scientific advice according to Article 115(5) of Regulation (EU) 2019/6, has concluded that none of these substances (currently not included in Regulation (EU) 1950/2006 as amended by Regulation (EU) 122/2013) should be included in the list. This recommendation is based on the following conclusions:

- In some cases, no clinical use could be identified for the substances in *Equidae*. With the exemption of metronidazole, the substances listed in Table 2 of the Annex to Commission Regulation (EU) 37/2010 cannot be considered as essential for the treatment of equine species because there are satisfactory alternative treatments for food-producing animals of the equine species (see report), and the substances are not considered to bring added clinical benefit compared to the existing alternative medicinal product(s) authorised for food producing animals of the equine species; the alternatives do yield equally satisfactory results in terms of successfully treating the animal, or avoiding unnecessary suffering of the animal.
- Metronidazole is proposed, from a clinical perspective, to be qualified as essential because it brings added clinical benefit compared to other treatment options for food-producing animals of the equine species for the treatment of anaerobic infections. Although pharmacokinetic parameters indicate a relatively rapid metabolism and some calculation is possible, the residue data for edible tissues are not available (i.e. studies were either not performed and/or data were not found). Furthermore, from a toxicological point of view, metronidazole is regarded as genotoxic and a potential human carcinogen. Therefore, the overall objective of maintaining a high level of consumer protection cannot be ensured when the substance is used for equine species even if a withdrawal period of six months is respected.

6. Conclusion

Based on the assessment and justifications presented in sections 4 and 5 of this scientific advice, the following list of essential substances is advised, along with the aimed indication, explanation of use, and identification of alternatives:

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Note substances identified with an [a] are those for which no satisfactory alternative treatment was identified; [b] means the substance brings added clinical benefit compared to other treatment options identified. Further details in section 4.

i. Anaesthetics

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Oxybuprocaine [a]	local topical anaesthesia for use in eyes	none identified	wide clinical experience
Prilocaine [b]	local topical anaesthesia prior to intravenous injection or catheterisation	lidocaine	in specific preparations (eutectic mixture of local anaesthetics), for topical application to skin; can be used to facilitate intravenous injection or catheterisation

ii. Analgesics

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Bromfenac [b]	treatment of uveitis and ocular inflammation	systemic NSAIDs (e.g. flunixin); topical (ocular) ketorolac	topical NSAIDs may result in less patient discomfort, reduced postoperative inflammation, prevention of miosis, and improvements in visual acuity in the early postoperative period
Fentanyl [b]	multimodal approach for moderate to severe acute painful conditions	butorphanol, morphine	produces better analgesia than certain other opioids and can be used for very painful conditions; recognized value for use in multi-modal approaches
Ketorolac [b]	treatment of eye pain and inflammation	systemic NSAID therapy (e.g. flunixin)	formulated for local application
Methocarbamol [b]	as part of treatment protocols in severe painful muscle spasms/muscle inflammation conditions	systemic NSAIDs (e.g. flunixin)	potent skeletal muscle relaxation; specific action on the internuncial neurons of the spinal cord to reduce acute skeletal muscle spasms without a concomitant alteration in muscle tone

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Morphine [b]	analgesia	butorphanol, fentanyl	more potent than other analgesics
Triamcinolone Acetonide [b]	for the treatment of joint inflammation	methylprednisolone	less harmful effects on cartilage metabolism

iii. Antimicrobials

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
i. Antibiotics			
Amikacin [b]	for the treatment of septicemia in horses and foals	gentamicin, ceftiofur	better safety profile in the target animal
Azithromicin [b]	to treat *Rhodococcus equi* infections susceptible to azithromicin	clarithromycin, erythromycin, gamithromycin, tulathromycin, doxycycline	added clinical benefit in cases of *R. equi* infections in foals that can be resolved as monotherapy or in combination with doxycycline only
Clarithromycin [b]	to treat *Rhodococcus equi* infections susceptible to clarithromycin	azithromycin, erythromycin, gamithromycin, tulathromycin, doxycycline	more active against *R. equi* in vitro than erythromycin or azithromycin; achieves greater concentrations in pulmonary epithelial lining fluid and alveolar macrophages than either erythromycin or azithromycin, though the half-life is shorter
Fusidic acid [b]	topical treatment of eye infections caused by gram-positive bacteria susceptible to fusidic acid	ofloxacin, moxifloxacin	broad spectrum for treatment of gram-positive infections; primary choice in superficial, uncomplicated corneal ulcers and acute conjunctivitis in horses
Moxifloxacin [b]	topical treatment of external eye infections caused by gram-positive cocci, gram-negative, atypical and anaerobic bacteria such as *P. aeruginosa* species susceptible to moxifloxacin	ofloxacin	advantageous pharmacokinetic profile; spectrum of activity includes gram-positive cocci and anaerobic bacteria that may be resistant to other quinolones
Ofloxacin [b]	treatment of external eye infections caused by gram-positive and gram-negative micro-organisms susceptible to ofloxacin	moxifloxacin	clinical experience; penetrates the entire cornea up to the anterior chamber of the eye
Polymyxin B [b]	for the treatment of bacterial keratitis, topical use	ofloxacin, moxifloxacin	effective alternative to systemic treatments; different mechanism of action to other topical alternatives
ii. Antifungals			
Amphotericin B [a]	treatment of fungal pneumonia, systemic use	none identified	treatment of choice
Miconazole [b]	for the treatment of fungal infection of the eye	natamycin, nystatin, voriconazole	broad spectrum of activity; less irritant compared to other topical antifungals
Nystatin [b]	for the treatment of fungal and yeast	miconazole	treatment of choice for yeast infections

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
	infections of the eye and genital tract		
Voriconazole [b]	treatment of fungal keratitis, topical use	miconazole	broad spectrum of activity
iii. Antivirals			
Acyclovir [b]	to treat cases of equine herpes virus infection associated with complications, topical use only	ganciclovir	treatment of choice for ocular ulcers when the implication of a viral pathogen is suspected
Ganciclovir [b]	to treat cases of equine herpes virus infection associated with complications, topical use	acyclovir, valacyclovir	wealth of evidence for the treatment of different virus-types causing herpetic infections
Valacyclovir [b]	to treat cases of equine herpes virus infections, oral use	acyclovir	better pharmacokinetic profile and a different route of administration

iv. Substances for respiratory disorders

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Ambroxol [b]	stimulation of surfactant in premature foals	steroids, bromhexine, dembrexine, surfactant transfer from healthy donor	preferred clinical choice for premature foal
Fluticasone [b]	for control of allergic pulmonary disease including mild to moderate cases of equine asthma and subtypes via inhalation	beclomethasone	inhalation leads to less adreno-cortical suppression, quicker rebound after therapy ends and fewer systemic side effects than systemic corticosteroid therapy because of its limited systemic absorption; especially indicated for control of mild-moderate and refractory severe asthma as well as long-term maintenance therapy
Ipratropium bromide [b]	as a bronchodilator in horses with mild-moderate asthma	clenbuterol	anticholinergic action, as an alternative to beta-agonists
Oxymetazoline [b]	treatment of nasal oedema	phenylephrine	α-adrenoceptor agonist with strong vasoconstrictive properties and longer acting effect
Phenylephrine [b]	treatment of nasal oedema	oxymetazoline	reduces the need for insertion of nasal tubes during recovery

v. Substances for cardiology

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Amiodarone [b]	systemic and oral treatment of atrial fibrillation, supraventricular and ventricular tachycardias	quinidine sulphate/gluconate, sotalol, verapamil	different mode of action: class III anti-dysrhythmic

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Propafenone [b]	treatment of ventricular tachycardia and tachyarrhythmia	quinidine sulphate/gluconate	different mode of action: sodium channel antagonist that decreases heart excitability
Quinapril [a]	treatment of heart failure; cardiovascular protection in horses with atrial fibrillation (AF) or mitral regurgitation (MR)	none identified	different mode of action: ACE inhibitor
Quinidine sulphate/gluconate [b]	treatment of cardiac arrhythmias	amiodarone, sotalol, verapamil	treatment of choice for atrial fibrillation
Sotalol [b]	long-term treatment of cardiac arrhythmias	amiodarone, quinidine sulphate/gluconate	more suitable in horses requiring long-term anti-arrhythmic therapy; less adverse events than amiodarone
Verapamil [b]	treatment of supraventricular arrhythmias	amiodarone, quinidine sulphate/gluconate, sotalol	different mode of action: calcium channel blocker

vi. Substances for diagnostic procedures

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Barium sulfate [a]	enhanced gastrointestinal tract visualization during radiographic examinations	none identified	no satisfactory alternative treatment for enhanced gastrointestinal tract visualisation during radiographic examinations
Fluorescein [b]	diagnostic tool for corneal keratitis or ulceration, topical use	Rose Bengal	diagnostic tool of choice when a viral culture is needed afterwards
Iohexol [a]	radiographic contrast agent for lower urinary tract studies, arthrography, myelography, sino- or fistulography and dacryocystography	none identified	non-ionic, water-soluble contrast agent
Phenylephrine [a]	grass sickness diagnosis	none identified	ancillary diagnostic approach to equine grass sickness polyneuropathy
Rose Bengal [b]	diagnostic tool for corneal keratitis or ulceration, topical use	fluorescein	diagnostic tool of choice for diagnosing eye keratitis/ulcers
Thyrotropin releasing hormone [a]	diagnosis of pars pituitary intermedia dysfunction	none identified	no satisfactory alternatives for diagnosis of pars pituitary intermedia dysfunction

vii. Substances for gastrointestinal disorders

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Metoclopramide [b]	for the treatment of post-operative ileus	intravenous fluid substitution, painkillers (e.g. flunixin), lidocaine	prokinetic drug
Misoprostol [b]	for the treatment of gastric glandular disease and colitis	omeprazole, sucralfate	superior to omeprazole for the treatment of equine gastric glandular disease
Phenylephrine [a]	treatment of splenic entrapment	none identified	clinical value in the resolution of splenic

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
			entrapment; causes a dose-dependent splenic contraction
Ranitidine [b]	treatment of gastric ulcers in critically ill neonates, intravenous use	omeprazole	the intravenous route of administration brings added clinical benefit over other oral antiulcer medications
Sucralfate [b]	treatment and prevention of gastric ulcers in horses	omeprazole	different mode of action than omeprazole (mucosal adherent), which provides physical lesion stabilisation

viii. Substances for metabolic disorders

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Insulin [b]	as an aid in the treatment of hyperlipaemia unresponsive to glucose therapy or severe hyperlipemia, used in combination with glucose and other therapies; for the diagnosis of metabolic disorders (e.g. insulin resistance associated with equine metabolic syndrome or pars pituitary intermedia dysfunction)	low-molecular weight heparin can be used for cases of hyperlipemia	insulin is the preferred clinical choice
Pergolide[57] [a]	symptomatic treatment of pituitary pars intermedia dysfunction in horses (PPID, also known as Equine's Cushing disease)	none identified	no therapeutic alternatives available; improves clinical condition

ix. Substances for musculoskeletal disorders

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Atracurium [b]	to induce muscle paralysis under general anaesthesia	cisatracurium, guaifenesin	brings added clinical benefit in horses under general anaesthesia in cases where increased muscle relaxation is necessary such as ophthalmic surgeries, certain orthopaedic repairs and when deep access to the abdominal cavity is needed.
Cisatracurium [b]	to induce muscle paralysis under general anaesthesia	atracurium, guaifenesin	brings added clinical benefit in horses under general anaesthesia in cases where increased muscle relaxation is necessary such as ophthalmic surgeries, certain orthopaedic

[57] Please refer to the section 'Points for further consideration' within this scientific advice for additional scientific considerations regarding this substance. It is proposed that despite the substance fulfilling the criteria established in the European Commission's request, it is not listed.

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
			repairs and when deep access to the abdominal cavity is needed.
Dantrolene sodium [b]	prevention of rhabdomyolysis; prevention of malignant hyperthermia during anaesthesia	NSAIDs, intravenous fluids, vitamin E/selenium	efficacious as preventative, inhibiting the release of calcium from the sarcoplasmic reticulum and thus causing dissociation of excitation-contraction coupling
Edrophonium [a]	to reverse the effects of atracurium muscle paralysis	none identified	cholinesterase inhibitor, essential for reversal of neuromuscular blockade; least side effects of the cholinesterase inhibitors in horses
Guaifenesin [b]	for induction and maintenance of general anaesthesia in field conditions	atracurium, cisatracurium	particularly indicated in field (non-hospital) conditions where anaesthesia may be necessary; the reduced cardiopulmonary depressive effects facilitate safe anaesthesia without advanced monitoring equipment or mechanical ventilation

x. Substances for nervous system disorders

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Diazepam [a]	short-term anti-convulsant for treatment of seizures	none identified	second-generation antiseizure

xi. Substances for ophthalmology

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Acetazolamide [b]	treatment of glaucoma, oral use	phenylephrine	its mechanism of action as carbonic anhydrase inhibitor
Cyclopentolate [b]	mydriatic agent	atropine, phenylephrine	induces significant mydriasis without affecting tear production, intraocular pressure, digestive function (i.e. gut motility and faeces production), or heart rate
Cyclosporine A [b]	for the treatment of autoimmune diseases of the eye	topical steroids	immunosuppressive effect by inhibiting T-lymphocyte proliferation and reducing cytokine gene expression
Phenylephrine [b]	treatment of glaucoma and epiphora	atropine and tropicamide	it does not (or only slightly) increase intra ocular pressure
Synephrine [b]	for the treatment of the mucous membranes of the eye as a decongestant	phenylephrine, tetryzoline	fast local effect; may enhances penetration local therapy, providing synergistic effects with

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
			e.g. local antimicrobial therapy
Tetryzoline [b]	for the treatment of the mucous membranes of the eye as a decongestant	phenylephrine, synephrine	fast local effect
Timolol maleate [b]	treatment of glaucoma, topical use	acetazolamide	its specific mode of action as a non-selective beta-adrenergic receptor blocking agent, provides for an important therapeutic choice in the treatment of glaucoma
Triamcinolone acetonide [b]	treatment of recurrent uveitis in cases that are refractory to other treatments	atropine, tropicamide	effective, low morbidity treatment in refractory cases to other treatments
Tropicamide [b]	treatment of recurrent uveitis	atropine, cyclopentolate, triamcinolone acetonide	rapid onset of action

xii. Substances for sedation and premedication (and antagonism)

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Acepromazine [b]	for a multimodal approach for tranquilisation and premedication in combination with other sedatives	detomidine, romifidine, xylazine, diazepam	the mode of action of acepromazine and its unique quality of sedation cannot be produced by alpha-2 agonist sedatives or benzodiazepines
Atipamezole [a]	for reversal of α-2 agonists	none identified	reverses sedative and analgesic effects and adverse cardiovascular reactions
Dexmedetomidine [b]	sedation or general anaesthesia as part of partial or total intravenous anaesthesia protocols	detomidine, romifidine, xylazine, diazepam	the most selective alpha2 agonist; short half-life and rapid redistribution, which particularly favour its use as a continuous rate infusion
Diazepam [b]	for premedication and induction of anaesthesia, mild tranquilisation with minimal cardiovascular and respiratory side effects	acepromazine, detomidine, romifidine, xylazine	the mode of action (at GABA receptor) provides unique tranquilisation without cardiorespiratory depression that cannot be produced by α-2 agonist sedatives (detomidine, romifidine and xylazine) or acepromazine
Flumazenil [a]	intravenous reversal agent for benzodiazepine effect during recovery from Total Intravenous Anaesthesia (TIVA) techniques	none identified	antagonist that competitively inhibits the benzodiazepine binding site at the GABA receptor
Naloxone [a]	reversal of opioid effects during emergencies	none identified	no alternatives available
Propofol [b]	as an intravenous anaesthetic, for induction of anaesthesia in foals	isoflurane	improvement in cardiovascular stability and quality of recovery over inhalation anaesthesia in foals

xiii. Substances for systemic disorders

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Allopurinol [b]	neonatal ischaemia reperfusion injury	vitamin E	different mode of action in inhibiting the formation of reactive oxygen species (ROS) than vitamin E
Dalteparin [b]	anticoagulant	heparin	reduction in molecular size is associated with a loss of thrombin inhibitory activity, but conversely an increase in FXa inhibition compared to unfractionated heparin
Dobutamine [b]	to manage hypotension under general anaesthesia	ephedrine	first-line medication for the treatment of hypotension in adult equines under general anaesthesia
Dopamine [a]	as part of a treatment protocol for only acute kidney injury/renal failure	none identified	low doses have been shown to cause renal vasodilation, increased renal blood flow, and increased urine production without systemic cardiovascular effects in conscious healthy horses
Ephedrine [b]	to treat hypotension under general anaesthesia	dobutamine	used to treat hypotension in adult equines under general anaesthesia where dobutamine is ineffective. Different mode of action to dobutamine with a more direct effect on cardiac contractility
Gelatinpolysuccinate [b]	to address long-term hypovolaemia resulting from conditions like e.g. low albumin	crystalloids	colloids are larger molecules compared to crystalloids (smaller molecules that stay longer in the intravascular space), which is an advantage for correcting hypovolemia from e.g. hypoalbuminemia
Glycopyrrolate [b]	to treat and prevent bradycardia	atropine	minimal central effect; suitable in conscious horses, before and after anaesthesia
Noradrenaline/ norepinephrine [b]	for treatment of early septic shock; for support of cardiovascular function in critically ill foals	dobutamine, dopamine	in compromised (sick) foals it is generally the only catecholamine effective in treatment of hypotension
Vasopressin [b]	treatment of circulatory collapse in foals and adult horses	epinephrine, dopamine, dobutamine	alternative in cases where standard catecholamine therapies like dopamine, dobutamine, epinephrine are ineffective or require potentiation to restore vascular tone in refractory vasodilatory shock states

xiv. Substances for tumours

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Imiquimod [a]	treatment of sarcoids	none identified	current research suggests that equine sarcoids likely result from a complex interaction including host immune system disfunction

xv. Z. Miscellaneous

Active substance	Indication(s):	Identification of alternatives:	Explanation of use / specific advantages:
Cetirizine [b]	for the treatment of conditions where an antihistamine is deemed necessary	chlorphenamine	second-generation histamine-1 (H1) receptor inverse agonists are alternatives with fewer CNS (sedative) side effects
Domperidone [b]	treatment of agalactia/dysgalactia in mares	sulpiride	its ability to stimulate prolactin secretion in situations of dopaminergic inhibition
Sulpiride [b]	treatment of agalactia/dysgalactia in mares	domperidone	its ability to stimulate prolactin secretion in situations of dopaminergic inhibition

7. Annex(es)

7.1. Tabulated summary of studies investigating the analgesic effect of phenylbutazone and considered for the assessment presented in section 4.2.3

Reference	Horses	Experimental model	Dose[58]	Parameters	Outcome
Toutain et al., 1994	5	Induced arthritis (Carpal joint)	PBZ 4mg/kg or Fluni 1mg/kg; IV, single dose	Local skin temp., stride length, carpal flexion, circum. Of inflamed joint.	Significantly better than saline; non-significant for clinical parameters
Mills et al., 1996	9	Induced soft tissue inflammation (Freud's adjuvant)	PBZ 4.4mg/kg, or PBZ 8.8mg/kg; IV, single dose repeated after 5 weeks	n.a.	Administration of PBZ would not affect the plasma kinetics of a subsequent dose
Erkert et al., 2005	12	Horses with navicular syndrome	PBZ 4.4 mg/kg, or Fluni 1.1 mg/kg; IV q24h for 4 days; crossover design	AAEP scale (modified); force plate	PBZ and Fluni better than placebo; non-significant force plate values between PBZ and Fluni
Hu et al., 2005	9	Natural occurring chronic forelimb lameness	PBZ 4.4mg/kg, or PBZ 8.8mg/kg; IV q24h for 4 days; crossover design	Lameness scores	Higher PBZ dosage not associated with greater analgesic effects
Foreman et al., 2008	8	Experimental pain model (HBS-adjustable heart bar shoe)	PBZ 4.4 mg/kg; IV, single dose; crossover design	Lameness scores	PBZ significantly better than placebo
Keegan et al., 2008	29	Natural occurring lameness (fore/hind limbs) of heterogenous origins	PBZ 2.2mg/kg, or PBZ 2.2mg/kg plus Fluni 1.1 mg/kg; PO for 5 days; crossover design	Computer assisted kinematic study on treadmill	Combo significantly better than PBZ alone; PBZ alone non-significant lameness improvement
Foreman et al., 2010	6	Experimental pain model (HBS-adjustable heart bar shoe)	PBZ 4.4 mg/kg, or Fluni 1.1 mg/kg; IV, single dose; crossover design	AAEP scale	Single dose PBZ or Fluni demonstrated efficacy vs. placebo; PBZ score decreased slightly earlier than for Fluni ($p<0.05$)
Foreman and Ruemmler, 2011	8	Experimental pain model (HBS-adjustable heart bar shoe)	PBZ 4.4 mg/kg, or Fluni 1.1 mg/kg, or PBZ+Fluni; IV, single dose; crossover design	AAEP scale	PBZ+Fluni not more efficacious than PBZ or Fluni alone; faster onset of effect PBZ (20 mins)
Banse and Cribb, 2017	16	Experimental pain model (HBS-adjustable heart bar shoe); experimental pain model (SYN-LPS synovitis)	PBZ 4.4 mg/kg, or Melox 0.6 mg/kg; PO single dose; crossover design	AAEP scale; pain scores	HBS: PBZ more effective than Melox; SYN: Melox more effective than PBZ
Submitted (unpublished) study, 2024	10	Natural occurring lameness (fore/hind limbs) of heterogenous origins	PBZ 4.4 mg/kg, or Fluni 1.1 mg/kg, or Melox 0.6 mg/kg; PO, for 2 days; crossover design	AAEP scale	PBZ faster onset of effects; PBZ residual effects with after 24h

[58] Legend: 'Fluni' is flunixin; 'Melox' is meloxicam; and 'PBZ' is phenylbutazone.

7.2. Estimation of the risk that could arise from the possible presence of residues after the six-month withdrawal period indicated by Article 115(5) of Regulation (EU) 2019/6

7.2.1. Amphotericin B

No pharmacokinetic information or depletion studies are available in horses. The available knowledge from laboratory animals indicates that amphotericin B residues could occur after a six-month withdrawal period in the horse. Therefore, an estimation of the possible presence of residues after a six-month withdrawal period after the treatment is applied as presented below. The risk estimation is made under the following uncertainties and assumptions:

- There is no experience with the application of the liposomal form of amphotericin B in horse, but its use on horses cannot be ruled out,
- the maximum individual dosage in equine species is assumed to be ca. 1 mg/kg (e.g. Stewart et al., 2009), and the maximum cumulative reported dosage is up to 6.75 mg/kg, when administered at increasing doses for three days (0.3-0.6 mg/kg), followed by 4 days without treatment and subsequently at a dose of 0.6 mg/kg every other day, for 5 weeks (Cornick et al., 1990),
- for worst case also a treatment over 30 days is taken into account resulting in possibly cumulative up to 27 mg/kg (Stewart et a., 2008; Chaffin et al., 1995)
- amphotericin B disposition in tissues of mice extrapolated to a tissue concentration at day 180 day is used as surrogate to represent the residue situation in horse tissues after a 6-months withdrawal period,
- for the latter the assumption is made that the concentrations in mice tissue fall exponentially, i.e. that all measured values are already in the elimination phase;
- in the absence of adequate TRVs from studies in laboratory animals, a low human therapeutic dose was used for comparison with the potential consumer exposure and for calculating the margin of exposure.

Estimation:

1. Assumptions for a worst-case residue depletion:

As previously indicated, no pharmacokinetic information or depletion studies are available in horses. In the absence of data as well as of any (validated) PBPK modelling, from Gangneux et al. (1996) measured disposition data in mice for the conventional and liposomal amphotericin B formulation is used (see table below). Using a linear regression, the concentration in liver and spleen was extrapolated to day 180. In liver, for the individual dose of 0.8 mg/kg and cumulative dose of 4.8 mg/kg in mice, respectively, concentrations were no longer detected by day 103 at the latest. For the individual dose of 5 mg/kg and cumulative dose of 30 mg/kg in mice, respectively, the residue in liver was estimated to be lower than in spleen (0.1 mg/kg for the early liposomal formulation, and 0.2 mg/kg for the delayed liposomal formulation), thus the result for spleen was indicative only.

Experimental design and formulation type	Adm.	Individual dose (mg/kg)	Accumulative dose (mg/kg)	Frequency	Day after treatment	Liver mean (µg/g)	Spleen mean (µg/g)	Spleen mean day 180 (µg/g)
Early; deoxycholate amphotericin B	iv	0.8	4.8	6 times treated early day 7 to 17 after infection	43	ND	[…]	
					103		ND	

Experimental design and formulation type	Adm.	Individual dose (mg/kg)	Accumulative dose (mg/kg)	Frequency	Day after treatment	Liver mean (µg/g)	Spleen mean (µg/g)	Spleen mean day 180 (µg/g)
Delayed; deoxycholate amphotericin B	iv	0.8	4.8	6 times treated delayed day 60 to 70	2	[…]	4.53	
					55	ND	0.11	2E-05
Early; liposomal amphotericin B	iv	0.8	4.8	6 times treated early day 7 to 17	3	[…]	23.84	0.03
					43	[…]	5.48	
					103	ND	0.53	
Delayed; liposomal amphotericin B	iv	0.8	4.8	6 times treated delayed day 60 to 70	2	[…]	5.92	0.0002
					55	ND	0.29	
Early; liposomal amphotericin B	iv	5	30	6 times treated early day 7 to 17	3	209.7	98.8	0.4
					43	55.94	28.72	
					103	2.9	4.3	
Delayed liposomal amphotericin B	iv	5	30	6 times treated delayed day 60 to 70	2	189.01	65.1	0.7
					55	25.48	16.6	

The concentration-time function was assumed to be exponential, i.e. according to the formula concentration = a*exp(-k*time). The constants a and k were calculated from the measured concentrations using linear regression after log transformation, so that the concentration on day 180 could be extrapolated. In order not to create a false impression of accuracy, all results were rounded to one leading decimal place. All calculations were carried out in Excel.

Note that this is a rough, pragmatic approach, so it can only be expected that the results are approximately of the estimated magnitude.

After the extrapolation to 180 days using three points in time, the estimate for the early applied liposomal amphotericin B cumulative 4.8 mg/kg in mice resulted in 0.03 µg/g (mg/kg) in spleen and for the early applied liposomal amphotericin B cumulative 30 mg/kg in mice resulted in 0.4 µg/g (mg/kg) in spleen. The measured data based on three points in time were regarded more adequate as a residue estimation.

2. Toxicological reference values available:

In the absence of adequate NOAEL, for the proposed estimation, the lowest human dose i.v. 0.25 mg/kg bw/d was used as toxicological reference value (TRV).

3. Consumer exposure:

Based on the worst-case residue calculation above (see point 1), consumers could be exposed to 0.03 mg amphotericin B/kg edible tissue or 0.4 mg amphotericin B/kg edible tissue depending on the worst-case dosage of a treated horse at the end of the withdrawal period. A human consumer could thus be exposed to 0.00025 mg amphotericin B/kg bw/d or 0.0033 mg amphotericin B/kg bw/d, if a 60 kg person ingests 0.5 kg meat and offal. As indicated above, this value has been obtained from a number of assumptions and with high uncertainties due to the lack of appropriate data.

Oral absorption is regarded to be 1%, i.e. internal exposure is estimated to be 0.0000025 mg/kg bw/d or 0.000033 mg/kg bw/d.

4. Margin of exposure:

The resulting margin of exposure MoE (table) is based on the human therapeutic internal dose based on the assumption of the absence of side effects at the human i.v. therapeutic dose.

TRV descriptor	TRV value (mg/kg bw/day)	Accumulative dose (mg/kg)3	Consumer exposure (mg/kg bw/day)	Resulting MOE*	Required MOE
Lowest human therapeutic dose	0.25	4.8	0.0000025	100,000	>30[1, 2]
Lowest human therapeutic dose	0.25	30	0.000033	7501	>30[1, 2]

* MoE=TRV/exposure
[1] Intra-species factor only: F=10; an additional inter-species factor to extrapolate from laboratory animals to humans is not required.
[2] As a LOEL is assumed as TRV than the required MoE should be higher than only the intra-species factor, i.e. implementing the additional factor F=3 which is the same factor when deriving a NOEL from a LOEL (LOEL/3 = NOEL).
[3] According to administrated accumulative dose in mice.

5. Considerations on the assumptions and uncertainties of this estimation:

- The use of a human therapeutic dose as a surrogate of the lowest observed (adverse) effect (LO(A)EL) is generally considered inadequate in consumer exposure calculations because the most sensitive endpoints actually relevant for consumer risk assessment could be even below this level. In addition, the product information of some human medicinal products notes that the side effects occur even at the lowest human therapeutic use.
- Insufficiency of data is not considered in the estimation so far (by introducing an additional uncertainty factor in the required MoE).

Conclusion:

The resulting MoE is regarded sufficient so that it can be concluded that from amphotericin B, although residues might be expected after a six-months withdrawal period, it can be accepted that amphotericin B 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.

7.2.2. Dorzolamide

No pharmacokinetic information or depletion studies are available in horses. The available knowledge from humans indicates that dorzolamide accumulates in the red blood cells and can produce side effects, and that even low concentrations in plasma have a long elimination half-life close to the proposed six-month withdrawal period in the horse. Therefore, an estimation of the possible presence of residues after a six-month withdrawal period is applied to a treated horse as presented below. The risk estimation is made under the following uncertainties and assumptions:

- Even distribution of residues in the treated animal,
- Same half-life in horses as that reported in humans,
- Treatment of monoliteral glaucoma,
- In absence of adequate TRVs for consumer exposure, use of the human therapeutic dose as a surrogate for deriving a LOEL for systemic side effects.

Estimation:

1. Assumptions for a worst-case residue depletion:

A worst-case treatment of monoliteral glaucoma is proposed, consisting of 0.25 ml of 2% dorzolamide every 8 hours for 14 days. This might result in 210 mg dorzolamide being administered topically to the treated animal.

A worst-case absorption rate of 33% is proposed, noting the absorption rates in humans were reported to be up to 33% (Martens-Lobenhoffer and Banditt, 2002). This would lead to 69.3 mg dorzolamide would be systemically absorbed in the treated animal.

An even distribution in the treated animal is assumed.

As previously indicated, no pharmacokinetic information or depletion studies are available in horses. In the absence of data as well as of any (validated) PBPK modelling to be used, the human elimination half-life of 4 months (i.e. 120 days) after treatment is used (Biollaz et al., 1995; Balfour and Wilde, 1997).

An estimation of the remaining active substance after 6-months (i.e. 180 days) was conducted using an *ad hoc* formula[59]. The result obtained suggests residues might remain after 180 days: of 24.5 mg dorzolamide/animal, or 0.061 mg/kg bw if an even distribution is assumed in a (worst-case) 400 kg horse; with the same approach, a 99.9% elimination is achieved around 40 months.

2. Toxicological reference values available:

Toxicological data is reported in the summary of product characteristics of some human medicinal products. Developmental studies in laboratory animals (rabbits and rats) retained the same NOAEL of 1 mg/kg bw day due to vertebral malformations and decreased foetal body weight in rabbits, and reduced birth weight, reduced weight gain, and a slight delay in postnatal development in rats. The lowest NOAEL for maternal toxicity derived from a developmental toxicity study in rats was 0.1 mg/kg bw/day, due to reduced body weight gain.

In clinical studies performed in humans a dose of 4 mg dorzolamide/day was used without systemic side effects being reported (Martens-Lobenhoffer and Banditt, 2002). This dose also proposed in the summary of product characteristics of some human medicinal products. For a 60 kg human, this dose equals to 0.066 mg/kg bw/day, which is assumed to be the LOEL for systemic side effects. However, it is also noted that the summary of product characteristics of some human medicinal products report, from clinical studies, that the inhibition of carbonic anhydrase (including CA-II) was even observed below the therapeutic dose to produce pharmacological effect on renal and respiratory function (i.e. 2 mg twice a day), though no signs of metabolic acidosis or serum electrolyte changes were observed.

Such a LOEL of 0.066 mg/kg bw/day is below the lowest observed toxicological NOAEL of 0.1 mg/kg bw/day reported in human medicinal products for maternal toxicity from a developmental toxicity study in rats. Therefore, for the proposed estimation, the toxicological reference value (TRV) is derived based on the lowest human dose as indicated above (in the absence of suitable human data).

However, for such human based TRV to be relevant for the proposed estimation, only the dose reaching systemic circulation is relevant (to be comparable with any exposure to residues after ingestion of foodstuffs). As indicated in the literature, only a percentage of the topical dose applied is absorbed systemically. Martens-Lobenhoffer and Banditt (2002) suggested up to 33 % absorption rate of a 4 mg/person dose. Using 33 % absorption this results in a dose of 1.3 mg/person (0.022 mg/kg bw/day), which is the dose regarded as being the lowest known to produce

[59] Ad hoc formula provided by the BVL (German Competent Authority) for estimating residues: Cumulated dose over whole treatment duration [mg/animal] *systemic availability [%]/100*0.5^(proposed withdrawal period [d]/plasma elimination half-life [d]), i.e. 210*0.33*0.5^(180/120)

pharmacological effect and yet no systemic side effects, i.e. LOEL surrogates for the proposed estimation.

3. Consumer exposure:

Based on the worst-case residue calculation above (see point 1), consumers could be exposed to 0.061 mg dorzolamide/kg edible tissue of a treated horse at the end of the withdrawal period. A human consumer could thus be exposed to 0.0005 mg dorzolamide/kg bw day, if a 60 kg person ingests 0.5 kg meat and offal. As indicated above, this value has been obtained from a number of assumptions and with high uncertainties due to the lack of appropriate data.

4. Margin of exposure:

The resulting margin of exposure MoE (table) is based on the human therapeutic internal dose based on the assumption of the absence of side effects at the human ocular therapeutic dose.

TRV descriptor	TRV value (mg/kg bw/day)	Consumer exposure (mg/kg bw/day)	Resulting MOE*	Required MOE
Lowest human therapeutic dose (ocular) without systemic side effects, assuming 33% systemic availability	0.022	0.0005	44	>30[1, 2]

* MoE=TRV/exposure
[1] Intra-species factor only: F=10; an additional inter-species factor to extrapolate from laboratory animals to humans is not required.
[2] As a LOEL is assumed as TRV than the required MoE should be higher than only the intra-species factor, i.e. implementing the additional factor F=3 which is the same factor when deriving a NOEL from a LOEL (LOEL/3 = NOEL).

5. Considerations on the assumptions and uncertainties of this estimation:

- As indicated above, this scenario represents treatment of monoliteral glaucoma; bilateral glaucoma, though less common, cannot be excluded.
- The assumption of even distribution of residues in the animal may result in an underestimation of the real residue levels in edible tissues.
- The half-live in horses might be different from that in humans.
- The use of a human therapeutic dose with no side effects observed as a surrogate of the lowest observed (adverse) effect (LO(A)EL) is generally considered inadequate in consumer exposure calculations because the most sensitive endpoints actually relevant for consumer risk assessment could be even below this level. In addition, the product information of some human medicinal products notes that the inhibition of carbonic anhydrase (including CA-II) was below the level believed to be necessary for a pharmacologic effect on renal and respiratory functions.
- Insufficiency of data is not considered in the estimation so far (by introducing an additional uncertainty factor in the required MoE).

Conclusion:

Considering the available information and the long half-life (≥ 120 days) in humans, in the absence of pharmacokinetic information or depletion data in horses and using a toxicological reference value (TRV) based on the human therapeutic use, not enough margin of safety (MoE) is achieved for dorzolamide when used as proposed. The resulting MoE only just reaches the required MoE and an underestimation of the exposure proposed cannot be excluded, even when based on a worst-case residue depletion with available data so far. Therefore, it can be concluded that dorzolamide can pose an unacceptable risk for consumers when used in food-producing animals of the equine species and a six-month withdrawal period is respected.

7.2.3. Brinzolamide

No pharmacokinetic information or depletion studies are available in horses. The available knowledge from humans indicates that brinzolamide accumulates in the red blood cells and can produce side effects, and that even low concentrations in plasma have a long elimination half-life close to the proposed six-month withdrawal period in the horse. Therefore, an estimation of the possible presence of residues after a six-month withdrawal period is applied to a treated horse as presented below. The risk estimation is made under the following uncertainties and assumptions:

- Even distribution of residues in the treated animal,
- Same half-life in horses as that reported in humans,
- Treatment of monoliteral glaucoma,
- In absence of adequate TRVs for consumer exposure, use of the human therapeutic dose as a surrogate for deriving a LOEL for systemic side effects.

Estimation

1. Assumptions for a worst-case residue depletion:

A worst-case treatment of monoliteral glaucoma is proposed, consisting of one drop of 1% brinzolamide (containing 309 µg brinzolamide; summary of product characteristics in human medicinal product) every 12 hours for 14 days. This might result in 8.652 mg brinzolamide being administered topically to the treated animal.

A worst-case absorption rate of 70% is proposed, noting the absorption rates in rats were reported to be 70% (Hall et al., 1999). This would lead to 6.056 mg brinzolamide would be systemically absorbed in the treated animal.

An even distribution in the treated animal is assumed.

As previously indicated, no pharmacokinetic information or depletion studies are available in horses. In the absence of data as well as of any (validated) PBPK modelling to be used, the human elimination half-life of 24 weeks (168 d) after treatment is used (summary of product characteristics in human medicinal product).

An estimation of the remaining active substance after 6-months (i.e. 180 days) was conducted using an *ad hoc* formula[60]. The result obtained suggests residues might remain after 180 days: of 2.88 mg brinzolamide/animal, or 0.0072 mg/kg bw if an even distribution is assumed in a (worst-case) 400 kg horse; with the same approach, a 99.9% elimination is achieved around 55 months.

2. Toxicological reference values available:

Toxicological data is reported in the summary of product characteristics of some human medicinal products. The lowest maternal NOAEL was seen in the developmental study in rabbits (1 mg/kg bw/d; maternal weight loss) whereas in rats the maternal NOAEL was 2 mg/kg bw/d. The lowest NOAEL for developmental toxicity can be regarded to be 2 mg/kg bw/d from the developmental study in rats (decreases in foetal weights).

The lowest human dose without side effects for one eye is calculated to be 185.4 µg brinzolamide/day. The calculation is based on the information that even a 0.3 % solution has a significant

[60] Ad hoc formula provided by the BVL (German Competent Authority) for estimating residues: Cumulated dose over whole treatment duration [mg/animal] *systemic availability [%]/100*0.5^(proposed withdrawal period [d]/plasma elimination half-life [d]), i.e. 8.652*0.7*0.5^(180/168)

pharmacological effect (Silver, 2000) but is assumed to be without side effects and based on 309 µg brinzolamide in the available 1% human medicinal product. For a 60 kg human, this dose equals to 3.09 µg/kg bw/day, which is assumed to be the LOEL for systemic side effects.

Such a LOEL of 3.09 µg/kg bw/day is below the lowest observed toxicological NOAEL of 1 mg/kg bw/day reported in human medicinal products for maternal toxicity from a developmental toxicity study in rabbits. Therefore, for the proposed estimation, the toxicological reference value (TRV) is derived based on the lowest human dose as indicated above (in the absence of suitable human data).

However, for such human based TRV to be relevant for the proposed estimation, only the dose reaching systemic circulation is relevant (to be comparable with any exposure to residues after ingestion of foodstuffs). As indicated in the literature, only a percentage of the topical dose applied is absorbed systemically and the only information is given for rats (70 %; Hall et al., 1999). Using 70 % absorption this results in a dose of 0.00216 mg/kg bw/day, which is the dose regarded as being the lowest known to produce pharmacological effect and yet no systemic side effects, i.e. LOEL surrogates for the proposed estimation.

3. Consumer exposure:

Based on the worst-case residue calculation above (see point 1), consumers could be exposed to 0.0072 mg brinzolamide/kg edible tissue of a treated horse at the end of the withdrawal period. A human consumer could thus be exposed to 0.00006 mg brinzolamide/kg bw day, if a 60 kg person ingests 0.5 kg meat and offal. As indicated above, this value has been obtained from a number of assumptions and with high uncertainties due to the lack of appropriate data.

4. Margin of exposure:

The resulting margin of exposure MoE (table) is based on the human therapeutic internal dose based on the assumption of the absence of side effects at the human ocular therapeutic dose.

TRV descriptor	TRV value (mg/kg bw/day)	Consumer exposure (mg/kg bw/day)	Resulting MOE*	Required MOE
Lowest human therapeutic dose (ocular) without systemic side effects, assuming 70% systemic availability	0.00216	0.00006	36	>30[1, 2]

* MoE=TRV/exposure
[1] Intra-species factor only: F=10; an additional inter-species factor to extrapolate from laboratory animals to humans is not required.
[2] As a LOEL is assumed as TRV than the required MoE should be higher than only the intra-species factor, i.e. implementing the additional factor F=3 which is the same factor when deriving a NOEL from a LOEL (LOEL/3 = NOEL).

5. Considerations on the assumptions and uncertainties of this estimation:

- As indicated above, this scenario represents treatment of monoliteral glaucoma; bilateral glaucoma, though less common, cannot be excluded.
- The assumption of even distribution of residues in the animal may result in an underestimation of the real residue levels in edible tissues.
- The half-live in horses might be different from that in humans.
- The use of a human therapeutic dose with no side effects observed as a surrogate of the lowest observed (adverse) effect (LO(A)EL) is generally considered inadequate in consumer exposure calculations because the most sensitive endpoints actually relevant for consumer risk assessment could be even below this level.

- Insufficiency of data is not considered in the estimation so far (by introducing an additional uncertainty factor in the required MoE).

Conclusion:

Considering the available information and the long half-life (≥ 168 days) in humans, in the absence of pharmacokinetic information or depletion data in horses and using a toxicological reference value (TRV) based on the human therapeutic use, not enough margin of safety (MoE) is achieved for brinzolamide when used as proposed. The resulting MoE only just reaches the required MoE and an underestimation of the exposure proposed cannot be excluded, even when based on a worst-case residue depletion with available data so far. Therefore, it can be concluded that brinzolamide can pose an unacceptable risk for consumers when used in food-producing animals of the equine species and a six-month withdrawal period is respected.

7.3. Considerations regarding consumer safety for chloramphenicol

For **chloramphenicol**, the CVMP noted in its summary report (i.e. EPMAR)

- that no adequate reproductive toxicity studies were available,
- that in teratogenicity studies (in rats and rabbits) the substance did not cause teratogenic effects but caused a high incidence of foetal deaths even at the lowest dose level tested,
- that almost all in vitro mutagenicity assays showed positive results and that available in vivo mutagenicity assays with the substance provided equivocal results,
- that three metabolites were also shown to be mutagenic in an in vitro assay,
- that carcinogenicity tests in experimental animals were inadequate, and
- that historical epidemiological data in humans was associated with induction of blood dyscrasias, particularly aplastic anaemia.

The Committee concluded that no ADI could be estimated because of the inability to identify a threshold level for the induction of aplastic anaemia in humans, its positive genotoxicity results in in vitro and in vivo test systems, the lack of an adequate carcinogenicity study, the lack of a NOEL for foetotoxicity, and the lack of an adequate reproductive toxicity study.

Regarding residue depletion, no radiolabelled depletion studies were available, and it was not possible to determine the concentration of total residues during drug depletion; percentages of parent drug and metabolite(s) residues could not be ascertained, and there was insufficient information to confirm a marker residue.

The Committee, therefore, concluded that no MRL values could be elaborated (EMA, 1996b).

As concluded in part 'A' of this assessment, chloramphenicol is not proposed to be qualified as essential, nor as bringing added clinical benefit for *Equidae*. However, since it was proposed by stakeholders to be included in the list, the most recent scientific evidence is discussed hereinafter. While this expert group has no mandate to question the conclusion previously reached by CVMP, as indicated in the European Commission's mandate, any concerns related to consumer protection merits further consideration.

According to the International Agency for Research on Cancer (IARC) chloramphenicol is considered "possibly carcinogenic to humans" based on the evidence from animal studies and limited evidence for humans related to aplastic anaemia and thus leukaemia (IARC, 1987). The World Health Organisation (WHO) in its most recent assessment update for chloramphenicol, based on the JECFA report in 2004, indicated that, there was still no safe level of residues of chloramphenicol or its metabolites in food that represents an acceptable risk to consumers. No ADI and no MRLs can be allocated to this substance because of aplastic anaemia (only observed in humans) with an unknown threshold and due to its genotoxicity (JECFA, 2004; FAO, 2011). Thus, residues of chloramphenicol in food are best prevented by not using chloramphenicol in food-producing animals (FAO, 2021). Lately, the United States National Toxicology Program (NTP) has classified chloramphenicol as falling into the group of "reasonably anticipated to be human carcinogens" (NTP, 2021).

At the EU level, a comprehensive report was published by the European Food Safety Authority (EFSA) in 2014 for deriving a laboratory analytical reference point for action since chloramphenicol is regularly detected in food of animal origin under National Residue Control Plans and occurs also naturally in plant-based foods (EFSA, 2014; Baynes et al., 2016; see also Commission Regulation (EU)

2019/1871[61]). Using the status of the pharmacokinetic, toxicological and human study data described in this report as a starting point, a bibliographic search has been performed looking for any new findings since 2014.

Toxicology

Chloramphenicol is a nitrophenyl that is fairly lipophilic (octanol/water partition coefficient 1.14). The solubility in water is 2.5 g/L at 25°C (Drugbank, 2023). No published literature since 2014 regarding studies on repeat-dose toxicity, genotoxicity, carcinogenicity or toxicity to reproduction could be identified.

In 2014, the Panel on Contaminants in the Food Chain (CONTAM) at EFSA assessed the toxicological data available (both from animals and humans) and concluded that deriving a health-based guidance value was not appropriate for chloramphenicol. Nevertheless, aplastic anaemia in humans as well as reproductive and liver toxicity in animals were envisaged as providing a basis for the pragmatic risk characterisation that was conducted with the aim of dealing with contaminated foodstuffs. Within this process the CONTAM panel could not assess carcinogenicity due to lack of data from long-term studies. Regarding the genotoxic potential of chloramphenicol, positive in vitro findings in mammalian cells and from in vivo studies were noted. It was determined that the genotoxic test results are likely to be dependent on the metabolic competence of the exposed organism(s) as there are certain metabolites of higher toxicity (EFSA, 2014). The NTP also reported that experimental animal studies were identified to be inadequate for assessing carcinogenicity (NTP, 2021). However, Suarez-Torres et al. (2021) proposed a model for prediction of carcinogenicity based on the positive mutagenicity/genotoxicity findings as well as carcinogenicity findings in mice and found a positive predictive value of 91.5% for chloramphenicol being a human carcinogen.

Thus, there is still a need for further information on the carcinogenicity, the mechanisms underlying the genotoxic effects, the potential formation of reactive intermediates which could result in residues in foods of animal origin, the occurrence of toxic/genotoxic metabolites as well as the formation of bound residues in edible tissues of food-producing animals (EFSA, 2014). Regarding aplastic anaemia in humans, the EFSA panel has commented on the human studies that "*[w]hile in case studies it has been clearly demonstrated that chloramphenicol exposure can cause aplastic anaemia, a relationship could not be established in epidemiological studies. The CONTAM Panel noted that the design of such studies, in particular retrospective studies, appears not to be appropriate to detect such a relationship due to the low incidence of aplastic anaemia and the idiosyncratic nature of the disease. A positive association of chloramphenicol exposure with an increased risk of developing leukaemia was reported in one study but not observed in subsequent studies*" (EFSA, 2014). Furthermore, the NTP classification was justified as based on limited evidence of carcinogenicity from studies in humans (NTP, 2021).

Pharmacokinetics in horses

Topical application to the eye is regarded to be the main administration route in horses for the proposed use in the survey.

Although chloramphenicol is, in general, rapidly and almost completely absorbed after oral as well as after intramuscular administration (bioavailability 70-80%), it may to some extent be also systemically absorbed after topical application to the eye (Drugbank, 2023). However, the actual extent of systematic absorption from ocular administration remains unclear (absorption route may be via the *ductus nasolacrimalis*). A transition to the oral route was also assumed by EFSA (EFSA, 2014). In the

[61] Commission Regulation (EU) 2019/1871 of 7 November 2019 on reference points for action for non-allowed pharmacologically active substances present in food of animal origin and repealing Decision 2005/34/EC

EU, chloramphenicol is classified as being well absorbed orally and distributed throughout the body for the management of residues in food of animal origin. (EFSA, 2018). In horses, chloramphenicol is rapidly and extensively absorbed (e.g. 83% absorption in foals after oral or intramuscular administrations) and widely distributed to tissues (volume of distribution being 1.4 l/kg bw in adult horses, 1.6 l/kg bw in neonatal foals). Chloramphenicol has medium to low plasma binding (30-53%). The substance crosses the placenta and the blood-brain barrier and is present in the mare's milk. In horses, chloramphenicol is strongly hepatically metabolised with only 5-15% excreted unchanged mainly via the renal route. There is a lack of information on tissue distribution in equine species (EFSA, 2014).

Chloramphenicol has a short half-life of 1.8 hours in adult horses (following single intragastric administration of 50 mg/kg bw) and 1.44 hours in foals (following oral administration of 50 mg/kg bw) (EFSA, 2014). Recent published literature following oral administration of the same 50 mg/kg bw dose in adult horses, either as single dose (McElligott et al., 2017; Patel et al., 2019) or at six-hour intervals over 4 days (Estell et al., 2020), confirms the short plasma half-life elimination of chloramphenicol in horses, although high individual animal variability was observed (Estell et al., 2020).

Residue depletion in horses

There is no tissue disposition or depletion data for horses described in the EFSA assessment nor elsewhere in the scientific literature available. As previously concluded by the WHO, the available depletion study data to identify a suitable marker residue in cattle and pigs was regarded as insufficient by the EFSA panel (EFSA, 2014). Recently published literature on residue depletion in one-day-old chickens individually orally treated with chloramphenicol at a dose of 100 mg/kg bw/day for three consecutive days is noted (Rejtharová et al., 2017).

Evaluation for chloramphenicol

As concluded for in part 'A' of this assessment, chloramphenicol is not proposed to be qualified as essential, nor as bringing added clinical benefit for *Equidae*. From the toxicological data available, no health-based guidance value could be established by the CVMP as well as other authorities, due to its genotoxicity based on relevant positive findings and aplastic anaemia that develops in rare cases in humans. Such conclusions have not changed in light of newly available evidence. Pharmacokinetic data in horses suggest that chloramphenicol is rapidly and extensively absorbed and widely distributed to tissues. The short half-life may indicate that the six-month withdrawal period set by Regulation (EU) 2019/6 could constitute a sufficient safety span; however, the toxicological properties, as well as the absence of data on tissue distribution and residue depletion data for chloramphenicol and its metabolites preclude any conclusions on an absence of risk for consumers. Thus, from a consumer safety perspective, it is also not recommended to include this substance on the list since it cannot be concluded that the overall objective of maintaining a high level of consumer protection can be ensured when a withdrawal period for equine species of six months is respected.

References

References are presented separately for each assessment section.

Anaesthetics

Al-Musawi, A., Matar, K., Kombian, S., Andersson, L., 'Blood concentration of prilocaine and lidocaine after the use of topical anesthesia (Oraqix®) in lacerated wounds', Dental Traumatology, 2016, Vol. 32, pp. 502-506.

Aronson, J.-K., In Elsevier B.V. (Eds.), 'Meyler's Side Effects of Drugs: The International Encyclopedia of Adverse Drug Reactions and Interactions', Newness, Edition 15, 2015, pp. 921-924.

Behne, M., Wilke, H.-J., Harder, S., 'Clinical pharmacokinetics of sevoflurane', Clin Pharmacokinet, Jan 1999, Vol. 36(1), pp. 13-26.

Breu, A., Rosenmeier, K., Kujat, R., Angele, P., Zink, W., 'The cytotoxicity of bupivacaine, ropivacaine, and mepivacaine on human chondrocytes and cartilage', Anesth Analg, 2013, Vol. 117, pp. 514-522.

Dugdale, A.-H., Beaumont, G., Bradbrook, C., Gurney, M., In Wiley-Blackwell (Eds.), 'Veterinary anaesthesia: principles to practice', Edition 2, Jul 2020.

Erkert, R.-S., Macallister, C.-G., Campbell, G., Payton, M.-E., Shawley, R., Clarke, C.-R., 'Comparison of topical lidocaine/prilocaine anesthetic cream and local infiltration of 2% lidocaine for episioplasty in mares', J Vet Pharmacol Ther, Jun 2005, Vol. 28(3), pp. 299-304.

Gazarian, M., Taddio, A., Klein, J., Kent, G., Koren, G., 'Penile absorption of EMLA cream in piglets: implications for use of EMLA in neonatal circumcision', Biol Neonate, 1995, Vol. 68(5), pp. 334-41.

Grosenbaugh, D.-A., Muir, W.-W., 'Cardiorespiratory effects of sevoflurane, isoflurane, and halothane anesthesia in horses', American journal of veterinary research, 1998, Vol. 59(1), pp 101-106.

Hansen, T.-G., 'Ropivacaine: a pharmacological review', Expert review of neurotherapeutics, 2004, Vol. 4(5), pp. 781-791.

Harcourt, M.-M., Smith, R.-L., Hosgood, G., 'Duration of skin desensitisation following palmar digital nerve blocks with lidocaine, bupivacaine, mepivacaine and prilocaine', Aust Vet J, Dec 2021, Vol. 99(12), pp. 541-546.

Hopster, K., Müller, C., Hopster-Iversen, C., Stahl, J., Rohn, K., Kästner, S., 'Effects of dexmedetomidine and xylazine on cardiovascular function during total intravenous anaesthesia with midazolam and ketamine and recovery quality and duration in horses', Veterinary anaesthesia and analgesia, 2014, Vol. 41(1), pp. 25-35.

IARC International Agency for Research on Cancer, 'Some aromatic aromatic amines, organic dyes, and related exposures', Monographs on the evaluation of carcinogenic risks to humans, 2010, Vol. 99.

Jayaram, P., Kennedy, D.-J., Yeh, P., Dragoo, J., 'Chondrotoxic effects of local anesthetics on human knee articular cartilage: a systematic review', PMR, 2019, Vol. 11, pp. 379-400.

Kasuya, F., Igarashi, K., Fukui, M., 'Metabolism of benoxinate in humans', J Pharm Sci, 1987a, Vol. 76(4), pp. 303-5.

Kasuya, F., Igarashi, K., Fukui, M., 'Oxybuprocaine and five metabolites simultaneously determined in urine by gas chromatography and gas chromatography-mass spectrometry after extraction with Extrelut®', Clin Chem, 1987b, Vol. 33(5).

Lelescu, C.-A., UrdăCimpean, A.-E., Dumitras, D.-A., Taulescu, M., Muresan, C., 'Effects of 0.4% oxybuprocaine hydrochloride ophthalmic solution and 1% ropivacaine hydrochloride on corneal sensitivity in rats', PloS ONE, 2020, Vol. 15(11).

Lingjun, l., Cai, B., Hongyang, L., Wei, J., Tao, L., Pengcheng, M., 'Dermal effects and pharmacokinetic evaluation of the lidocaine/prilocane cream in healthy Chinese volunteers', BMC Pharmacol Toxicol, 2023, Vol. 24(1), p. 51.

McGee, H.-T., Fraunfelder, F.-W., 'Toxicities of topical ophthalmic anesthetics', Expert Opin Drug Saf, 2007, Vol. 6(6), pp. 637-40.

Richter, E., 'Biomonitoring of human exposure to arylamines', Front Biosci Elite, 2015, Vol. 7(2), pp. 193-207.

Robinson, A., Christopher Sun, T., Uquillas, E., 'In horses undergoing volatile anaesthesia, is recovery quality superior with sevoflurane compared to isoflurane?', Veterinary Evidence, 2023, Vol. 8(1).

Schumacher, J., Boone, L., 'Local anaesthetics for regional and intra-articular analgesia in the horse', Equine Veterinary Education, 2021, Vol. 33(3), pp. 159-168.

Sheikh, N.-K., Dua, A., 'Procaine', StatPearls, Treasure Island (FL), 2023. Accessed September 2023, available from: https://www.ncbi.nlm.nih.gov/books/NBK551556/

Söbbeler, F.-J., Kästner, S.-B., 'Effects of transdermal lidocaine or lidocaine with prilocaine or tetracaine on mechanical superficial sensation and nociceptive thermal thresholds in horses', Vet Anaesth Analg, Mar 2018, Vol. 45(2), pp. 227-233.

Steffey, E.-P., 'Recent advances in inhalation anesthesia', Vet Clin North Am Equine Pract, 2002, Vol. 18(1), pp. 159-168.

Scriba, G.-K.-E., In Sweetman C., (Ed.), 'Martindale: The Complete Drug Reference', Pharmaceutical Press, London, 2009, Edition 36.

Van der Meer, A.-D., Burm, A.-G.-L., Stienstra, R., Van Kleef, J.-W., Vletter, A.-A., Olieman, W., 'Pharmacokinetics of prilocaine after intravenous administration in volunteers', Anesthesiology, 1999, Vol. 90, pp. 988-92.

White, K.-L., Hird, J.-F.-R., Taylor, P.-M., 'Sevoflurane or isoflurane anaesthesia? A prospective, randomised blinded clinical trial in horses undergoing elective surgery', Vet Rec, Aug 2021, Vol. 189(3), p. e507.

Analgesics

Al-Hasani, R., Bruchas, M.-R., 'Molecular mechanisms of opioid receptor-dependent signaling and behavior', Anesthesiology, 2011, Vol. 115(6), pp. 1363-1381.

Allbaugh, R.-A., 'Equine recurrent uveitis: A review of clinical assessment and management', Equine Veterinary Education, 2017, Vol. 29(5), pp. 279-288.

Allen, J.-S., Campbell, J.-A., Cariello, N.-F., Kutz, S.-A., Thilagar, A., Xu, J., Ham, A.-L., Mitchell, A.-D., 'Genetic toxicology of remifentanil, an opiate analgesic', Teratog Carcinog Mutagen, 2003, Suppl 1, pp. 137-149.

Argenti, D., Jensen, B.-K., Hensel, R., Bordeaux, K., Schleimer, R., Bickel, C., Heald, D., 'A mass balance study to evaluate the biotransformation and excretion of [14C]-triamcinolone acetonide following oral administration', J Clin Pharmacol, 2000, Vol. 40(7), pp. 770-780.

Baldo, B.-A., 'Opioid analgesic drugs and serotonin toxicity (syndrome): mechanisms, animal models, and links to clinical effects', Arch Toxicol, 2018, Vol. 92(8), pp. 2457-2473.

Banse, H., Cribb, A.-E., [UCVM Class of 2016] 'Comparative efficacy of oral meloxicam and phenylbutazone in 2 experimental pain models in the horse', The Canadian veterinary journal [In French: La revue veterinaire Canadienne], 2017, Vol. 58(2), pp. 157-167.

Benner, K.-W., Spencer H.-D., 'Meperidine restriction in a pediatric hospital', J Pediatr Pharmacol Ther, 2011, Vol. 16(3), pp. 185-190.

Bertone, J., Horspool, L.-J.-I., In Cole, C., Bentz, B., Maxwell, L., (Eds.), 'Equine Clinical Pharmacology', Saunders Ltd., Edition 1, 2004.

Bianco, A.-W., Constable, P.-D., Cooper, B.-R., Taylor, S.-D., 'Pharmacokinetics of ketorolac tromethamine in horses after intravenous, intramuscular, and oral single-dose administration', J Vet Pharmacol Ther, 2016, Vol. 39(2), pp. 167-75.

Blobaum, A.-L., Marnett, L.-J., 'Structural and functional basis of cyclooxygenase inhibition', Journal of medicinal chemistry, 2007, Vol. 50(7), pp. 1425-1441.

Booth, N.-H., 'Nonnarcotic analgesics', In Booth, N.-H., McDonald, L.-E., (Eds.), Veterinary Pharmacology and Therapeutics, Iowa State University Press, Ames, Iowa, 1988, Edition 6, pp. 337-338.

Bowen, I.-M., Redpath, A., Dugdale, A., Burford, J.-H., Lloyd, D., Watson, T., Hallowell, G.-D., 'BEVA primary care clinical guidelines: Analgesia', Equine Vet J, 2020, Vol. 52(1), pp. 13-27.

Buck, M.-L., 'Is Meperidine the Drug That Just Won't Die?' J Pediatr Pharmacol Ther, 2011, Vol. 16(3), pp. 167-169.

Clotz, M.-A., Nahata, M.-C., 'Clinical uses of fentanyl, sufentanil, and alfentanil', Clin Pharm, 1991, Vol. 10(8), pp. 581-593.

Comer, S.-D., Cahill, C.-M., 'Fentanyl: Receptor pharmacology, abuse potential, and implications for treatment', Neurosci Biobehav Rev, 2019, Vol. 106, pp. 49-57.

DePriest, A.-Z., Puet, B.-L., Holt, A.-C, Roberts, A., Cone, E.-J., 'Metabolism and Disposition of Prescription Opioids: A Review', Forensic Sci Rev, 2015, Vol. 27(2), pp. 115-145.

Derendorf, H., Hochhaus, G., Rohatagi, S., Möllmann, H., Barth, J., Sourgens, H., Erdmann, M., 'Pharmacokinetics of triamcinolone acetonide after intravenous, oral and inhaled administration', J Clin Pharmacol, 1995, Vol. 35(3), pp. 302-305.

Devine, E.-P., KuKanich, B., Beard, W.-L., 'Pharmacokinetics of intramuscularly administered morphine in horses', J Am Vet Med Assoc, 2013, Vol. 243(1), pp. 105-112.

Drugbank, Bromfenac [Updated 3 Feb 2023]. Accessed June 2024, available from: https://go.drugbank.com/drugs/DB00963

Duz, M., Marshall, J.-F., Parkin, T.-D., 'Proportion of nonsteroidal anti-inflammatory drug prescription in equine practice', Equine veterinary journal, 2019, Vol. 51(2), pp. 147-153.

ECHA, Registration Dossier – Ketorolac. Accessed January 2024a, available from: https://echa.europa.eu/de/registration-dossier/-/registered-dossier/19373

ECHA, Substance Info Card – 9-α-fluoro-11-β,21-dihydroxy-16-α,17-α-isopropylidenedioxypregna-1,4-diene-3,20-dione. Accessed April 2024b, available from: https://echa.europa.eu/substance-information/-/substanceinfo/100.000.863

EFSA, Panel on Contaminants in the Food Chain (CONTAM), 'Scientific Opinion on the risks for public health related to the presence of opium alkaloids in poppy seeds', EFSA Journal, 2011, Vol. 9(11).

Erkert, R.-S., MacAllister, C.-G., Payton, M.-E., Clarke, C.-R., 'Use of force plate analysis to compare the analgesic effects of intravenous administration of phenylbutazone and flunixin meglumine in horses with navicular syndrome', American journal of veterinary research, 2005, Vol. 66(2), pp. 284-288.

Flood, J., Stewart, A.-J., 'Non-Steroidal Anti-Inflammatory Drugs and Associated Toxicities in Horses', Animals: an open access journal from MDPI, 2022, Vol. 12(21), p. 2939.

Foreman, J.-H., Barange, A., Lawrence, L.-M., Hungerford, L.-L., 'Effects of single-dose intravenous phenylbutazone on experimentally induced, reversible lameness in the horse', Journal of veterinary pharmacology and therapeutics, 2008, Vol. 31(1), pp. 39-44.

Foreman, J.-H., Grubb, T.-L., Inoue, O.-J, Banner, S.-E., Ball, K.-T., 'Efficacy of single-dose intravenous phenylbutazone and flunixin meglumine before, during and after exercise in an experimental reversible model of foot lameness in horses', Equine Vet J Suppl, 2010, Vol. 38, pp. 601-605.

Foreman, J.-H., Ruemmler, R., 'Phenylbutazone and flunixin meglumine used singly or in combination in experimental lameness in horses', Equine veterinary journal, 2011, Vol. 43(40), pp. 12-17.

Fornasari, D., 'Pharmacotherapy for Neuropathic Pain: A Review', 2017, Pain Ther, Vol. 6(1), pp. 25–33.

French, K., Pollitt, C.-C., Pass, M.-A., 'Pharmacokinetics and metabolic effects of triamcinolone acetonide and their possible relationships to glucocorticoid-induced laminitis in horses', J Vet Pharmacol Therap, 2000, Vol. 23(5), pp. 287-292.

Frisbie, D.-D., Kawcak, C.-E., Trotter, G.-W., Powers, B.-E., Walton, R.-M., McIlwraith, C.-W., 'Effects of triamcinolone acetonide on an in vivo equine osteochondral fragment exercise model', Equine Vet J, 1997, Vol. 29(5), pp. 349–359.

Frisbie, D.-D., Kawcak, C.-E., Baxter, G.-M., Trotter, G.-W., Powers, B.-E., Lassen, E.-D., McIlwraith, C.-W., 'Effects of 6α-methylprednisolone acetate on an equine osteochondral fragment exercise model', Am J Vet Res, 1998, Vol. 59(12), pp. 1619–1628.

Gagnon, N.-A., Hartley, C., Gilger, B.-C., 'Efficacy and safety of suprachoroidal triamcinolone injection in horses with poorly responsive equine recurrent uveitis', Vet Ophthalmol, 2021, Vol. 24(3), pp. 308-312.

Hamamoto-Hardman, B.-D., Steffey, E.-P., Seminoff, K., McKemie, D.-S., Kass, P., Knych, H.-K., 'Preliminary study of the pharmacokinetics, tissue distribution, and behavioral and select physiological effects of morphine 6-glucuronide (M6G) following intravenous administration to horses', Can J Vet Res, 2022, Vol. 86(3), pp. 172-180.

Hamamoto-Hardman, B.-D., Steffey, E.-P., Weiner, D., McKemie, D.-S., Kass, P., Knych, H.-K., 'Pharmacokinetics and selected pharmacodynamics of morphine and its active metabolites in horses after intravenous administration of four doses', J Vet Pharmacol Ther, 2019, Vol. 42(4), pp. 401-410.

Hashemi, M., Zali, A., Golmakani, E., Delshad, M.-H., Shadnoush, M., Akbari, M.-E., 'Efficacy, safety, and tolerability of sublingual fentanyl orally disintegrating tablet in the treatment of breakthrough cancer pain: a randomized, double-blind, placebo-controlled study', Daru, 2021, Vol. 29(1), pp. 51-59.

Haussler, K.-K., 'Managing back pain', In Sprayberry, K.-A., Robinson, N.-E., (Eds.), Robinson's Current Therapy in Equine Medicine, WB Saunders, St. Louis, Missouri, 2015, Edition 7, pp. 92- 96.

Hoy, S.-M., 'Bromfenac Ophthalmic Solution 0.07%: A Review of Its Use After Cataract Surgery', Clinical drug investigation, 2015, Vol. 35(8), pp. 525-529.

Hu, H.-H., MacAllister, C.-G., Payton, M.-E., Erkert, R.-S., 'Evaluation of the analgesic effects of phenylbutazone administered at a high or low dosage in horses with chronic lameness', Journal of the American Veterinary Medical Association, 2005, Vol. 226(3), pp. 414-417.

Jacobs, C.-C, Schnabel, L.-V., McIlwraith, C.-W., Blikslager, A.-T., Non-steroidal anti-inflammatory drugs in equine orthopaedics, Equine Vet J, 2022, Vol. 54(4), pp. 636–648.

Jeon, J.-S., Kim, H., Jo, S., Sim, J., Kim, S.-K., 'Role of microsomal metabolism in bromfenac-induced cytotoxicity', Chemico-biological interactions, 2024, Vol. 391, p. 110903.

Johnson, R.-E., Strain, E.-C., Amass, L., 'Buprenorphine: how to use it right', Drug Alcohol Depend, 2003, Vol. 70(2), pp. S59-77.

Jung, H., Chae, H.-K., 'The safety and efficacy of methocarbamol as a muscle relaxant with analgesic action: analysis of current data', Innov Biosyst Bioeng, 2019, Vol. 3(4), pp. 201-211.

Jung, D., Mroszczak, E., Bynum, L., 'Pharmacokinetics of ketorolac tromethamine in humans after intravenous, intramuscular and oral administration', Eur J Clin Pharmacol, 1988, Vol. 35(4), pp. 423-5.

Jung, D., Mroszczak, E.-J., Wu, A., Ling, T.-L., Sevelius, H., Bynum, L., 'Pharmacokinetics of ketorolac and p-hydroxyketorolac following oral and intramuscular administration of ketorolac tromethamine', Pharm Res, 1989, Vol. 6(1), pp. 62-5.

Keegan, K.-G., Messer, N.-T., Reed, S.-K., Wilson, D.-A., Kramer, J., 'Effectiveness of administration of phenylbutazone alone or concurrent administration of phenylbutazone and flunixin meglumine to alleviate lameness in horses', American journal of veterinary research, 2008, Vol. 69(2), pp. 167-173.

Knych, H.-K., 'Nonsteroidal Anti-Inflammatory Drug use in Horses', Vet Clin North Am Equine Pract, 2017, Vol. 33(1), pp. 1-15.

Knych, H.-K., Vidal, M.-A., Casbeer, H.-C., McKemie, D.-S., 'Pharmacokinetics of triamcinolone acetonide following intramuscular and intra-articular administration to exercised Thoroughbred horses', Equine Vet J, 2013, Vol. 45(6), pp. 715-720.

Knych, H.-K., Steffey, E.-P., McKemie, D.-S., 'Preliminary pharmacokinetics of morphine and its major metabolites following intravenous administration of four doses to horses', J Vet Pharmacol Ther, 2014, Vol. 37(4), pp. 374-381.

Knych, H.-K., Stanley, S.-D., Seminoff, K.-N., Mckemie, D.-S., Kass, P.-H., 'Pharmacokinetics of methocarbamol and phenylbutazone in exercised Thoroughbred horses', J. Vet Pharmacol Ther, 2016, Vol. 39(5), pp. 469-477.

Knych, H.-K., Arthur, R.-M., McKemie, D.-S., Baden, R., Oldberg, N., Kass, P.-H., 'Pharmacokinetics of intravenous flumetasone and effects on plasma hydrocortisone concentrations and inflammatory mediators in the horse', Equine Vet J, 2019, Vol. 51(2), pp. 238-245.

Koupai-Abyazani, M.-R., Esaw, B., Laviolette, B., 'Determination of methocarbamol in equine serum and urine by high-performance liquid chromatography with ultraviolet detection and atmospheric pressure ionization-mass spectrometric confirmation', J Anal Toxicol, 1997, Vol. 21(4), pp. 301-5.

Kukkar, A., Bali, A., Singh, N., Jaggi, A.-S., 'Implications and mechanism of action of gabapentin in neuropathic pain', Arch Pharm Res, 2013, Vol. 36(3), pp. 237-251.

Latta, K.-S., Ginsberg, B., Barkin, R.-L., 'Meperidine: a critical review', Am J Ther, 2002, Vol. 9(1), pp. 53–68.

Lees, P., Toutain, P.-L., 'Pharmacokinetics, pharmacodynamics, metabolism, toxicology and residues of phenylbutazone in humans and horses', Veterinary journal (London, England: 1997), 2013, Vol. 196(3), pp. 294-303.

López-Sanromán, F.-J., Montes Freilich, G., Gómez-Cisneros, D., Izquierdo-Moreno, J., Varela del Arco, M., Manso-Díaz, G., 'Morphine with or without Acepromazine in Horses: A Kinematic Evaluation', Animals (Basel), 2022, Vol. 12(9), pp. 1193.

Löscher, W., 'Pharmaka mit Wirkung auf das Zentralnervensystem', In Löscher, W., Ungemach, F.-R., Kroker, E., (Eds.), Pharmakotherapie bei Haus- und Nutztieren, Enke Verlag, Stuttgart (D), 2010, Edition 8, pp. 64-133.

Lutfy, K., Cowan, A., 'Buprenorphine: a unique drug with complex pharmacology', Curr Neuropharmacol, 2004, Vol. 2(4), pp. 395-402.

Lutfy, K., Eitan, S., Bryant, C.-D., Yang, Y.-C., Saliminejad, N., Walwyn, W., Kieffer, B.-L., Takeshima, H., Carroll, F.-I., Maidment, N.-T., Evans, C.-J. 'Buprenorphine-induced antinociception is mediated by mu-opioid receptors and compromised by concomitant activation of opioid receptor-like receptors', J Neurosci, 2003, Vol. 23(32), pp. 10331-10337.

Macrì, A., Vagge, A., Salis, A., Fucile, C., Marini, V., Martelli, A., Giuffrida, S., Iester, M., Damonte, G., Mattioli, F., 'Bromfenac ophthalmic solution 0.09%: human aqueous humor concentration detected by high-performance liquid chromatography', International ophthalmology, 2017, Vol. 37(2), pp. 417-421.

Maneuf, Y.-P., Luo, Z.-D., Lee, K., 'Alpha2delta and the mechanism of action of gabapentin in the treatment of pain', Semin Cell Dev Biol, 2006, Vol. 17(5), pp. 565-570.

Maxwell, L.-K., Thomasy, S.-M., Slovis, N., Kollias-Baker, C., 'Pharmacokinetics of fentanyl following intravenous and transdermal administration in horses', Equine Vet J, 2003, Vol. 35(5), pp. 484-490.

McCormack, P.-L., 'Ketorolac 0.45% ophthalmic solution', Drugs Aging, Jul 2011, Vol. 28(7), pp. 583-9.

McIlwraith, C.-W., Lattermann, C., 'Intra-articular Corticosteroids for Knee Pain-What Have We Learned from the Equine Athlete and Current Best Practice', J Knee Surg, 2019, Vol. 32(1), pp. 9–25.

Mills, P.-C., Ng, J.-C., Auer, D.-E., 'The effect of inflammation on the disposition of phenylbutazone in thoroughbred horses', Journal of veterinary pharmacology and therapeutics, 1996, Vol. 19(6), pp. 475-481.

Möllmann, H., Rohdewald, P., Schmidt, E.-W., Salomon, V., Derendorf, H., 'Pharmacokinetics of triamcinolone acetonide and its phosphate ester', Eur J Clin Pharmacol, 1985, Vol. 29(1), pp. 85-89.

Muir, W., 'Recognizing and treating pain in horses', In Reed, S.-M., Bayly, W.-M., Sellon, D.-C., (Eds.), 'Equine Internal Medicine-E-Book', 2009, Edition 3, pp. 233.

Muir, W.-W., Sams, R.-A., Ashcraft, S., 'Pharmacologic and pharmacokinetic properties of methocarbamol in the horse', Am J Vet Res, 1984, Vol. 45(11), pp. 2256-2260.

Muir 3rd, W.-W., Sams, R.-A., Ashcraft, S., 'The pharmacology and pharmacokinetics of high-dose methocarbamol in horses', Equine Vet J Suppl, 1992, Vol. 11, pp. 41-44.

Mullen, K.-R., Schwark, W., Divers, T.-J., 'Pharmacokinetics of single-dose intragastric and intravenous pregabalin administration in clinically normal horses', Am J Vet Res, 2013, Vol. 74(7), pp. 1043-1048.

Nolan, A.-M., 'Pharmacology of analgesic drugs', In Flecknell, P.-A., Waterman-Pearson, A., (Eds.), 'Pain Management in Animals', WB Saunders, London (UK), 2000, Edition 1, pp. 21-52.

Pacifici, G.-M., 'Metabolism and pharmacokinetics of morphine in neonates: A review', Clinics (Sao Paulo), 2016, Vol. 71(8), pp. 474-480.

Papich, M.-G., (Ed.), Papich Handbook of Veterinary Drugs, Elsevier, 2021, Edition 5, pp. 363-365.

Papich, M.-G., (Ed.), Saunders Handbook of Veterinary Drugs: Small and Large Animal, Elsevier, 2020, Edition 5.

Plumb D.-C., (Ed.), Plumb's Veterinary Drug Handbook, Wiley-Blackwell, 2015, Edition 8, pp. 430-437.

Plumb, D.-C., (Ed.), Plumb's Veterinary Drug Handbook, Wiley-Blackwell, 2018, Edition 9.

Poth, M.-K.-M., McKemie, D.-S., Traynham, M., Kass, P.-H., Knych, H.-K., 'Concentrations, pharmacokinetics and selected pharmacodynamics of morphine and its active metabolites following oral administration to horses', J Vet Pharmacol Ther, 2023, Vol. 46(4), pp. 238-249.

Pruitt 3rd, B.-N., 'Methocarbamol suspension for the treatment of rhabdomyolysis in equines', Int J Pharm Compd, 2013, Vol. 17(5), pp. 384-387.

Riviere, J.-E., Papich, M.-G., Veterinary Pharmacology and Therapeutics, Wiley-Blackwell, 2017, Edition 10.

Rumpler, M.-J., Colahan, P., Sams, R.-A., 'The pharmacokinetics of methocarbamol and guaifenesin after single intravenous and multiple-dose oral administration of methocarbamol in the horse', J Vet Pharmacol Ther, 2014, Vol. 37(1), 25-34.

Shankar, L.-G., Odayappan, A., Shukla, A.-G., Ramaswamy, V.-C., Rengaraj, V., Srinivasan, K., 'Topical 0.1% Nepafenac versus 0.09% Bromfenac Eye Drops for Inflammation after Laser Peripheral Iridotomy: A Randomized Controlled Trial', Ophthalmology, Glaucoma, 2022, Vol. 5(5), pp. 516-524.

Sheppard, J.-D., 'Topical bromfenac for prevention and treatment of cystoid macular edema following cataract surgery: a review', Clin Ophthalmol, 2016, Vol. 10, pp. 2099-2111.

Sibrack, J., Patel, P., Hammer, R., 'Methocarbamol', StatPearls Publishing, Treasure Island (FL), 2024. Accessed June 2024; available from: https://www.ncbi.nlm.nih.gov/books/NBK565868/

Skjodt, N.-M., Davies, N.-M., 'Clinical pharmacokinetics and pharmacodynamics of bromfenac', Clinical pharmacokinetics, 1999, Vol. 36(6), pp. 399–408.

Soma, L.-R., Uboh, C.-E., You, Y., Guan, F., Boston, R.-C., 'Pharmacokinetics of intra-articular, intravenous, and intramuscular administration of triamcinolone acetonide and its effect on endogenous plasma hydrocortisone and cortisone concentrations in horses', Am J Vet Res, 2011, Vol. 72(9), pp. 1234-1242.

Sweetman, S.-C., (Ed.), Martindale: 'The Complete Drug Reference', Pharmaceutical Press, London, 2009, Edition 36.

Thomasy, S.-M., Slovis, N., Maxwell, L.-K., Kollias-Baker, C., 'Transdermal fentanyl combined with nonsteroidal anti-inflammatory drugs for analgesia in horses', J Vet Intern Med, 2004, Vol. 18(4), pp. 550-554.

Tobin, T., Chay, S., Kamerling, S., Woods, W.-E., Weckman, T.-J., Blake, J.-W., Lees, P., 'Phenylbutazone in the horse: a review', Journal of veterinary pharmacology and therapeutics, 1986, Vol. 9(1), pp. 1-25.

Toutain, P.-L., Autefage, A., Legrand, C., Alvinerie, M., 'Plasma concentrations and therapeutic efficacy of phenylbutazone and flunixin meglumine in the horse: pharmacokinetic/pharmacodynamic modelling', Journal of veterinary pharmacology and therapeutics, 1994, Vol. 17(6), pp. 459-469.

Trotter, G.-W., McIlwraith, C.-W., Yovich, J.-V., Norrdin, R.-W., Wrigley, R.-H., Lamar, C.-H., 'Effects of intra-articular administration of methylprednisolone acetate on normal equine articular cartilage', Am J Vet Res, 1991, Vol. 52(1), pp. 83–87.

Soma, L.-R., Uboh, C.-E., Maylin, G.-M., 'The use of phenylbutazone in the horse', Journal of veterinary pharmacology and therapeutics, 2012, Vol. 35(1), pp. 1-12.

Vadivelu, N., Gowda, A.-M., Urman, R.-D., Jolly, S., Kodumudi, V., Maria, M., Taylor, R.-Jr., Pergolizzi, J.-V.-Jr., 'Ketorolac tromethamine – routes and clinical implications', Pain Pract, Feb 2015, Vol. 15(2), pp. 175-93.

Waterbury, L.-D., Galindo, D., Villanueva, L., Nguyen, C., Patel, M., Borbridge, L., Attar, M., Schiffman, R.-M., Hollander, D.A., 'Ocular penetration and anti-inflammatory activity of ketorolac 0.45% and bromfenac 0.09% against lipopolysaccharide-induced inflammation', J Ocul Pharmacol Ther, 2011, Vol. 27(2), pp.173-178.

Wernecke, C., Braun, H.-J., Dragoo, J.-L., 'The Effect of Intra-articular Corticosteroids on Articular Cartilage: A Systematic Review', Orthop J Sports Med, 2015, Vol. 3(5), pp. 1–7.

Antimicrobials

Abdel Aziz, E., Abdel-Gelil, R., Ahmed, A., Essa, K., Hamed, E., 'Depletion of Amikacin Residues in Fresh, Boiled and Frozen Rabbit's Tissues Using High Performance Liquid Chromatography', Zagazig Veterinary Journal, 2022, Vol. 50(2), pp. 107-115.

Aboubakr, M., Elbadawy, M., Medhat, A., 'Pharmacokinetics, bioavilability and tissue residues of amikacin in broiler chickens', World Journal of Pharmacy and Pharmaceutical Sciences, 2016, Vol. 6(1), pp. 96-105.

Alcorn, J., McNamara, P.-J., 'Acyclovir, ganciclovir, and zidovudine transfer into rat milk', Antimicrob Agents Chemother, Jun 2002, Vol. 46(6), pp. 1831-6.

Al-Mola, G.-M., Al-Saad, K.-M., 'Comparative study of imidocarb, buparvarvaquone & diminazinein treatment of draught horses affected with equine babesiosis', Iraqi journal of Veterinary Sciences, 2006, Vol. 33(2), pp. 103-112.

Álvarez-Narváez, S., Giguère, S., Anastasi, E., Hearn, J., Scortti, M., Vázquez-Boland, J.-A., 'Clonal Confinement of a Highly Mobile Resistance Element Driven by Combination Therapy in Rhodococcus equi', mBio, 2019, Vol. 10(5), p. e02260-19.

Álvarez-Narváez, S., Berghaus, L.-J., Morris, E.-R.-A., Willingham-Lane, J.-M., Slovis, N.-M., Giguere, S., Cohen, N. D., 'A Common Practice of Widespread Antimicrobial Use in Horse Production Promotes Multi-Drug Resistance', Scientific reports, 2020, Vol. 10(1), p. 911.

Álvarez-Narváez, S., Giguère, S., Cohen, N., Slovis, N., Vázquez-Boland, J.-A., 'Spread of Multidrug-Resistant Rhodococcus equi, United States', Emerg Infect Dis, 2021, Vol. 27(2), pp. 529-537.

American Society of Health-System Pharmacists, 'AHFS Drug Information 2012', Edition 54, 2012.

Andersen, J.-T., Petersen, M., Jimenez-Solem, E., Broedbaek, K., Andersen, N.-L., Torp-Pedersen, C., Keiding, N., Poulsen, H.-E., 'Clarithromycin in early pregnancy and the risk of miscarriage and malformation: a register based nationwide cohort study', PloS One, 2013, Vol. 8(1), pp. e53327.

Anderson, R.-D., Griffy, K.-G., Jung, D., Dorr, A., Hulse, J.-D., Smith, R.-B., 'Ganciclovir absolute bioavailability and steady-state pharmacokinetics after oral administration of two 3000-mg/d dosing regimens in human immunodeficiency virus- and cytomegalovirus-seropositive patients', Clin Ther, 1995, Vol. 17(3), pp. 425-32.

Asaumi, R., Toshimoto, K., Tobe, Y., Hashizume, K., Nunoya, K.-I., Imawaka, H., Lee, W., Sugiyama, Y., 'Comprehensive PBPK Model of Rifampicin for Quantitative Prediction of Complex Drug-Drug Interactions: CYP3A/2C9 Induction and OATP Inhibition Effects', CPT: pharmacometrics & systems pharmacology, 2018, Vol. 7(3), pp. 186-196.

Avedissian, S.-N., Liu, J., Rhodes, N.-J., Lee, A., Pais, G.-M., Hauser, A.-R., Scheetz, M.-H., 'A Review of the Clinical Pharmacokinetics of Polymyxin B', Antibiotics (Basel), Mar 2019, Vol. 8(1), p. 31.

Azarvandi, A., Khosravi, A.-R., Shokri, H., Talebkhan Garoussi, M., Gharahgouzlou, F., Vahedi, G., Sharifzadeh, A., 'Presence and distribution of yeasts in the reproductive tract in healthy female horses', Equine veterinary journal, 2017, Vol. 49(5), pp. 614-617.

Aziza, A.-E.-I., El-Sherbeny, K.-M., 'Clarithromycin genotoxicity in mice', Cytologia, 2006, Vol. 71(1), pp. 5-10.

Azman, M., Sabri, A.-H., Anjani, Q.-K., Mustaffa, M.-F., Hamid, K.-A., 'Intestinal Absorption Study: Challenges and Absorption Enhancement Strategies in Improving Oral Drug Delivery', Pharmaceuticals (Basel), Aug 2022, Vol. 15(8), p. 975.

Balderson, D.-E., Cai, G., Fries, M.-A., Kleinman, D.-M., McLaughlin, M.-M., Trivedu, T.-M., Wuzerlmann, J.-I., Young, S.-B., 'A systematic review and meta-analysis to compare the efficacy of acyclovir 3% ophthalmic ointment to idoxuridine in curing herpetic keratitis by Day 7 of treatment', BMC Ophthalmol, 2015, Vol. 15, p. 42.

Barnett, K.-C., Crispin, S.-M., Matthews, A.-G., Lavach, J.-D., (Eds.), 'Equine Ophthalmology: An Atlas and Text', Saunders, Edinburgh, 2004, Edition 2, pp. 256.

Barton, M.-H., Parviainen, A., Norton, N., 'Polymyxin B protects horses against induced endotoxaemia in vivo', Equine veterinary journal, 2004, Vol. 36(5), pp. 397-401.

Beckmann, J., Kees, F., Schaumburger, J., Kalteis, T., Lehn, N., Grifka, J., Lerch, K., 'Tissue concentrations of vancomycin and Moxifloxacin in periprosthetic infection in rats', Acta orthopaedica, 2007, Vol. 78(6), pp. 766-773.

Benson, J.-M., Nahata, M.-C., 'Clinical use of systemic antifungal agents', Clin Pharm, Jun 1988, Vol. 7(6), pp. 424-38.

Berlin, S., Spieckermann, L., Oswald, S., Keiser, M., Lumpe, S., Ullrich, A., Grube, M., Hasan, M., Venner, M., Siegmund, W., 'Pharmacokinetics and Pulmonary Distribution of Clarithromycin and Rifampicin after Concomitant and Consecutive Administration in Foals', Mol Pharm, 2016, Vol. 13(3), pp. 1089-1099.

Berlin, S., Kirschbaum, A., Spieckermann, L., Oswald, S., Keiser, M., Grube, M., Venner, M., Siegmund, W., 'Pharmacological indices and pulmonary distribution of rifampicin after repeated oral administration in healthy foals', Equine Veterinary Journal, 2017, Vol. 49(5), pp. 618-623.

Berlin, S., Wallstabe, S., Scheuch, E., Oswald, S., Hasan, M., Wegner, D., Grube, M., Venner, M., Ullrich, A., Siegmund, W., 'Intestinal and hepatic contributions to the pharmacokinetic interaction between gamithromycin and rifampicin after single-dose and multiple-dose administration in healthy foals', Equine veterinary journal, 2018, Vol. 50(4), pp. 525-531.

Bobadilla-González, P., García-Menaya, J.-M., Cordobés-Durán, C., Pérez-Rangel, I., Sánchez-Vega, S., 'Generalized urticaria to fusidic acid', 2009, Allergy, Vol. 64(5), pp. 817-818.

Borchers, K., Ebert, M., Fetsch, A., Hammond, T., Sterner-Kock, A., 'Prevalence of equine herpesvirus type 2 (EHV-2) DNA in ocular swabs and its cell tropism in equine conjunctiva', Veterinary microbiology, 2006, Vol. 118(3-4), pp. 260-266.

Börjesson, S., Greko, C., Myrenås, M., Landén, A., Nilsson, O., Pedersen, K., 'A link between the newly described colistin resistance gene mcr-9 and clinical Enterobacteriaceae isolates carrying blaSHV-12 from horses in Sweden', Journal of global antimicrobial resistance, 2020, Vol. 20, pp. 285-289.

Brooks, D.-E., Andrew, S.-E., Denis, H.-M., Strubbe, D.-T., Biros, D.-J., Cutler, T.-J., 'Rose Bengal positive epithelial microerosions as a manifestation of equine keratomycosis', Veterinary ophthalmology, 2000, Vol. 3(2-3), pp. 83-86.

Brooks, D.-E., (Eds.), 'Ophthalmology for the Equine Practitioner', Teton Newmedia, Jackson (WY), 2nd Edition, 2008.

Bucki, E.-P., Giguère, S., Macpherson, M., Davis, R., 'Pharmacokinetics of once-daily amikacin in healthy foals and therapeutic drug monitoring in hospitalized equine neonates', Journal of veterinary internal medicine, 2004, Vol. 18(5), pp. 728-733.

Budde, J.-A., McCluskey, D.-M., (Eds.), 'Plumb's Veterinary Drug Handbook', Wiley-Blackwell, 2023, Edition 10, pp. 1568.

Burrows, G.-E., MacAllister, C.-G., Beckstrom, D.-A., Nick, J.-T., 'Rifampin in the horse: comparison of intravenous, intramuscular, and oral administrations', American Journal of Veterinary Research, 1985, Vol. 46(2), pp. 442-446.

Burrows, G.-E., MacAllister, C.-G., Ewing, P., Stair, E., Burrows, S.-L., 'Rifampin disposition in the horse: effects of repeated dosage of rifampin or phenylbutazone', Journal of Veterinary Pharmacology and Therapeutics, 1992, Vol. 15(3), pp. 305-308.

Burton, A.-J., Giguère, S., Sturgill, T.-L., Berghaus, L.-J., Slovis, N.-M., Whitman, J.-L., Levering, C., Kuskie, K.-R., Cohen, N.-D., 'Macrolide- and rifampin-resistant Rhodococcus equi on a horse breeding farm, Kentucky, USA', Emerg Infect Dis, 2013, Vol. 19(2), pp. 282-5.

Campbell, K.-B., 'Antimicrobial agents and torsades de pointes', In Tisdale, J.-E., (Ed.) Torsades de Pointes, Academic Press, 2022.

Carmichael, R.-J., Whitfield, C., Maxwell, L.-K., 'Pharmacokinetics of ganciclovir and valganciclovir in the adult horse', J vet Pharmacol Therap, 2013, Vol. 36, pp. 441-449.

Catry, B., Cavaleri, M., Baptiste, K., Grave, K., Grein, K., Holm, A., Jukes, H., Liebana, E., Lopez Navas, A., Mackay, D., Magiorakos, A.-P., Moreno Romo, M.-A., Moulin, G., Muñoz Madero, C., Matias Ferreira Pomba, M.-C., Powell, M., Pyörälä, S., Rantala, M., Ružauskas, M., Sanders, P., Torren Edo, J., 'Use of colistin-containing products within the European Union and European Economic Area (EU/EEA): development of resistance in animals and possible impact on human and animal health. International journal of antimicrobial agents', 2015, Vol. 46(3), pp. 297-306.

Caselli, E., D'Accolti, M., Soffritti, I., Piffanelli, M., Mazzacane, S., 'Spread of mcr-1-Driven Colistin Resistance on Hospital Surfaces, Italy', Emerg Infect Dis, Sep 2018, Vol. 24(9), pp. 1752-1753.

Chaffin, M.-K., Schumacher, J., McMullan, W.-C., 'Cutaneous pythiosis in the horse', Vet Clin North Am Equine Pract, 1995, Vol. 11, pp. 91-103.

Chaffin, M.-K., Cohen, N.-D., Martens, R.-J., Edwards, R.-F., Nevill, M., 'Foal-related risk factors associated with development of Rhodococcus equi pneumonia on farms with endemic infection', J Am Vet Med Assoc, 2003, Vol. 223(12), pp. 1791–1799.

Chalder, R.-H., Knott, T., Rushton, J.-O., Nikolic-Pollard, D., 'Changes in antimicrobial resistance patterns of ocular surface bacteria isolated from horses in the UK: An eight-year surveillance study (2012-2019)', Vet Ophthalmol, Nov 2020, Vol. 23(6), pp. 950-956.

Chang, C., Yang, X., Fahmi, O.-A., Riccardi, K.-A., Di, L., Obach, R.-S., 'An exposure-response analysis based on rifampin suggests CYP3A4 induction is driven by AUC: an in vitro investigation', Xenobiotica; the fate of foreign compounds in biological systems, 2017, Vol. 47(8), pp. 673-681.

Chopra, I., 'Mechanisms of resistance to fusidic acid in Staphylococcus aureus', J Gen Microbiol, 1976, Vol. 96(2), pp. 229-238.

Clode, A.-B., Davis, J.-L., Salmon, J., Michau, T.-M., Gilger, B.-C., 'Evaluation of concentration of voriconazole in aqueous humor after topical and oral administration in horses', American journal of veterinary research, 2006, Vol. 67(2), pp. 296-301.

Clode, A.-B., Davis, J.-L., Salmon, J., LaFevers, H., Gilger, B.-C., 'Aqueous humor and plasma concentrations of ciprofloxacin and moxifloxacin following topical ocular administration in ophthalmologically normal horses', Am J Vet Res, 2010, Vol. 71(5), pp. 564-569.

Cohen, N.-D., Chaffin, M.-K., Kuskie, K.-R., Syndergaard, M.-K., Blodgett, G.-P., Takai, S., 'Association of perinatal exposure to airborne Rhodococcus equi with risk of pneumonia caused by R equi in foals', Am J Vet Res, 2013, Vol. 74(1), pp. 102–109.

Cohen, N.-D., Slovis, N.-M., Giguère, S., Baker, S., Chaffin, M.-K., Bernstein, L.-R., 'Gallium maltolate as an alternative to macrolides for treatment of presumed Rhodococcus equi pneumonia in foals', J Vet Intern Med, 2015, Vol. 29(3), pp. 932-939.

Colitz, C.-M., Latimer, F.-G., Cheng, H., Chan, K.-K., Reed, S.-M., Pennick, G.-J., 'Pharmacokinetics of voriconazole following intravenous and oral administration and body fluid concentrations of voriconazole following repeated oral administration in horses', American journal of veterinary research, 2007, Vol. 68(10), pp. 1115-1121.

Cornick, J.-L., 'Diagnosis and treatment of pulmonary histoplasmosis in a horse', Cornell Vet, 1990, Vol. 87, pp. 97-103.

Czerwinski, S.-L., Lyon, A.-W., Skorobohach, B., Léguillette, R., 'Pharmacokinetic analysis of topical tobramycin in equine tears by automated immunoassay', BMC Vet Res, 2012, Vol. 8, pp. 141.

Davis, J.-L., Salmon, J.-H., Papich, M.-G., 'Pharmacokinetics of voriconazole after oral and intravenous administration to horses', American journal of veterinary research, 2006, Vol. 67(6), pp. 1070-1075.

De Broe, M.-E., Paulus, G.-J., Verpooten, G.-A., Roels, F., Buyssens, N., Wedeen, R., Van Hoof, F., Tulkens, P.-M., 'Early effects of gentamicin, tobramycin, and amikacin on the human kidney', Kidney international, 1984, Vol. 25(4), pp. 643-652.

De Kanter, J.-K., Peci, F., Bertrums, E., Rosendahl Huber, A., van Leeuwen, A., van Roosmalen,M.-J., Manders, F., Verheul, M., Oka, R., Brandsma, A.M., 'Antiviral treatment causes a unique mutational signature in cancers of transplantation recipients', Cell Stem Cell, 2021, Vol. 28, pp. 1726-1739.

DeManuelle, T.-C., Ihrke, P.-J., Brandt, C.-M., Kass, P.-H., Vulliet, P.-R., ,Determination of skin concentrations of enrofloxacin in dogs with pyoderma', Am J Vet Res, 1998, Vol. 59(12), pp. 1599-1604.

Dirikolu, L., Foreman, J.-H., Tobin, T., 'Current therapeutic approaches to equine protozoal myeloencephalitis', J Am Vet Med Assoc, Feb 2013, Vol. 242(4), pp. 482-91.

Dodiya, P.-G., Patel, J.-S., Parmar, V.-L., Kalariya, V.-A., Singh, V.-K., 'Clinical Management of Equine Piroplasmosis', Ind J Vet Sci and Biotech, 2022, Vol. 18(2), pp. 129-131.

Donnellan, C.-M., Marais, H.-J., 'Equine Piroplasmosis', in: Mair, T.-S., Hutchinson, R.-E., (Eds.), 'Infectious Disease of Horses', UK, 2009, EVJ Ltd, pp. 333-340.

Dória, R.-G., Freitas, S.-H., Linardi, R.-L., Mendonça, F.-S., Arruda, L.-P., Boabaid, F.-M., Valadão, C.-A., 'Treatment of pythiosis in equine limbs using intravenous regional perfusion of amphotericin B', Veterinary surgery, 2012, Vol. 41(6), pp. 759-765.

Dória, R.-G., Carvalho, M.-B., Freitas, S.-H., Laskoski, L.-M., Colodel, E.-M., Mendonça, F.-S., Silva, M.-A., Grigoletto, R., Fantinato-Neto, P., 'Evaluation of intravenous regional perfusion with amphotericin B and dimethylsulfoxide to treat horses for pythiosis of a limb', BMC veterinary research, 2015, Vol. 11, p. 152.

Doughty, M.-J., Dutton, G.-N., 'Fusidic acid viscous eyedrops–an evaluation of pharmacodynamics, pharmacokinetics and clinical use for UK optometrists', Ophthalmic Physiol Opt, 2006, Vol. 26(4), pp. 343-61.

Dowling, P.-M., 'Miscellaneous Antimicrobials: Ionophores, Nitrofurans, Nitroimidazoles, Rifamycins, Oxazolidinones, and Others', In Giguère, S., Prescott, J.-F., Baggot, J.-D., Walker, R.-D., Dowling, P.-M., (Eds.), 'Antimicrobial Therapy in Veterinary Medicine', Wiley-Blackwell, Ames, Iowa, 2006, Edition 4, pp. 285-300.

Dowling, P.-M., Wilson, R.-C., Tyler, J.-W., Duran, S.-H., 'Pharmacokinetics of ciprofloxacin in ponies', J Vet Pharmacol Ther, 1995, Vol. 18(1), pp. 7-12.

Drinkard, C.-R., Shatin, D., Clouse, J., 'Postmarketing surveillance of medications and pregnancy outcomes: clarithromycin and birth malformations', Pharmacoepidemiol Drug Saf, 2000, Vol. 9(7), pp. 549-56.

Drugbank, Moxifloxacin. Accessed April 2024, available from: https://go.drugbank.com/drugs/DB00218

Easton-Jones, C.-A., Madigan, J.-E., Barnum, S., Maxwell, L.-K., Taylor, S.-D., Arnesen, T., Pusterla, N., 'Effect of valacyclovir on EHV-5 viral kinetics in horses with equine multinodular pulmonary fibrosis', J Vet Intern Med, Sep 2018, Vol. 32(5), pp. 1763-1767.

Edington, N., Bridges, C.-G., Patel, J.-R., 'Endothelial cell infection and thrombosis in paralysis caused by equid herpesvirus-1: equine stroke', Archives of Virology, 1986, Vol. 90, pp. 111-124.

El-Say, K.-M., Megahed, M.-A., Abdalla, A., El-Sawy, H.-S., Afify, H., Ramadan, A.-A., Ahmed, T.-A., 'P-gp inhibition and enhanced oral bioavailability of amikacin Sulfate: A novel approach using Thiolated Chito-PEGylated Lipidic Hybrids', Int J Pharm, 2024, Vol. 10, pp. 658.

Enna, S.-J., Bylund, D.-B., 'xPharm: the comprehensive pharmacology reference', Elsevier, 2008.

Erol, E., Shaffer, C.-L., Lubbers, B.-V., 'Synergistic combinations of clarithromycin with doxycycline or minocycline reduce the emergence of antimicrobial resistance in Rhodococcus equi', Equine Vet J, 2021, Vol. 54(4), pp. 799-806.

Fairuz, S., Nair, R.-S., Billa, N., 'Orally Administered Amphotericin B Nanoformulations: Physical Properties of Nanoparticle Carriers on Bioavailability and Clinical Relevance', Pharmaceutics, Aug 2022, Vol. 14(9), p. 1823.

Fan, H., Li, L., Wijlaars, L., Gilbert, R.-E., 'Associations between use of macrolide antibiotics during pregnancy and adverse child outcomes: A systematic review and meta-analysis', PloS One, 2019, Vol. 14(2), pp. e0212212.

Faustino, C., Pinheiro, L., 'Lipid Systems for the Delivery of Amphotericin B in Antifungal Therapy', Pharmaceutics, Jan 2020, Vol. 12(1), p. 29.

Fines, M., Pronost, S., Maillard, K., Taouji, S., Leclercq, R., 'Characterization of mutations in the rpoB gene associated with rifampin resistance in Rhodococcus equi isolated from foals', J Clin Microbiol, 2001, Vol. 39(8), pp. 2784–278.

Fish, D.-N., Chow, A.-T., 'The clinical pharmacokinetics of levofloxacin', Clin Pharmacokinet, 1997, Vol. 32(2), pp. 101-19.

Fohner, A.-E., Sparreboom, A., Altman, R.-B., Klein, T.-E., 'PharmGKB summary: macrolide antibiotic pathway, pharmacokinetics/pharmacodynamics', Pharmacogenet Genomics, 2018, Vol. 27(4), pp. 164-167.

Foote, B.-C., Hendrix, D.-V.-H., Ward, D.-A., Rajeev, S., Smith, J.-S., Sun, Z., 'Monitoring trends in antimicrobial susceptibility patterns of bacterial isolates from horses with ulcerative keratitis', Equine Veterinary Education, 2023, Vol. 35(11), pp. 597-606.

Ford, M., Delaney, K.-A., Ling, L., Erickson, T., In Saunders (Eds.), 'Clinical Toxicology', Saunders, Edition 1, 2001, pp. 499-502.

Frank, L.-A., 'Clinical pharmacology of rifampin', Journal of the American Veterinary Medical Association, 1990, Vol. 197(1), pp. 114-117.

Galera, P.-D., Brooks, D.-E., 'Optimal management of equine keratomycosis', Veterinary Medicine: Research and Reports, 2012, Vol. 3, pp. 7–17.

Gangneux, J.-P., Sulahian, A., Garin, Y.-J., Farinotti, R., Derouin, F., 'Therapy of visceral leishmaniasis due to Leishmania infantum: experimental assessment of efficacy of AmBisome', Antimicrob Agents Chemother, May 1996, Vol. 40(5), pp. 1214-8.

Gardner, S.-Y., Davis, J.-L., Jones, S.-L., LaFevers, D.-H., Hoskins, M.-S., McArver, E.-M., Papich, M.-G., 'Moxifloxacin pharmacokinetics in horses and disposition into phagocytes after oral dosing', J Vet Pharmacol Ther, 2004, Vol. 27(1), pp. 57-60.

Garré, B., van der Meulen, K., Nugent, J., Neyts, J., Croubels, S., De Backer, P., Nauwynck, H., 'In vitro susceptibility of six isolates of equine herpesvirus 1 to acyclovir, ganciclovir, cidofovir, adefovir, PMEDAP and foscarnet', Veterinary Microbiology, 2007, Vol. 122, pp. 43-51.

Garré, B., Gryspeerdt, A., Croubels, S., De Backer, P., Nauwynck, H., 'Evaluation of orally administered valacyclovir in experimentally EHV1-infected ponies', Veterinary Microbiology, 2009, Vol. 135, pp. 214-221.

Gehring, R., Haskell, S.-R., Payne, M.-A., Craigmill, A.-L., Webb, A.-I., Riviere, J.-E., 'Aminoglycoside residues in food of animal origin', Journal of the American Veterinary Medical Association, 2005, Vol. 227(1), pp. 63-66.

Geist, M.-J., Egerer, G., Burhenne, J., Riedel, K.-D., Weiss, J., Mikus, G., 'Steady-state pharmacokinetics and metabolism of voriconazole in patients', The Journal of antimicrobial chemotherapy, 2013, Vol. 68(11), pp. 2592-2599.

Giguère, S., 'Treatment of Infections Caused by Rhodococcus equi', Vet Clin North Am Equine Pract, 2017, Vol. 33(1), pp. 67-85.

Giguère, S., Jacks, S., Roberts, G.-D., Hernandez, J., Long, M.-T., Ellis, C., 'Retrospective comparison of azithromycin, clarithromycin, and erythromycin for the treatment of foals with Rhodococcus equi pneumonia', J Vet Intern Med, 2004, Vol. 18(4), pp. 568–573.

Giguère, S., Cohen, N.-D., Chaffin, M.-K., Hines, S.-A., Hondalus, M.-K., Prescott, J.-F., Slovis, N.-M., Papich, 'Rhodococcus equi: clinical manifestations, virulence, and immunity', J Vet Intern Med, 2011a, Vol. 25(6), pp. 1221–1230.

Giguère, S., Cohen, N.-D., Chaffin, M.-K., Slovis, N.-M., Hondalus, M.-K., Hines, S.-A., Prescott, J.-F., 'Diagnosis, treatment, control, and prevention of infections caused by Rhodococcus equi in foals', J Vet Intern Med, 2011b, Vol. 25(6), pp. 1209–1220.

Giguère, S., Lee, E.-A., Guldbech, K.-M., Berghaus, L.-J., 'In vitro synergy, pharmacodynamics, and postantibiotic effect of 11 antimicrobial agents against Rhodococcus equi', Vet Microbiol, 2012, Vol. 160(1-2), pp. 207-213.

Gnann, J.-W., Barton, N.-H., Whitley, R.-J., 'Acyclovir: Mechanism of action, pharmacokinetics, safety and clinical applications', Pharmacotherapy, 1983, Vol. 3(5), pp. 275-83.

Goodman, L.-B., Loregian, A., Perkins, G.-A., Nugent, J., Buckles, E.-L., Mercorelli, B., Kydd, J.-H., Palu, G., Smith, K.-C., Osterrieder, N., Davis-Poynter, N., 'A point mutation in a herpesvirus polymerase determines neuropathogenicity', PloS Pathogens, 2007, Vol. 3, p. e160.

Goudah, A., 'Disposition kinetics of moxifloxacin in lactating ewes', Veterinary journal (London, England: 1997), 2008, Vol. 178(2), pp. 282-287.

Goudah, A., Abo El-Sooud, K., Shim, J.-H., Shin, H.-C., Abd El-Aty, A.-M., 'Characterization of the pharmacokinetic disposition of levofloxacin in stallions after intravenous and intramuscular administration', J Vet Pharmacol Ther, 2008, Vol. 31(5), pp. 399-405.

Grayson, M.-L., Cosgrove, S., Crowe, S., Hope, W., McCarthy, J., Mills, J., Mouton, J.-W., Paterson, D., (Eds.), 'Kucers' The Use of Antibiotics: A Clinical Review of Antibacterial, Antifungal, Antiparasitic, and Antiviral Drugs', CRC Press, 2018, Edition 7, pp. 4894.

Heel, R.-C., Brogden, R.-N., Pakes, G.-E., Speight, T.-M., Avery, G.-S., 'Miconazole: a preliminary review of its therapeutic efficacy in systemic fungal infections', Drugs, Jan 1980, Vol. 19(1), pp. 7-30.

Higgins, J.-C., Pusterla, N., 'Fungal Pneumonia in Horses', Clin Tech Equine Pract, 2006, Vol. 5, pp. 218-224.

Hildebrand, F., Venner, M. Giguère, S., 'Efficacy of gamithromycin for the treatment of foals with mild to moderate bronchopneumonia', J Vet Intern Med, 2015, Vol. 29(1), pp. 333-338.

Howe, D.-K., MacKay, R.-J., Reed, S.-M., 'Equine protozoal myeloencephalitis', Vet Clin North Am Equine Pract, Dec 2014, Vol. 30(3), pp. 659-75.

Huber, L., Giguère, S., Slovis, N.-M., Carter, C.-N., Barr, B.-S., Cohen, N.-D., Elam, J., Erol, E., Locke, S.-J., Phillips, E.-D., Smith, J.-L., 'Emergence of Resistance to Macrolides and Rifampin in Clinical Isolates of Rhodococcus equi from Foals in Central Kentucky, 1995 to 2017', Antimicrobial agents and chemotherapy, 2018, Vol. 63(1), p. e01714-18.

Huber, L., Giguère, S., Cohen, N.-D., Slovis, N.-M., Hanafi, A., Schuckert, A., Berghaus, L., Greiter, M., Hart, K.-A., 'Prevalence and risk factors associated with emergence of Rhodococcus equi resistance to macrolides and rifampicin in horse-breeding farms in Kentucky, USA', Vet Microbiol, 2019, Vol. 235, pp. 243-247.

Huttner, A., Harbath, S., 'Infectious diseases', Edition 4, 2017, Vol. 2, pp. 1277-1279.

IARC International Agency for Research on Cancer, 'Some antiviral and antineoplastic drugs, and other pharmaceutical agents', Monographs on the evaluation of carcinogenic risks to humans, 2000, Vol. 76.

IARC International Agency for Research on Cancer. List of Classifications. Accessed April 2024, available from: https://monographs.iarc.who.int/list-of-classifications/

Iatropoulos, M.-J., Jeffrey, A.-M., Enzmann, H.-G., von Keutz, E., Schlueter, G., Williams, G.-M., 'Assessment of chronic toxicity and carcinogenicity in an accelerated cancer bioassay in rats of moxifloxacin, a quinolone antibiotic', Experimental and toxicologic pathology : official journal of the Gesellschaft fur Toxikologische Pathologie, 2001, Vol. 53(5), pp. 345-357.

Isgren, C.-M., 'Improving clinical outcomes via responsible antimicrobial use in horses', Equine Veterinary Education, 2021, Vol. 34(1).

Isidori, M., Lavorgna, M., Nardelli, A., Pascarella, L., Parrella, A., 'Toxic and genotoxic evaluation of six antibiotics on non-target organisms', Sci Total Environ, 2005, Vol. 346(1-3), pp. 87-98.

Jacks, S.-S., Giguère, S., Nguyen, A., 'In vitro susceptibilities of Rhodococcus equi and other common equine pathogens to azithromycin, clarithromycin, and 20 other antimicrobials', Antimicrob Agents Chemother, 2003, Vol. 47(5), pp. 1742–1745.

Jacobsen, T., Sifontis, N., 'Drug interactions and toxicities associated with the antiviral management of cytomegalovirus infection', American Journal of Health-System Pharmacy, 2010, Vol. 67, pp. 1417-1425.

Jagannath, C., Wells, A., Mshvildadze, M., Olsen, M., Sepulveda, E., Emanuele, M., Hunter, R.-L.-Jr., Dasgupta, A., 'Significantly improved oral uptake of amikacin in FVB mice in the presence of CRL-1605 copolymer', Life Sci, 1999, Vol. 64(19), pp. 1733-8.

Jeffrey, A.-M., Shao, L., Brendler-Schwaab, S.-Y., Schlüter, G., Williams, G.-M., 'Photochemical mutagenicity of phototoxic and photochemically carcinogenic fluoroquinolones in comparison with the photostable moxifloxacin', Archives of toxicology, 2000, Vol. 74(9), pp. 555-559.

Jensen, R.-H., Astvad, K.-M., Silva, L.-V., Sanglard, D., Jørgensen, R., Nielsen, K.-F., Mathiasen, E.-G., Doroudian, G., Perlin, D.-S., Arendrup, M.-C., 'Stepwise emergence of azole, echinocandin and amphotericin B multidrug resistance in vivo in Candida albicans orchestrated by multiple genetic alterations', The Journal of antimicrobial chemotherapy, 2015, Vol. 70(9), pp. 2551-2555.

Kahn, S.-K., Cohen, N.-D., Bordin, A.-I., Coleman, M.-C., Heird, J.-C., Welsh Jr, T.-H., 'Transfusion of hyperimmune plasma for protecting foals against Rhodococcus equi pneumonia', Equine Vet J, 2023, Vol. 55(3), pp. 376-388.

Keller, R.-L., Hendrix, D.-V.-H., 'Bacterial isolates and antimicrobial susceptibilities in equine bacterial ulcerative keratitis (1993–2004)', Equine Vet J, 2005, Vol. 37(3), pp. 207-211.

Khan, F., Ismail, M., Khan, Q., Ali, Z., 'Moxifloxacin-induced QT interval prolongation and torsades de pointes: a narrative review', Expert opinion on drug safety, 2018, Vol. 17(10), pp. 1029-1039.

Kahn, S.-K., Cohen, N.-D., Bordin, A.-I., Coleman, M.-C., Heird, J.-C., Welsh, T.-H.-Jr., 'Transfusion of hyperimmune plasma for protecting foals against Rhodococcus equi pneumonia', Equine Vet J, 2023, Vol. 55(3), pp. 376-88.

King, D.-H., 'History, pharmacokinetics, and pharmacology of acyclovir', J Am Acad Dermatol, Jan 1988, Vol. 18(1 Pt 2), pp. 176-9.

Kornguth, M.-L., Kunin, C.-M., 'Distribution of gentamicin and amikacin in rabbit tissues', Antimicrob Agents Chemother, Jun 1977, Vol. 11(6), pp. 974-7.

Krause, K.-M., Serio, A.-W., Kane, T.-R., Connolly, L.-E., 'Aminoglycosides: An Overview', Cold Spring Harb Perspect Med, Jun 2016, Vol. 6(6), p. a027029.

Langtry, H.-D., Lamb, H.-M., 'Levofloxacin. Its use in infections of the respiratory tract, skin, soft tissues and urinary tract', Drugs, 1998, Vol. 56(3), pp. 487-515.

Laskin, O.-L., 'Clinical Pharmacokinetics of Acyclovir', Clinical Pharmacokinetics, 1983, Vol. 8, pp. 187-201.

Latimer, F.-G., Colitz, C.-M., Campbell, N.-B., Papich, M.-G., 'Pharmacokinetics of fluconazole following intravenous and oral administration and body fluid concentrations of fluconazole following repeated oral dosing in horses', American journal of veterinary research, 2001, Vol. 62(10), pp. 1606-1611.

LeBel, M., 'Ciprofloxacin: chemistry, mechanism of action, resistance, antimicrobial spectrum, pharmacokinetics, clinical trials, and adverse reactions', Pharmacotherapy, 1988, Vol. 8(1), pp. 3-33.

Leclere, M., Magdesian, K.-G., Cole, C.-A., Szabo, N.-J., Ruby, R.-E., Rhodes, D.-M., Edman, J., Vale, A., Wilson, W.-D., Tell, L.-A., 'Pharmacokinetics and preliminary safety evaluation of azithromycin in adult horses', J Vet Pharmacol Ther, 2012, Vol. 35(6), pp. 541-549.

Leigue, L., Montiani-Ferreira, F., Moore, B.-A., 'Antimicrobial susceptibility and minimal inhibitory concentration of Pseudomonas aeruginosa isolated from septic ocular surface disease in different animal species', Open Vet J, 2016, Vol. 6(3), pp. 215-222.

Liu, M., Chen, M., Yang, Z., 'Design of amphotericin B oral formulation for antifungal therapy', Drug delivery, 2017, Vol. 24(1), pp. 1-9.

Löscher, W., Ungemach, F.-R., Kroker, R., 'Pharmakotherapie bei Haus- und Nutztieren' [In German], Edition 8, Enke, Stuttgart, 2014.

MacKay, R.-J., 'Equine protozoal myeloencephalitis', Vet Clin North Am Equine Pract, Apr 1997, Vol. 13(1), pp. 79-96.

MacKay, R.-J., Howe, D.-K., 'Equine Protozoal Myeloencephalitis', Vet Clin North Am Equine Pract, Aug 2022, Vol. 38(2), pp. 249-268.

Mandell, G., in Benett, J.-E., Dolin, R., Blaser, M.-J., (Eds.), 'Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases', Elsevier Canada, Edition 9, North York, 2019.

Martins-Sorenson, N., Snesrud, E., Xavier, D.-E., Cacci, L.-C., Iavarone, A.-T., McGann, P., Riley, L.-W., Moreira, B.-M., 'A novel plasmid-encoded mcr-4.3 gene in a colistin-resistant Acinetobacter baumannii clinical strain', The Journal of antimicrobial chemotherapy, 2020, Vol. 75(1), pp. 60-64.

Matot, I., Pizov, R., 'Pulmonary extraction and accumulation of lipid formulations of amphotericin B', Critical care medicine, 2000, Vol. 28(7), pp. 2528-2532.

Maxwell, L.-K., Bentz, B.-G., Bourne, D.-W.-A., Erkert, R.-S., 'Pharmacokinetics of valacyclovir in the adult horse', J vet Pharmacol Therap, 2008, Vol. 31, pp. 312-320.

Maxwell, L.-K., Bentz, B.-G., Gilliam, L.-L., Ritchey, J.-W., Pusterla, N., Eberle, R., Holbrook, T.-C., McFarlane, D., Rezabek, G.-B., Meinkoth, J., Whitfield, C., Goad, C.-L., Allen, G.-P., 'Efficacy of the early administration of valacyclovir hydrochloride for the treatment of neuropathogenic equine herpesvirus type-1 infection in horses', American journal of veterinary research, 2017, Vol. 78(10), pp. 1126-1139.

McLeroth, P., Paduch, D.-A., Abt, M., Hughes, R., Moore, S., Mudie, N., 'Effects of valganciclovir on spermatogenesis in renal transplant patients – results of a multicenter prospective nonrandomized study', Transpl Int, Mar 2020, Vol. 33(3), pp. 310-320.

Medicine, Agricultural and Food Sciences, 'The Swedish Veterinary Association's Guidelines for the clinical use of antibiotics in the treatment of horses', 2019. Accessed April 2024; available from: https://www.semanticscholar.org/paper/The-Swedish-Veterinary-Association%E2%80%99s-Guidelines-for/edeba0ffbd08fee94f87ba71d6a60453f8275282

Mercer, M.-A., Davis, J.-L., McKenzie, H.-C., 'The Clinical Pharmacology and Therapeutic Evaluation of Non-Steroidal Anti-Inflammatory Drugs in Adult Horses', Animals: an open access journal from MDPI, 2023, Vol. 13(10), p. 1597.

Miller, W.-H., Miller, R.-L., 'Phosphorylation of acycovir diphosphate by cellular enzymes', Biochem Pharmacol, Dec 1982, Vol. 31(23), pp. 3879-84.

Mills, J., 'Idoxuridine', in Grayson, M.-L., Cosgrove, S., Crowe, S., Hope, W., McCarthy, J., Mills, J., Mouton, J.-W., Paterson, D., (Eds.), 'Kucers' The Use of Antibiotics', CRC Press, Boca Raton, Oct 2017, Edition 7, pp. 3634-3639.

Miranda, P., Krasny, H.-C., Page, D.-A., Elion, G.-B., 'The disposition of acyclovir in different species', J pharmacol Exp Ther, 1981, Vol. 219(2), pp. 309-15.

Mishra, R., Patra, B.-K., Das, S., 'Microbial profile of chronic eye infection in horses and its treatment', Intas Polivet, 2015, Vol. 16(2), pp. 397-398.

Mora-Pereira, M., Abarca, E.-M., Duran, S., Ravis, W., McMullen, R.-J., Fischer, B.-M., Lee, Y.-P., Wooldridge, A.-A., 'Sustained-release voriconazole-thermogel for subconjunctival injection in horses: ocular toxicity and in-vivo studies', BMC Vet Res, 2020, Vol. 16, p. 115.

Morresey, P.-R., Mackay, R.-J., 'Endotoxin-neutralizing activity of polymyxin B in blood after IV administration in horses', American journal of veterinary research, 2006, Vol. 67(4), pp. 642-647.

Morris, D.-D., Crowe, N., Moore, J.-N., 'Correlation of clinical and laboratory data with serum tumor necrosis factor activity in horses with experimentally induced endotoxemia', American journal of veterinary research, 1990, Vol. 51(12), pp. 1935-1940.

Morris, D.-D., Moore, J.-N., Crowe, N., 'Serum tumor necrosis factor activity in horses with colic attributable to gastrointestinal tract disease', American journal of veterinary research, 1991, Vol. 52(10), pp. 1565-1569.

Moskaluk, A.-E., VandeWoude, S., 'Current Topics in Dermatophyte Classification and Clinical Diagnosis', Pathogens, Aug 2022, Vol. 11(9), p. 957.

Muscatello, G., Leadon, D.-P., Klayt, M., Ocampo-Sosa, A., Lewis, D.-A., Fogarty, U., Buckley, T., Gilkerson, J.-R., Meijer, W.-G., Vazquez-Boland, J.-A., 'Rhodococcus equi infection in foals: the science of 'rattles'', Equine Vet J, 2007, Vol. 39(5), pp. 470-478.

Nation, R.L., 'Polymyxins', in Kucers (Eds.), 'Kucers' The Use of Antibiotics: A Clinical Review of Antibacterial, Antifungal, Antiparasitic, and Antiviral Drugs', CRC Press, Edition 7, 2018.

Newman, J.-C., Prange, T., Jennings, S., Barlow, B.-M., Davis, J.-L., 'Pharmacokinetics of tobramycin following intravenous, intramuscular, and intra-articular administration in healthy horses', J Vet Pharmacol Ther, 2013, Vol. 36(6), pp. 532-541.

O'Brien, J.-J., Campoli-Richards, D.-M., 'Acyclovir. An updated review of its antiviral activity, pharmacokinetic properties and therapeutic efficacy', Drugs, Mar 1989, Vol. 37(3), pp. 233-309.

O'Neil, M.-J., Smith, A., Heckelman, P.-E., Budavari, S., (Eds.), 'The Merck Index', Merck & Co Inc, Whitehouse Station, NJ, 2001, Edition 13, pp. 2562.

Orsini, J.-A., Soma, L.-R., Rourke, J.-E., Park, M., 'Pharmacokinetics of amikacin in the horse following intravenous and intramuscular administration', Journal of veterinary pharmacology and therapeutics, 1985, Vol. 8(2), pp. 194-201.

Orsini, J.-A., Park, M.-I., Spencer, P.-A., 'Tissue and serum concentrations of amikacin after intramuscular and intrauterine administration to mares in estrus', The Canadian veterinary journal [La revue veterinaire Canadienne], 1996, Vol. 37(3), pp. 157-160.

Paegelow, J.-L., Schoonover, M.-J., Young, J.-M., 'Pharmacokinetics of amikacin after intravenous, intra-articular, and combined intravenous and intra-articular administration in healthy neonatal foals', J Vet Intern Med, 2024, Vol. 38(3), pp. 1825-1834.

Papich, M.-G., (Ed.) 'Moxifloxacin' In 'Saunders Handbook of Veterinary Drugs', Elsevier, 2016, Edition 4.

Papich, M.-G., (Ed.), Papich Handbook of Veterinary Drugs, Elsevier, 2021, Edition 5.

Papich, M.-G., Riviere, J.-E., 'Chloramphenicol and derivatives, macrolides, lincosamides, fluoroquinolones, and miscellaneous antimicrobials', in Riviere, J.-E., Papich, M.-G., Adams, H.-R., (Eds.), 'Veterinary Pharmacology and Therapeutics', Wiley-Blackwell, 2009, Edition 9.

Passler, N.-H., Chan, H.-M., Stewart, A.-J., Duran, S.-H., Welles, E.-G., Lin, H.-C., Ravis, W.-R., 'Distribution of voriconazole in seven body fluids of adult horses after repeated oral dosing', Journal of veterinary pharmacology and therapeutics, 2010, Vol. 33(1), pp. 35-41.

Patel, H.-B., Mody, S.-K., Patel, H.-B., Patel, V.-A., Patel, U.-D., 'Disposition Kinetic of Moxifloxacin following Intravenous, Intramuscular, and Subcutaneous Administration in Goats', ISRN Vet Sci, Dec 2011, p. 584342.

Pearce, J.-G., Naunton, M., Maddess, T., 'A Literature-Based Review and Analysis of the Pharmacodynamics of the Dose Frequency of Topical 0.3% Ciprofloxacin and 0.3% Ofloxacin in the Day-1 Treatment of Bacterial Keratitis', J Ocul Pharmacol Ther, 2023, Vol. 39(1), pp. 17-26.

Peiró, J.-R., Barnabé, P.-A., Cadioli, F.-A., Cunha, F.-Q., Lima, V.-M., Mendonça, V.-H., Santana, A.-E., Malheiros, E.-B., Perri, S.-H., Valadão, C.-A., 'Effects of lidocaine infusion during experimental endotoxemia in horses', Journal of veterinary internal medicine, 2010, Vol. 24(4), pp. 940-948.

Pescovitz, M.-D., 'Absence of teratogenicity of oral ganciclovir used during early pregnancy in a liver transplant recipient', Transplantation, Mar 1999, Vol. 67(5), pp. 758-9.

Peters, J., Block, W., Oswald, S., Freyer, J., Grube, M., Kroemer, H.-K., Lämmer, M., Lütjohann, D., Venner, M., Siegmund, W., 'Oral absorption of clarithromycin is nearly abolished by chronic comedication of rifampicin in foals', Drug Metab Dispos, 2011, Vol. 39(9), pp. 1643-1649.

Peters, J., Eggers, K., Oswald, S., Block, W., Lütjohann, D., Lämmer, M., Venner, M., Siegmund, W., 'Clarithromycin is absorbed by an intestinal uptake mechanism that is sensitive to major inhibition by rifampicin: results of a short-term drug interaction study in foals', Drug Metabo Dispos, 2012, Vol. 40(3), pp. 522–528.

Plempel, M., 'Pharmacokinetics of imidazole antimycotics', Postgraduate medical journal, 1979, Vol. 55(647), pp. 662-666.

Plumb D.-C., (Ed.), 'Plumb's Veterinary Drug Handbook', Wiley-Blackwell, 2015, Edition 8.

Pietsch, F., Bergman, J.-M., Brandis, G., Marcusson, L.-L., Zorzet, A., Huseby, D.-L., Hughes, D., 'Ciprofloxacin selects for RNA polymerase mutations with pleiotropic antibiotic resistance effects', J Antimicrob Chemother, 2017, Vol. 72(1), pp. 75-84.

Pinto, N., Schumacher, J., Taintor, J., Degraves, F., Duran, S., Boothe, D., 'Pharmacokinetics of amikacin in plasma and selected body fluids of healthy horses after a single intravenous dose', Equine veterinary journal, 2011, Vol. 43(1), pp. 112-116.

Price Jr, F.-W., Whitson, W.-E., Collins, K.-S., Gonzales, J.-S., 'Corneal tissue levels of topically applied ciprofloxacin', Cornea, 1995, Vol. 14(2), pp. 152-156.

Posch, W., Blatzer, M., Wilflingseder, D., Lass-Flörl, C., 'Aspergillus terreus: Novel lessons learned on amphotericin B resistance', Medical mycology, 2018, Vol. 56(1), pp. 73-82.

Prades, M., Brown, M.-P., Gronwall, R., Houston, A.-E., 'Body fluid and endometrial concentrations of ketoconazole in mares after intravenous injection or repeated gavage', Equine veterinary journal, 1989, Vol. 21(3), pp. 211-214.

PubChem. Bethesda (MD), National Library of Medicine (US), National Center for Biotechnology Information, 2004. PubChem Compound Summary for Moxifloxacin. Accessed April 2024, available from: https://pubchem.ncbi.nlm.nih.gov/compound/Moxifloxacin#section=Absorption-Distribution-and-Excretion

PubChem. Bethesda (MD), National Library of Medicine (US), National Center for Biotechnology Information, 2004. PubChem Compound Summary for CID 4993, Pyrimethamine. Accessed May 2024, available from: https://pubchem.ncbi.nlm.nih.gov/compound/Pyrimethamine

PubChem. Bethesda (MD), National Library of Medicine (US), National Center for Biotechnology Information, 2024. PubChem Compound Summary for CID 71616, Voriconazole. Accessed May 2024; available from: https://pubchem.ncbi.nlm.nih.gov/compound/Voriconazole

Rashid, H., Chaudhry, M., Rashid, H., 'Comparative efficacy of diminazene diaceturate and diminazene aceturate for the treatment of babesiosis in horses', Trop Anim Health Prod, 2008, Vol. 40, pp. 463-467.

Reed, S.-M., Saville, W.-J., 'Equine protozoal encephalomyelitis', Proceedings 42nd Annu Meet Am Assoc Equine Pract, 1996, pp. 75–79.

Reed, S.-M., Furr, M., Howe, D.-K., Johnson, A.-L., MacKay, R.-J., Morrow, J.-K., Pusterla, N., Witonsky, S., 'Equine Protozoal Myeloencephalitis: An Updated Consensus Statement with a Focus on Parasite Biology, Diagnosis, Treatment, and Prevention', J Vet Intern Med, 2016, Vol. 30(2), pp. 491-502.

Renard, L., Sanders, P., Laurentie, M., 'Pharmacocinétique de la colistine sulfate administrée par voies intraveineuse et intramusculaire chez le veau [Pharmacokinetics of colistin sulfate administered by intravenous and intramuscular routes in the calf]', Annales de recherches veterinaires [Annals of veterinary research], 1991, Vol. 22(4), pp. 387-394.

Reuveni, H., Bull, C.-O., Landry, M.-L., Milstone, L.-M., Schwartz, P.-M., 'Antiviral activity of 5-iodo-2'-deoxyuridine and related drugs in human keratinocytes infected in vitro with herpes simplex virus type-1', Skin Pharmacol, 1991, Vol. 4(4), pp. 291-7.

Risovic, V., Boyd, M., Choo, E., Wasan, K.-M., 'Effects of lipid-based oral formulations on plasma and tissue amphotericin B concentrations and renal toxicity in male rats', Antimicrob Agents Chemother, Oct 2003, Vol. 47(10), pp. 3339-42.

Riviere, J.-E., Papich, M.-G., Veterinary Pharmacology and Therapeutics, Wiley-Blackwell, 2017, Edition 10.

Robert, P.-Y., Tassy, A., Bioavailability of antibiotics, J Fr Ophtalmol, 2000, Vol. 23(5), pp. 510-513.

Rodvold, K.-A., 'Clinical pharmacokinetics of clarithromycin', Clin Pharmacokinet, 1999, Vol. 37(5), pp. 385-98.

Romero, B., Iglesias-García, M., Roquet, I., Fragoso, J., Martín-Cuervo, M., 'Intravenous ganciclovir as treatment of Multinodular pulmonary fibrosis (EHV-5) in a horse', In: Proceedings of 16th WEVA Congress, 2019, Verona (Italy). Poster.

Rubio-Martínez, L.-M., Elmas, C.-R., Black, B., Monteith, G., 'Clinical use of antimicrobial regional limb perfusion in horses: 174 cases (1999–2009)', J Am Vet Med Assoc, 2012, Vol. 241(12), pp. 1650-1658.

Rutenberg, D., Venner, M., Giguère, S., 'Efficacy of Tulathromycin for the Treatment of Foals with Mild to Moderate Bronchopneumonia', J Vet Intern Med, 2017, Vol. 31 (3), pp. 901-906.

Saijonmaa-Koulumies, L., Parsons, E., Lloyd, D.-H., 'Elimination of Staphylococcus intermedius in healthy dogs by topical treatment with fusidic acid', J Small Anim Pract, 1998, Vol. 39(7), pp. 341-347.

Scholar, E., 'Moxifloxacin', in Enna, S.-J., Bylund, D.-B., (Eds.) 'xPharm: The Comprehensive Pharmacology Reference', Elsevier, 2008, pp. 1-6.

Scholz, I., Oberwittler, H., Riedel, K.-D., Burhenne, J., Weiss, J., Haefeli, W.-E., Mikus, G. 'Pharmacokinetics, metabolism and bioavailability of the triazole antifungal agent voriconazole in relation to CYP2C19 genotype', British journal of clinical pharmacology, 2009, Vol. 68(6), pp. 906-915.

Schutte, J.-G., Van de Ingh, T.-S.-G.-M., 'Microphthalmia, brachygnathia superior, and palatocheiloschisis in a foal associated with griseofulvin administration to the mare during early pregnancy', Vet Quart, 1997, Vol. 19, p. 58.

Schwarz, B., Anen, C., van den Hoven, R., 'Preliminary data of a retrospective study on neurological side effects after administration of polymyxin B to endotoxaemic horses', Equine Vet J, 2013, Vol. 45, pp. 18-19.

Scicluna, C., 'Clinical evaluation of lithium thenoate to treat respiratory disease in horses', Proceedings of the 5th World Equine Airways Symposium (WEAS), Calgary, Canada, 2013.

Scotty, N.-C., Brooks, D.-E., Schuman Rose, C.-D., 'In vitro efficacy of an ophthalmic drug combination against corneal pathogens of horses', Am J Vet Res, 2008, Vol. 69(1), pp. 101-107.

Scriba, G.-K.-E., In Sweetman C., (Eds.), 'Martindale: The Complete Drug Reference', Pharmaceutical Press, London, 2009, Edition 36.

Serrano, D.-R., Lalatsa, A., 'Oral amphotericin B: The journey from bench to market', J Drug Deliv Sci Tech, 2017, Vol. 42, pp. 75-83.

Siefert, H.-M., Domdey-Bette, A., Henninger, K., Hucke, F., Kohlsdorfer, C., Stass, H.-H., 'Pharmacokinetics of the 8-methoxyquinolone, moxifloxacin: a comparison in humans and other mammalian species', The Journal of antimicrobial chemotherapy, 1999a, Vol. 43(B), pp. 69-76.

Siefert, H.-M., Kohlsdorfer, C., Steinke, W., Witt, A., 'Pharmacokinetics of the 8-methoxyquinolone, moxifloxacin: tissue distribution in male rats', The Journal of antimicrobial chemotherapy, 1999b, Vol. 43(B), pp. 61-67.

Sizar, O., Rahman, S., Sundareshan, V., 'Amikacin', StatPearls, Treasure Island (FL), 2023. Accessed June 2024, available from: https://www.ncbi.nlm.nih.gov/books/NBK430908/

Stahlmann, R., Lode, H., 'Antibiotika und Chemotherapeutika – antiinfektiöse Therapie', In Aktories, K., Förstermann, U., Hofmann, F., Starke, K., (Eds.), 'Allgemeine und spezielle Pharmakologie und Toxikologie', Urban and Fischer, München, 2005, Edition 9, pp. 784-902.

Stass, H., Kubitza, D., 'Pharmacokinetics and elimination of moxifloxacin after oral and intravenous administration in man', The Journal of antimicrobial chemotherapy, 1999, Vol. 43(B), pp. 83-90.

Stewart, A.-J., Welles, E.-G., Salazar, T., 'Fungal Infections of the Upper Respiratory Tract', Compendium Equine, pp. 208-219.

Stewart, A.-J., Salazar, T., Waldridge, B.-M., Schumacher, J., Welles, E.-G., Hanson, R.-R., Sartin, E.-A., Lenz, S.-D., Holland, M., 'Multimodal treatment of recurrent sinonasal cryptococcal granulomas in a horse', J Am Vet Med Assoc, 2009, Vol. 235, pp. 723-730.

Suarez-Mier, G., Giguère, S., Lee, E.-A., 'Pulmonary disposition of erythromycin, azithromycin, and clarithromycin in foals', J Vet Pharmacol Ther, 2007, Vol. 30(2), pp. 109-115.

Tadros, E.-M., Frank, N., 'Effects of continuous or intermittent lipopolysaccharide administration for 48 hours on the systemic inflammatory response in horses', American journal of veterinary research, 2012, Vol. 73(9), pp. 1394-1402.

Taintor, J., Schumacher, J., DeGraves, F., 'Comparison of amikacin concentrations in normal and inflamed joints of horses following intra-articular administration', Equine Vet J, 2006, Vol. 38(2), pp. 189-191.

Theuretzbacher, U., Ihle, F., Derendorf, H., 'Pharmacokinetic/pharmacodynamic profile of voriconazole', Clinical pharmacokinetics, 2006, Vol. 45(7), pp. 649-663.

Thieulent, C.-J., Sutton, G., Toquet, M.-P., Fremaux, S., Hue, E., Fortier, C., Pléau, A., Deslis, A., Abrioux, S., Guitton, E., 'Oral Administration of Valganciclovir Reduces Clinical Signs, Virus Shedding and Cell-Associated Viremia in Ponies Experimentally Infected with the Equid Herpesvirus-1 C2254 Variant', Pathogens, 2022, Vol. 11(5), p. 539.

Thorn, P., Johansen, S., 'Pharmacokinetic investigation of fusidic acid 1% viscous eye drops in healthy volunteers', Eur J Ophthalmol, 1997, Vol. 7(1), pp. 9-12.

Tomi, M., Nishimura, T., Nakashima, E., 'Mother-to-fetus transfer of antiviral drugs and the involvement of transporters at the placental barrier', J Pharm Sci, 2011, Vol. 100, pp. 3708-3718.

Vanderhaeghe, H., Vandijck, P., Desomer, P., 'Identity of Ramycin with Fusidic Acid', Nature, 1965, Vol. 205, pp. 710-711.

Van Spijk, J.-N., Beckmann, K., Wehrli Eser, M., Boxler, M., Stirn, M., Rhyner, T., Kaelin, D., Saleh, L., Schoster, A., 'Adverse effects of polymyxin B administration to healthy horses', J Vet Intern Med, 2022, Vol. 36(4), pp. 1525-1534.

Vardanyan, R., Hruby, V., (Eds.), 'Synthesis of best-seller drugs', Academic press, 2016, Edition 1, pp. 868.

Varshney, A., Ansari, Y., Zaidi, N., Ahmad, E., Badr, G., Alam, P., Khan, R.-H., 'Analysis of binding interaction between antibacterial ciprofloxacin and human serum albumin by spectroscopic techniques', Cell Biochem Biophys, 2014, Vol. 70(1), pp. 93-101.

Venner, M., Peters, J., Höhensteiger, N., Schock, B., Bornhorst, A., Grube, M., Adam, U., Scheuch, E., Weitschies, W., Rosskopf, D., Kroemer, H.-K., Siegmund, W., 'Concentration of the macrolide antibiotic tulathromycin in broncho-alveolar cells is influenced by comedication of rifampicin in foals', Naunyn-Schmiedeberg's archives of pharmacology, 2010, Vol. 381(2), pp. 161-169.

Venner, M., Rödiger, A., Laemmer, M., Giguère, S., 'Failure of antimicrobial therapy to accelerate spontaneous healing of subclinical pulmonary abscesses on a farm with endemic infections caused by Rhodococcus equi', Vet J, 2012, Vol. 192(3), pp. 293-298.

Venner, M., Astheimer, K., Lämmer, M., Giguère, S., 'Efficacy of mass antimicrobial treatment of foals with subclinical pulmonary abscesses associated with Rhodococcus equi', J Vet Intern Med, 2013a, Vol. 27(1), pp. 171-176.

Venner, M., Credner, N., Lämmer, M., Giguère, S., 'Comparison of tulathromycin, azithromycin and azithromycin-rifampin for the treatment of mild pneumonia associated with Rhodococcus equi', Vet Rec, 2013b, Vol. 173(16), pp. 397.

Vercruysse, E.-M., Narinx, F.-P., Rives, A.-C.-M., Sauvage, A.-C., Grauwels, M.-F., Monclin, S.-J., 'Equine ulcerative keratitis in Belgium: Associated bacterial isolates and in vitro antimicrobial resistance in 200 eyes', Vet Ophthalmol, 2022, Vol. 25(5), pp. 326–337.

VIDAL Compendium, la base de référence sur les medicaments [In French]. Accessed June 2024; available from: https://www.vidal.fr/medicaments.html

Villarino, N., Martín-Jiménez, T., 'Pharmacokinetics of macrolides in foals', J Vet Pharmacol Ther, 2012, Vol. 36(1), pp. 1-13.

Vissani, M.-A., Perglione, C.-O., Zabal, O., Alvarez, G., Thiry, E., Barrandeguy, M., Parreño, V.-J., 'Topical Ganciclovir Reduces Viral Excretion in Mares With Equine Coital Exanthema', Equine Vet Sci, 2020, Vol. 94, p. 103199.

Vogelsinger, H., Weiler, S., Djanani, A., Kountchev, J., Bellmann-Weiler, R., Wiedermann, C.-J., Bellmann, R., 'Amphotericin B tissue distribution in autopsy material after treatment with liposomal amphotericin B and amphotericin B colloidal dispersion', The Journal of antimicrobial chemotherapy, 2006, Vol. 57(6), pp. 1153-1160.

Volmer, A.-A., Szpilman, A.-M., Carreira, E.-M., 'Synthesis and biological evaluation of amphotericin B derivatives', Natural product reports, 2010, Vol. 27(9), pp. 1329-1349.

Von Keutz, E., Schlüter, G., 'Preclinical safety evaluation of moxifloxacin, a novel fluorchinolone', Journal of Antimicrobial Chemotherapy, 1999, Vol. 43(B), pp. 91-100.

Wagstaff, A.-J., Faulds, D., Goa, K.-L., 'Acyclovir. A reappraisal of its antiviral activity, pharmacokinetic properties and therapeutic efficacy', Drugs, 1994, Vol. 47(1), pp. 153-205.

Wang, C., Feng, Y., Liu, L., Wei, L., Kang, M., Zong, Z., 'Identification of novel mobile colistin resistance gene mcr-10', Emerging microbes & infections, 2020, Vol. 9(1), pp. 508-516.

Wang, X., Mohammad, I.-S., Fan, L., Zhao, Z., Nurunnabi, M., Sallam, M.-A., Wu, J., Chen, Z., Yin, L., He, W., 'Delivery strategies of amphotericin B for invasive fungal infections', Acta Pharm Sin B, Aug 2021, Vol. 11(8), pp. 2585-2604.

Wehrli, W., Knüsel, F., Schmid, K., Staehelin, M., 'Interaction of rifamycin with bacterial RNA polymerase', Proceedings of the National Academy of Sciences of the United States of America, 1968, Vol. 61(2), pp. 667-673.

Werners, A.-H., 'Treatment of endotoxaemia and septicaemia in the equine patient', Journal of veterinary pharmacology and therapeutics, 2017, Vol. 40(1), pp. 1-15.

Westermeyer, H.-D., Hendrix, D.-V.-H., Ward, D.-A., Cox, S.-K., 'Tear, cornea, and aqueous humor concentrations of ciprofloxacin and moxifloxacin after topical ocular application in ophthalmologically normal horses', Am J Vet Res, 2011, Vol. 72(3), pp. 398-403.

Wetzig, M., Venner, M., Giguère, S., 'Efficacy of the combination of doxycycline and azithromycin for the treatment of foals with mild to moderate bronchopneumonia', Equine Vet J, 2020, Vol. 52(4), pp. 613-619.

WHO Access, Watch, Reserve (AwaRe) classification of antibiotics for evaluation and monitoring of use, 2023. Accessed June 2024; available from: https://www.who.int/publications/i/item/WHO-MHP-HPS-EML-2023.04

WHO, World Health Organization, 'WHO List of Medically Important Antimicrobials: a risk management tool for mitigating antimicrobial resistance due to non-human use', Geneva: World Health

Organization, 2024. Accessed May 2024; available from: https://cdn.who.int/media/docs/default-source/gcp/who-mia-list-2024-lv.pdf

Williams, K.-J., 'Experimental induction of pulmonary fibrosis in horses with the gammaherpesvirus equine herpesvirus 5', PloS One, 2013, Vol. 8, p. e777754.

Wilson, W.-D., Spensley, M.-S., Baggot, J.-D., Hietala, S.-K., 'Pharmacokinetics, bioavailability, and in vitro antibacterial activity of rifampin in the horse', American Journal of Veterinary Research, 1988, Vol. 49(12), pp. 2041-2046.

Wise, L.-N., Ueti, M.-W., Kappmeyer, L.-S., 'In vitro activity of ponazuril against Theileria equi', Vet Parasitol, 2012, Vol. 185, pp. 282-285.

Wise, L.-N., Kappmeyer, L.-S., Mealey, R.-H., Knowles, D.-P., 'Review of Equine Piroplasmosis', J Vet Intern Med, 2013, Vol. 27, pp. 1334-1346.

Womble, A.-Y., Giguère, S., Lee, E.-A., Vickroy, T.-W., 'Pharmacokinetics of clarithromycin and concentrations in body fluids and bronchoalveolar cells of foals', Am J Vet Res, 2006, Vol. 67(10), pp. 1681-6.

Womble, A.-Y, Giguère, S., Lee, E.-A., 'Pharmacokinetics of oral doxycycline and concentrations in body fluids and bronchoalveolar cells of foals', Journal of Veterinary Pharmacology and Therapeutics, 2007, Vol. 30(3), pp. 187-193.

Xu, C., Li, C.-Y., Kong, A.-N., 'Induction of phase I, II and III drug metabolism/transport by xenobiotics', Archives of Pharmacal Research, 2005, Vol. 28(3), pp. 249-268.

Yamori, S., Ichiyama, S., Shimokata, K., Tsukamura, M., 'Bacteriostatic and bactericidal activity of antituberculosis drugs against Mycobacterium tuberculosis, Mycobacterium avium–Mycobacterium intracellulare complex and Mycobacterium kansasii in different growth phases', Microbiol Immunol, 1992, Vol. 36, pp. 361-368.

Yamshchikov, A.-V., Schuetz, A., Lyon, G.-M., 'Rhodococcus equi infection', Lancet Infect Dis, 2010, Vol. 10(5), pp. 350–359.

Yun, B., Zhang, T., Azad, M.-A.-K., Wang, J., Nowell, C.-J., Kalitsis, P., Velkov, T., Hudson, D.-F., Li, J., 'Polymyxin B causes DNA damage in HK-2 cells and mice', Archives of toxicology, 2018, Vol. 92(7), pp. 2259-2271.

Yun, B., Zhang, T., Azad, M.-A.-K., Wang, J., Nowell, C.-J., Kalitsis, P., Velkov, T., Hudson, D.-F., Li, J., 'Polymyxin B causes DNA damage in HK-2 cells and mice'. Erratum in: Arch Toxicol, Jul 2018, Vol. 92(7), pp. 2273-2274.

Zaugg, J.-L., Lane, V.-M., 'Evaluations of buparvaquone as a treatment for equine babesiosis (Babesia equi)', Am J Vet Res, May 1989, Vol. 50(5), pp. 782-5.

Zaugg, J.-L., Lane, V.-M., 'Efficacy of buparvaquone as a therapeutic and clearing agent of Babesia equi of European origin in horses', Am J Vet Res, Aug 1992, Vol. 53(8), pp. 1396-9.

Zavascki, A.-P., Goldani, L.-Z., Cao, G., Superti, S.-V., Lutz, L., Barth, A.-L., Ramos, F., Boniatti, M. M., Nation, R.-L., Li, J., 'Pharmacokinetics of intravenous polymyxin B in critically ill patients', Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 2008, Vol. 47(10), pp. 1298-1304.

Zhao, X., Yuan, Y., Shao, Q., Qiao, H., 'Simultaneous Determination of Moxifloxacin Hydrochloride and Dexamethasone Sodium Phosphate in Rabbit Ocular Tissues and Plasma by LC-MS/MS: Application for Pharmacokinetics Studies', Molecules (Basel, Switzerland), 2022, Vol. 27(22), p. 7934.

Ziegler, A.-L., Freeman, C.-K., Fogle, C.-A., Burke, M.-J., Davis, J.-L., Cook, V.-L., Southwood, L.-L., Blikslager, A.-T., 'Multicentre, blinded, randomised clinical trial comparing the use of flunixin meglumine with firocoxib in horses with small intestinal strangulating obstruction', Equine veterinary journal, 2019, Vol. 51(3), pp. 329-335.

Zobba, R., Ardu, M., Niccolini, S., 'Clinical and laboratory findings in equine piroplasmosis', J Eq Vet Sci, 2008, Vol. 28, pp. 301-308.

Substances for respiratory disorders

Allegra, L., Bossi, R., Braga, P., 'Influence of surfactant on mucociliary transport', Eur J Respir Dis, 1985, Vol. 67(142), pp. 71-76.

Allen, A., Bareille, P.-J., Rousell, V.-M., 'Fluticasone furoate, a novel inhaled corticosteroid, demonstrates prolonged lung absorption kinetics in man compared with inhaled fluticasone propionate', Clin Pharmacokinet, 2013, Vol. 52, pp. 37–42.

Almadhoun, K., Sharma, S., 'Bronchodilators', StatPearls, Treasure Island (FL), 2023. Accessed September 2023, available from: https://pubmed.ncbi.nlm.nih.gov/30085570/

Biggadike, K., Uings, I., Farrow, S.-N., 'Designing corticosteroid drugs for pulmonary selectivity', Proc Am Thoracic Soc, 2004, Vol. 1, pp. 352–355.

Biggadike, K., 'Fluticasone furoate/fluticasone propionate – different drugs with different properties', The Clinical Respiratory Journal, Blackwell Publishing Ltd., 2011. Letter to the Editor.

Brittain, H.-G., 'Profiles of drug substances, excipients and related methodology', 2003, Vol. 30, pp. 117-122.

Dempsey, O.-J., Coutie, W.-J.-R., Wilson, A.-M., Williams, P., Lipworth, B.-J., 'Evaluation of the buccal component of systemic absorption with inhaled fluticasone propionate', Thorax, 1999, Vol. 54, pp. 614-617.

Disse, B.-G., Ziegler, H.-W., 'Pharmacodynamic mechanism and therapeutic activity of ambroxol in animal experiments', Respiration, 1987, Vol. 51(1), pp. 15-22.

Druce, H.-M., Ramsey, D.-L., Karnati, S., Carr, A.-N., 'Topical nasal decongestant oxymetazoline (0.05%) provides relief of nasal symptoms for 12 hours', Rhinology, 2018, Vol. 54(4), pp. 343-350.

Drugbank, Ambroxol [Updated 3 Feb 2023]. Accessed July 2023a, available from: https://go.drugbank.com/drugs/DB06742

Drugbank, Ipratropium [Updated 7 Jul 2023]. Accessed July 2023b, available from: https://go.drugbank.com/drugs/DB00332

Duvivier, D.-H., Votion, D., Vandenput, S., Art, T., Lekeux, P., 'Airway response of horses with COPD to dry powder inhalation of ipratropium bromide', Vet J, 1997, Vol. 154(2), pp. 149-53.

Duvivier, D.-H., Bayly, W.-M., Votion, D., Vandenput, S., Art, T., Farnir, F., Lekeux, P., 'Effects of inhaled dry powder ipratropium bromide on recovery from exercise of horses with COPD', Equine Vet J, 1999, Vol. 31(1), pp. 20-4.

ECHA, Substance Info Card – Ambroxol. Accessed July 2023a, available from: https://echa.europa.eu/de/substance-information/-/substanceinfo/100.038.621

ECHA, Substance Info Card – Fluticasone furoate. Accessed July 2023b, available from: https://echa.europa.eu/de/substance-information/-/substanceinfo/100.158.130

ECHA, Substance Info Card – Fluticasone propionate. Accessed July 2023c, available from: https://echa.europa.eu/de/substance-information/-/substanceinfo/100.129.097

ECHA C&L. Notified Classification and Labelling – Oxymetazoline. Accessed August 2023, available from: https://echa.europa.eu/de/information-on-chemicals/cl-inventory-database/-/discli/details/51171

EMA/168579/2015, 'Ambroxol and bromhexine expectorants: safety information to be updated', available from: Ambroxol and bromhexine-containing medicines – referral | European Medicines Agency (europa.eu).

EMEA/MRL/080/96-FINAL, 'Committee for Veterinary Medicinal Products. Dembrexine Summary Report'.

EMEA/MRL/503/98-FINAL, 'Committee for Veterinary Medicinal Products. Bromhexine Summary Report'.

EMEA/MRL/517/98-FINAL, 'Committee for Veterinary Medicinal Products. Atropine Summary Report'.

Förster, H.-J., Kramer, I., Pook, K.-H., Wahl, D., 'Studies on the pharmacokinetics and biotransformation of ipratropium bromide in the rat and dog', Arzneimittelforschung, 1976, Vol. 26(5A), pp. 992-1005.

Giguere, S., Viel, L., Lee, E., MacKay, R.-J., Hernandez, J., Franchini, M., 'Cytokine induction in pulmonary airways of horses with heaves and effect of therapy with inhaled fluticasone propionate', Vet Immunol Immunopathol, 2002, Vol. 85(3-4), pp. 147-158.

Hammer, R., Bozler, G., Jauch, R., Koss, F.-W., 'Ambroxol, comparative studies of pharmacokinetics and biotransformation in rat, rabbit, dog and man', Arzneimittel-Forschung (Drug research), 1978, Vol. 28(5A), pp. 899-903.

Hogger, P., Rohdewald, P., 'Binding kinetics of fluticasone propionate to the human glucocorticoid receptor', Steroids, 1994, Vol. 59(10), pp. 597-602.

Hohlfeld, J., Fabel, H., Hamm, H., 'The role of pulmonary surfactant in obstructive airways disease', Eur Respir J, Feb 1997, Vol. 10(2), pp. 482-91.

Hug, F., 'Molecular modelling analysis of the metabolism of ambroxol', Journal of Pharmacology and Toxicology, 2008, Vol. 3(2), pp. 147-152.

Johnson, M., 'Development of fluticasone propionate and comparison with other inhaled corticosteroids', J Allergy Clin Immunol, 1998, Vol. 101(4 Pt 2), pp. 434-439.

Lavoie, J.-P., 'Is the time primed for equine asthma?', Equine Vet. Educ., 2015, Vol. 27(5), pp. 225-226.

Leung, S.-Y., Eynott, P., Nath, P., Chung, K.-F., 'Effects of ciclesonide and fluticasone propionate on allergen-induced chronic airway inflammation and bronchial hyperreactivity in sensitized Brown-Norway rats', Eur Respir J, 2003, Vol. 22(45), p. 1820.

Leung, S.-Y., Eynott, P., Nath, P., Chung, K.-F., 'Effects of ciclesonide and fluticasone propionate on allergen-induced airway inflammation and remodelling features' J Allergy Clin Immunol, 2005, Vol. 115(5), pp. 989-96.

Liu, F., Zhou, Q., Wang, H., Fu, H., Li, Y., Tao, M., Luo, H., Cao, Y., 'Efficacy and safety of oxymetazoline for the treatment of rosacea: A meta-analysis', J Cosmet Dermatol, 2023, Vol. 22(9), pp. 2408-2419.

Löscher, W., Richter, A., 'Lehrbuch der Pharmakologie und Toxikologie für die Veterinärmedizin', Enke, Stuttgart, Edition 4, 2016, p. 274.

Lusuardi, M., Capelli, A., Carli, S., Tacconi, M.-T., Salmona, M., Donner, C.-F., 'Role of surfactant in chronic obstructive pulmonary disease: therapeutic implications', Respiration, 1992, Vol. 59(1), pp. 28-32.

Khan, S.-A., 'Decongestants (Toxicity)', MSD Manual Veterinary Manual. Revised August 2014, modified Nov 2022. Accessed August 2023, available from: https://www.msdvetmanual.com/toxicology/toxicities-from-human-drugs/decongestants-toxicity?query=oxymetazoline.

Mackie, A.-E., McDowall, J.-E., Falcoz, C., Ventresca, P., Bye, A., Daley-Yates, P.-T., 'Pharmacokinetics of Fluticasone Propionate Inhaled via the Diskhaler and Diskus Powder Devices in Healthy Volunteers', Clin Pharmacokinet, 2000, Vol. 39(1), pp 23-30.

Mahajan, M.-K., Uttamsingh, V., Daniels, J.-S., Gan, L.-S., LeDuc, B.-W., Williams, D.-A., 'In vitro metabolism of oxymetazoline: evidence for bioactivation to a reactive metabolite', Drug Metab Dispos, 2011, Vol. 39(4), pp. 693-702.

Malerba, M., Ragnoli, B., 'Ambroxol in the 21st century: pharmacological and clinical update', Expert Opin Drug Metab Toxicol, 2008, Vol. 4(8), pp. 1119-1129.

Massey, K.-L., Gotz, V.-P., 'Ipratropium bromide', Drug Intell Clin Pharm, 1985, Vol. 19(1), pp. 5-12.

McEvoy, G.-K., 'American Hospital Formulary Service', AHFS Drug Information, American Society of Health-System Pharmacists, Bethesda (MD), 2006, p. 2825.

McGorum, B.-C., Nicholas, D.-R., Foster, A.-P., Shaw, D.-J., Pirie, R.-S., 'Bronchodilator activity of the selective muscarinic antagonist revatropate in horses with heaves', Vet J, 2013, Vol. 195(1), pp. 80-5.

Minto, C., Li, B., Tattam, B., Brown, K., Seale, J.-P., Donnelly, R., 'Pharmacokinetics of epimeric budesonide and fluticasone propionate after repeat dose inhalation – intersubject variability in systemic absorption from the lung', Br J Clin Pharmacol, 2000, Vol. 50, pp. 116-124.

Nave, R., Hummel, R.-P., Wohlsen, A., 'The active metabolite of ciclesonide, desisobutyryl-ciclesonide, forms highly lipophilic fatty acid conjugates in precision-cut rat lung slices', Am J Resp Crit Care Med, 2004, p. 169.

Robertson, B., 'Pharmacological stimulation of surfactant secretion and surfactant replacement', Eur J Respir Dis, 1985, Vol. 67(142), pp. 63-70.

Sastre, J., 'Pharmacology of fluticasone propionate', J Investig Allergol Clin Immunol, 1997, Vol. 7(5), pp. 382-384.

Seki, T., Matsumura, R., Kohei, H., 'A clinico-pharmacological study on trans-4-(2-Amino-3,5 dibrornobenzylamino)cyclohexanol hydrochloride', Jap J Clin Therap, 1977, Vol. 8, pp. 25-31.

Spadijer Mirkovic, C., Peric, A., Vukomanovic Durdevic, B., Vojvodic, D., 'Effects of Fluticasone Furoate Nasal Spray on Parameters of Eosinophilic Inflammation in Patients With Nasal Polyposis and Perennial Allergic Rhinitis', Ann Otol Rhinol Laryngol, 2017, Vol. 126(8), pp. 573-580.

Uboh, C.-E., Rudy, J.-A., Soma, L.-R., Fennell, M., 'Characterization of bromhexine and ambroxol in equine urine: effect of furosemide on identification and confirmation', Journal of Pharmaceutical & Biomedical Analysis, 1991, Vol. 9(1), pp. 33-39.

Weiser, T., 'Comparison of the effects of four Na+ channel analgesics on TTX-resistant Na+ currents in rat sensory neurons and recombinant Nav1.2 channels', Neurosci Lett, 2006, Vol. 395, pp. 179-84.

Yang, Y.-G., Song, L.-X., Jiang, N., Xu, X.-T., Di, X.-H., Zhang, M., 'Pharmacokinetics of ambroxol and clenbuterol tablets in healthy Chinese volunteers', Int J Clin Exp Med, 2015, Vol. 8(10), pp. 18744-18750.

Yasir, M., Goyal, A., Sonthalia, S., 'Corticosteroid Adverse Effects', StatPearls, Treasure Island (FL), 2023 [Updated 3 Jul 2023]. Accessed September 2023, available from: https://www.ncbi.nlm.nih.gov/books/NBK531462

Yokota, S., Hikasa, Y., Shimura, I., Kusunose, S., 'Effects of imidazoline and nonimidazoline alpha-adrenergic agents, including xylazine, medetomidine, yohimbine, tolazoline, and atipamezole, on aggregation of bovine and equine platelets', Am J Vet Res, Mar 2013, Vol. 74(3), pp. 395-402.

Zavattini, G., 'Ambroxol e stimolazione del surfattante polmonare [Ambroxol and stimulation of the pulmonary surfactant]', Acta Otorhinolaryngol Ital, 1987, Vol. 7(16), pp. 33-8.

Substances for cardiology

Abd-Allah, E.-R., Amin, S., El Ghareeb, A.-E.-W., Badawy, M.-A., 'Effect of Rythmol (propafenone HCl) administration during pregnancy in Wistar rats', J Biochem Mol Toxicol, 2022, Vol. 36(8).

Antonaccio, M.-J., Gomoll, A., 'Pharmacology, pharmacodynamics and pharmacokinetics of sotalol', Am J Cardiol, 1990, Vol. 65(2), pp. 12A-21A.

Bachmakov, I., Rekersbrink, S., Hofmann, U., Eichelbaum, M., Fromm M.-F., 'Characterisation of (R/S)-propafenone and its metabolites as substrates and inhibitors of P-glycoprotein', Naunyn Schmiedebergs Arch Pharmacol, 2005, Vol. 371(3), pp. 195-201.

Barrell, E.-A., Fiorezna, L.-R., Gillian, A.-P., Kraus, M.-S., 'ECG of the Month', 2015, Vol. 247(7).

Bouckaert, S., Voorspoels, J., Vandenbossche, G., Deprez, P., Remon, J.-P., 'Effect of drug formulation and feeding on the pharmacokinetics of orally administered quinidine in the horse', J Vet Pharmacol Ther, 1994, Vol. 17(4), pp. 275-278.

Broux, B., De Clercq, D., Decloedt, A., De Baere, S., Devreese, M., Van Der Vekens, N., Ven, S., Croubels, S., van Loon, G., 'Pharmacokinetics of intravenously and orally administered sotalol hydrochloride in horses and effects on surface electrocardiogram and left ventricular systolic function', Vet J, 2016, Vol. 208, pp. 60-64.

Broux, B., De Clercq, D., Decloedt, A., Vera, L., Devreese, M., Gehring, R., Croubels, S., van Loon, G. 'Pharmacokinetics and electrophysiological effects of sotalol hydrochloride in horses', Equine Vet J, 2018, Vol. 50(3), pp. 377-383.

Buchholz, B.-M., Murdock, A., Bayly, W.-M., Sides, R.-H., 'Effects of intravenous aminocaproic acid on exercise-induced pulmonary haemorrhage (EIPH)', Equine Vet J Suppl, 2010, Vol. 38, pp. 256-260.

Burns, J.-J., MacMillan, K.-M., John, E.-E., 'Retrospective review of atrial fibrillation in Standardbred racehorses at a tertiary care facility in Atlantic Canada', Can Vet J, 2022, Vol. 63(10), pp. 1051–1056.

Busse, D., Fromm, M.-F., Mörike, K., Drescher, S., Kühlkamp, V., Eichelbaum, M., 'Disposition and pharmacologic effects of R/S-verapamil in patients with chronic atrial fibrillation: an investigation comparing single and multiple dosing', Clin Pharmacol Ther, 2001, Vol. 69(5), pp. 324-332.

Carstensen, H., Hesselkilde, E.-Z., Fenner, M., Loft-Andersen, A.-V., Flethøj, M., Kanters, J.-K., Sattler, S.-M., Tfelt-Hansen, J., Pehrson, S., Jespersen, T., Buhl, R., 'Time-dependent antiarrhythmic effects of flecainide on induced atrial fibrillation in horses', J Vet Intern Med, 2018, Vol. 32(5), pp. 1708-1717.

Colley, J.-C., Edwards, J.-A., Heywood, R., Purser, D., 'Toxicity studies with quinine hydrochloride', Toxicology, 1989, Vol. 54(2), pp. 219-226.

Davis, J.-L., Kruger, K., LaFevers, D.-H., Barlow, B.-M., Schirmer, J.-M., Breuhaus, B.-A. 'Effects of quinapril on angiotensin converting enzyme and plasma renin activity as well as pharmacokinetic parameters of quinapril and its active metabolite, quinaprilat, after intravenous and oral administration to mature horses', Equine Vet J, 2014, Vol. 46(6), pp. 729-733.

De Clercq, D., van Loon, G., Baert, K., De Backer, P., Deprez, P., 'Treatment with amiodarone of refractory ventricular tachycardia in a horse', J Vet Intern Med, 2007, Vol. 21(4), pp. 878-880.

De Clercq, D., van Loon, G., Baert, K., Tavernier, R., Croubels, S., De Backer, P., Deprez, P., 'Intravenous amiodarone treatment in horses with chronic atrial fibrillation', Vet J, 2006, Vol. 172(1), pp. 129-134.

De Clercq, D., van Loon, G., Tavernier, R., Verbesselt, R., Deprez, P., 'Use of intravenous propafenone in horses with chronic atrial fibrillation', 47th BEVA Congress, Liverpool, 2008.

De Clercq, D., van Loon, G., Tavernier, R., Verbesselt, R., Deprez, P., 'Use of propafenone for conversion of chronic atrial fibrillation in horses', Am J Vet Res, 2009, Vol. 70(2), pp. 223-227.

Deedwania, P.-C., 'Sotalol Is More Powerful Than Propranolol in Suppressing Complex Ventricular Arrhythmias', J Cardiovasc Pharmacol Ther, 1997, Vol. 2(4), pp. 259-272.

Dembek, K.-A., Hurcombe, S.-D.-A., Schober, K.-E., Toribio, R.-E., 'Sudden death of a horse with supraventricular tachycardia following oral administration of flecainide acetate', J Vet Emerg Crit Care (San Antonio), 2014, Vol. 24(6), pp. 759-763.

De Oliveira, J.-T., da Silva Barbosa, M.-C., de Camargos, L.-F., Gomes da Silva, I.-V., de Pilla Varotti, F., da Silva, L.-M., Marmo Moreira, L., Pereira Lyon, J., da Silva Vieira dos Santos, V.-J., Vieira dos Santos, F., 'Digoxin reduces the mutagenic effects of mitomycin C in human and rodent cell lines', Cytotechnology, 2017, Vol. 69, pp. 699-710.

Fahie, S., Cassagnol, M., 'Verapamil', StatPearls, Treasure Island (FL), February 2023, available from: https://www.ncbi.nlm.nih.gov/books/NBK538495/.

Florek, J.-B., Lucas, A., Girzadas, D., 'Amiodarone', Treasure Island (FL), November 2023, available from: https://www.ncbi.nlm.nih.gov/books/NBK482154/.

Gehlen, H., Vieht, J.-C., Stadler, P., 'Effects of the ACE inhibitor quinapril on echocardiographic variables in horses with mitral valve insufficiency', J Vet Med A Physiol Pathol Clin Med, 2003, Vol. 50(9), pp. 460-465.

Grandi, E., Ripplinger, C.-M., 'Antiarrhythmic mechanisms of beta blocker therapy', Pharmacol Res, 2019, Vol. 146.

Harron, D.-W., Brogden, R.-N., 'Propafenone. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in the treatment of arrhythmias', Drugs, 1987, Vol. 34(6), pp. 617-647.

Hinchcliff, K.-W., McKeever, K.-H., Muir 3rd, W.-W., 'Hemodynamic effects of atropine, dobutamine, nitroprusside, phenylephrine, and propranolol in conscious horses', J Vet Intern Med, 1991, Vol. 5(2), pp. 80-86.

IARC International Agency for Research on Cancer. Some drugs and herbal products / IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. IARC monographs on the evaluation of carcinogenic risks to humans, Lyon, France, 2013, Vol. 108.

Kaplan, H.-R., Taylor, D.-G., Olson, S.-C., Andrews, L.-K., 'Quinapril–a preclinical review of pharmacology, pharmacokinetics, and toxicology', Angiology, 1989, Vol. 40 (4 Pt 2), pp. 335-350.

Kieback, A.-G., Felix, S.-B., Reffelmann, T., 'Quinaprilat: a review of its pharmacokinetics, pharmacodynamics, toxicological data and clinical application', Expert Opin Drug Metab Toxicol, 2009, Vol. 5(10), pp. 1337-1347.

Kpaeyeh Jr, J.-A., Wharton, J.-M., 'Sotalol', Card Electrophysiol Clin, 2016, Vol. 8(2), pp. 437-452.

McGuirk, S-M., Muir, W.-W., Sams, R.-A., 'Pharmacokinetic analysis of intravenously and orally administered quinidine in horses', Am J Vet Res, 1981, Vol. 42(6), pp. 938-942.

McGurrin, M.-K.-J., 'The diagnosis and management of atrial fibrillation in the horse', Vet Med (Auckl), 2015, Vol. 6, pp. 83-90.

Muir, W.-W., McGuirk, S.-M., 'Pharmacology and pharmacokinetics of drugs used to treat cardiac disease in horses', Vet Clin North Am Equine Pract, 1985, Vol. 1(2), pp. 335-352.

Muir 3rd, W.-W., Reed, S.-M., McGuirk, S.-M., 'Treatment of atrial fibrillation in horses by intravenous administration of quinidine', J Am Vet Med Assoc, 1990, Vol. 197(12), pp. 1607-1610.

Münzner, R., Renner, H.-W., 'Mutagenicity testing of quinine with submammalian and mammalian systems', Toxicology, 1983, Vol. 26(2), pp. 173-178.

Narayana, S.-K., Woods, D.-R., Boos, C.-J., 'Management of amiodarone-related thyroid problems', Ther Adv Endocrinol Metab, 2011, Vol. 2(3), pp. 115-126.

Ochs, H.-R., Greenblatt, D.-J., Woo, E., 'Clinical pharmacokinetics of quinidine', Clin Pharmacokinet, 1980, Vol. 5(2), pp. 150-168.

Olson, S.-C., Horvath, A.-M., Michniewicz, B.-M., Sedman, A.-J., Colburn, W.-A., Welling, P.-G., 'The clinical pharmacokinetics of quinapril', Angiology, 1989, Vol. 40 (4 Pt 2), pp. 351-359.

Papich, M.-G., (Ed.), 'Papich Handbook of Veterinary Drugs', Elsevier, 2021, Edition 5.

Plumb D.-C., (Ed.), 'Plumb's Veterinary Drug Handbook', Wiley-Blackwell, 2015, Edition 8.

Puigdemont, A., Arboix, M., Gaspari, F., Bortolotti, A., Bonati, M., 'In-vitro plasma protein binding of propafenone and protein profile in eight mammalian species', Res Commun Chem Pathol Pharmacol, 1989, Vol. 64(3), pp. 435-440.

Puigdemont, A., Riu, J.-L., Guitart, R., Arboix, M., 'Propafenone kinetics in the horse. Comparative analysis of compartmental and noncompartmental models', J Pharmacol Methods, 1990, Vol. 23(2), pp. 79-85.

Redpath, A., Bowen, M., 'Cardiac Therapeutics in Horses', Vet Clin North Am Equine Pract, 2019, Vol. 35(1), pp. 217-241.

Reef, V.-B., Reimer, J.-M., Spencer, P.-A., 'Treatment of atrial fibrillation in horses: new perspectives', J Vet Intern Med, 1995, Vol. 9(2), pp. 57-67.

Robinson, S.-J., Feary, D.-J., 'Sudden death following oral administration of flecainide to horses with naturally occurring atrial fibrillation', The Australian Equine Veterinarian, 2008, Vol. 27(3), pp. 49-51.

Sedigh-Ardekani, M., Saadat, I., Saadat, M., 'Evaluation of chromosome aberrations induced by digoxin in Chinese hamster ovary cells', EXCLI Journal, 2013, Vol. 12, pp. 523-527.

Sköld, A.-C., Danielsson, B.-R., 'Developmental toxicity in the pregnant rabbit by the class III antiarrhythmic drug sotalol', Pharmacol Toxicol, 2001, Vol. 88(1), pp. 34-39.

Thompson, H., 'Procainamide', Treasure Island (FL), May 2023, available from: https://www.ncbi.nlm.nih.gov/books/NBK557788/.

Van Loon, G., Blissitt, K.-J., Keen, J.-A., Young, L.-E., 'Use of intravenous flecainide in horses with naturally-occurring atrial fibrillation', Equine Vet J, 2004, Vol. 36(7), pp. 609-614.

Whelchel, D.-D., Tennent-Brown, B.-S., Coleman, A.-E., Rapoport, G.-S., Blas-Machado, U., Maisenbacher, H.-W., Credille, B.-C., Giguere, S., 'Treatment of supraventricular tachycardia in a horse', J Vet Emerg Crit Care (San Antonio), 2017, Vol. 27(3), pp. 362-368.

Substances for diagnostic procedures

ATSDR Agency for Toxic Substances and Disease Registry, 'Toxicological profile for barium and barium compounds', 2007. Accessed November 2023, available from: https://www.atsdr.cdc.gov/toxprofiles/tp24.pdf

Barry, R.-E., Behrendt, W.-A., 'Studies on the pharmacokinetics of fluorescein and its dilaurate ester under the conditions of the fluorescein dilaurate test', Arzneimittelforschung, 1985, Vol. 35(3), pp. 644-648.

Beech, J., Boston, R., Lindborg, S., 'Comparison of cortisol and ACTH responses after administration of thyrotropin releasing hormone in normal horses and those with pituitary pars intermedia dysfunction', J Vet Intern Med, 2011, Vol. 25(6), pp. 1431–1438.

Bhoelan, B.-S., Stevering, C.-H., van der Boog, A.-T.-J., van der Heyden, M.-A.-G., 'Barium toxicity and the role of the potassium inward rectifier current', Clin Toxicol (Phila), 2014, Vol. 52(6), pp. 584-593.

Brooks, D.-E., Andrew, S.-E., Denis, H.-M., Strubbe, D.-T., Biros, D.-J., Cutler, T.-J., 'Rose Bengal positive epithelial microerosions as a manifestation of equine keratomycosis', Veterinary ophthalmology, 2000, Vol. 3(2-3), pp. 83-86.

Burnett, C.-M., Goldenthal, E.-I., 'The teratogenic potential in rats and rabbits of D&C Yellow No. 8', Fd Chem Toxic, 1986, Vol. 24(8), pp. 819-823.

Cheng, W., Zhao, F., Tang, C.-Y., Li, X.-W., Luo, M., Duan, S.-B., 'Comparison of iohexol and iodixanol induced nephrotoxicity, mitochondrial damage and mitophagy in a new contrast-induced acute kidney injury rat model', Arch Toxicol, 2018, Vol. 92(7), pp. 2245–2257.

Clavel, J.-P., Lorillot, M.-L., Buthiau, D., Gerbet, D., Heitz, F., Galli, A., 'Absorption intestinale du baryum lors d'explorations radiologiques [Intestinal absorption of barium during radiological studies]', Therapie, 1987, Vol. 42(2), pp. 239-243.

Daniel, A.-J., Judy, C.-E., Rick, M.-C., Saveraid, T.-C., Herthel, D.-J., 'Comparison of radiography, nuclear scintigraphy, and magnetic resonance imaging for detection of specific conditions of the distal tarsal bones of horses: 20 cases (2006–2010)', J Am Vet Med Assoc, 2012, Vol. 240(9), pp. 1109-1114.

Drugbank, Iohexol [Updated 21 March 2024]. Accessed March 2024, available from: https://go.drugbank.com/drugs/DB01362

ECHA C&L. Notified Classification and Labelling – Barium Sulfate. Accessed November 2023a, available from: https://echa.europa.eu/de/information-on-chemicals/cl-inventory-database/-/discli/details/175971

ECHA, Registration Dossier – Barium Sulfate. Accessed November 2023b, available from: https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15235

ECHA, Registration Dossier – Iohexol. Accessed November 2023c, available from: https://echa.europa.eu/de/registration-dossier/-/registered-dossier/6709

ECHA, Substance Info Card – Barium Sulfate. Accessed November 2023d, available from: https://echa.europa.eu/de/substance-information/-/substanceinfo/100.171.057

ECHA C&L. Notified Classification and Labelling – Fluorescein sodium. Accessed January 2024a, available from: https://echa.europa.eu/de/information-on-chemicals/cl-inventory-database/-/discli/details/12467

ECHA, Registration Dossier – Fluorescein sodium. Accessed January 2024b, available from: https://echa.europa.eu/de/registration-dossier/-/registered-dossier/17278/1/1

Eco SSL Ecological Soil Screening Levels, 'Ecological Screening Levels for Barium', 2005. Accessed November 2023, available from: https://rais.ornl.gov/documents/eco-ssl_barium.pdf

EMEA/MRL/580/99-FINAL, 'Committee for Veterinary Medicinal Products. Barium Selenate Summary Report'.

Feenstra, R.-P., Tseng, S.-C.-G., 'Comparison of fluorescein and rose bengal staining', Ophthalmology, 1992, Vol. 99(4), pp. 606–617.

Grotte, D., Mattox, V., Brubaker, R., 'Fluorescent, physiological and pharmacokinetic properties of fluorescein glucuronide', Exp Eye Res, 1985, Vol. 40(1), pp. 23-33.

Hahn, C.-N., Mayhew, I.-G., 'Phenylephrine eyedrops as a diagnostic test in equine grass sickness', Vet rec, 2000, Vol. 147(21), pp. 603-606.

Haria, M., Brogden, R.-N., 'Iohexol: A review of its pharmacological properties and use as a contrast medium in myelography and neuroangiography', Cns Drugs, 1997, Vol. 7, pp. 229-255.

IARC International Agency for Research on Cancer, Monographs on the evaluation of carcinogenic risks to humans, 1982, Vol. 1-29 (Suppl. 4). Accessed January 2024, available from: https://publications.iarc.fr/136

IARC International Agency for Research on Cancer. List of Classifications. Accessed January 2024, available from: https://monographs.iarc.who.int/list-of-classifications/

Johnston, H., Pojana, G., Zuin, S., Jacobsen, N.-R., Møller, P., Loft, S., Semmler-Behnke, M., McGuiness, C., Balharry, D., Marcomini, A., Wallin, H., Kreyling, W., Donaldson, K., Tran, L., Stone, V., 'Engineered nanomaterial risk. Lessons learnt from completed nanotoxicology studies: potential solutions to current and future challenges', Crit Rev Toxicol, 2013, Vol. 43(1), pp. 1-20.

Kawada, T.-K., McLeod, D.-C., 'Iohexol and iopamidol: second-generation nonionic radiographic contrast media', Drug Intell Clin Pharm, 1985, Vol. 19(7-8), pp. 525-529.

Kirberger, R.-M., Cassel, N., Carstens, A., Goddard, A., 'The effects of repeated intravenous iohexol administration on renal function in healthy beagles – a preliminary report', Acta Vet Scand, 2012, Vol. 54(1).

Konduru, N., Keller, J., Ma-Hock, L., Gröters, S., Landsiedel, R., Donaghey, T.-C., Brain, J.-D., Wohlleben, W., Molina, R.-M., 'Biokinetics and effects of barium sulfate nanoparticles', Part Fibre Toxicol, 2014, Vol. 11.

Krishna, S., Jaiswal, A.-K., Gupta, M., Sharma, D.-K., Ali, Z., 'Barium Poisoning with Analytical Aspects and its Management', Int J Adv Res Med Chem, 2020, Vol. 2(1), pp. 20-27.

Lamb, J.-T., 'Iohexol vs. iopamidol for myelography', Investigative Radiology, 1985, Vol. 20(1), pp. S37-S43.

Landsiedel, R., Ma-Hock, L., Hofmann, T., Wiemann, M., Strauss, V., Treumann, S., Wohlleben, W., Gröters, S., Wiench, K., van Ravenzwaay, B., 'Application of short-term inhalation studies to assess the inhalation toxicity of nanomaterials', Part Fibre Toxicol, 2014, Vol. 11.

Loza, K., Föhring, I., Bünger, J., Westphal, G.-A., Köller, M., Epple, M., Sengstock, C., 'Barium sulfate micro- and nanoparticles as bioinert reference material in particle toxicology', Nanotoxicology, 2016, Vol. 10(10), pp. 1492-1502.

Masereeuw, R., Moons, M.-M., Smits, P., Russel, F.-G.-M., 'Glomerular filtration and saturable absorption of iohexol in the rat isolated perfused kidney', Br J Phamacol, 1996, Vol. 119(1), pp. 57-64.

Mauras, Y., Allain, P., Roques, M.-A., Caron, C., 'Etude de l'absorption digestive du baryum après l'administration orale du sulfate de baryum pour exploration radiologique [Digestive absorption of barium after oral administration of barium sulfate for a radiologic study]', Therapie, 1983, Vol. 38(1), pp. 109-111.

McCauley, P.-T., Washington, I.-S., 'Barium bioavailability as the chloride, sulfate, or carbonate salt in the rat', Drug Chem Toxicol, 1983, Vol. 6(2), pp. 209-217.

McEnerney, J.-K., Wong, W.-P., Peyman, G.-A., 'Evaluation of the teratogenicity of fluorescein sodium', Am J Ophthalmol, 1977. Vol. 84(6), pp. 847-850.

Mizutani, T., 'Toxicity of xanthene food dyes by inhibition of human drug-metabolizing enzymes in a noncompetitive manner', J Environ Public Health, 2009, p. 953952.

Molina, R.-M., Konduru, N.-V., Queiroz, P.-M., Figueroa, B., Fu, D., Ma-Hock, L., Groeters, S., Schaudien, D., Brain, J.-D., 'Fate of Barium Sulfate Nanoparticles Deposited in the Lungs of Rats', Sci Rep, 2019, Vol. 9(1), p. 8163.

Monga, V., Meena, C.-L, Kaur, N., Jain, R., 'Chemistry and biology of thyrotropin-releasing hormone (TRH) and its analogs', Curr Med Chem, 2008, Vol. 15(26), pp. 2718-2733.

Møss, J., Bundgaard, H., 'Kinetics and pattern of degradation of thyrotropin-releasing hormone (TRH) in human plasma', Pharmaceutical Research, 1990, Vol. 7(7), pp. 751-755.

Murube, J., 'Fluorescein: the most commonly used surfocular vital stain', Ocul Surf, Jul 2013, Vol. 11(3), pp. 144-149.

Nazarov, P.-A., Kirsanov, R.-S., Denisov, S.-S., Khailova, L.-S., Karakozova, M.-V., Lyamzaev, K.-G., Korshunova, G.-A., Lukyanov, K.-A., Kotova, E.-A., Antonenko, Y.-N., 'Fluorescein Derivatives as Antibacterial Agents Acting via Membrane Depolarization', Biomolecules, Feb 2020, Vol. 10(2), p. 309.

Nestmann, E.-R., Kowbel, D.-J., Ellenton, J., 'Mutagenicity in Salmonella of fluorescent dye tablets used in water tracing', Water Research, 1980, Vol. 14(7), pp. 901-902.

NCBI National Center for Biotechnology Information. PubChem Compound Summary for CID 24414 – Barium Sulfate. Accessed October 2023, available from: https://pubchem.ncbi.nlm.nih.gov/compound/Barium-Sulfate

NTP National Toxicology Program, 'NTP Toxicology and Carcinogenesis Studies of Barium Chloride Dihydrate (CAS No. 10326-27-9) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies)', Natl Toxicol Program Tech Rep Ser, 1994, Vol. 432, pp. 1-285.

Oskarsson, A., 'Chapter 4 – Barium', In Nordberg, G.-F., Costa, M., (Eds.), 'Handbook on the Toxicology of Metals', Academic Press, 2021, Edition 5, pp. 91-100.

Pirie, R.-S., Jago, R.-C., Hudson, N.-P., 'Equine grass sickness', Equine veterinary journal, 2014, Vol. 46(5), pp. 545-553.

Safran, M., Wu, C.-F., Matys, R., Alex, S., Emerson, C.-H., 'The pharmacokinetics of thyrotropin-releasing hormone (TRH) and deamido-TRH in the rat', Endocrinology, 1984, Vol. 115(3), pp. 1031-1037.

SCHER Scientific Committee on Health and Environmental Risks, 'Assessment of the Tolerable Daily Intake of Barium', 2012. Accessed January 2024, available from: https://ec.europa.eu/health/scientific_committees/environmental_risks/docs/scher_o_161.pdf

Schroeder, H.-A., Tipton, I.-H., Nason, A.-P., 'Trace metals in man: strontium and barium', J Chronic Dis, 1972, Vol. 25(9), pp. 491-517.

Seto, C., Araie, M., Takase, M., 'Study of fluorescein glucuronide. II. A comparative ocular kinetic study of fluorescein and fluorescein glucuronide', Graefes Arch Clin Exp Ophthalmol, 1986, Vol. 224(2), pp. 113-117.

Shevchenko, K.-V., Vyunova, T.-V., Radilov, A.-S., Andreeva, L.-A., Nagaev, I.-Yu., Shevchenko, V.-P., Rembovsky, V.-R., Myasoedov, N.-F., 'Penetration of thyroliberin in the blood and brain regions at intranasal or intravenous administration', Dokl Biochem Biophys, 2015, Vol. 465, pp. 436-439.

Shaw, D.-D., Kido, D.-K., Stroshane, R.-M., Palace, G.-P., Sahler, L., 'Pharmacokinetics and excretion of iohexol after lumbar myelography in man', Invest Radiol, 1985, Vol. 20(6), pp. 632-637.

Sowden, E., Pirie, A., 'Barium and strontium concentrations in eye tissue', Biochem J, 1958, Vol. 70(4), pp. 716-717.

Thomas, R.-G., Ewing, W.-C., Catron, D.-L., McClellan, R.-O., 'In vivo solubility of four forms of barium determined by scanning techniques', Am Ind Hyg Assoc J, 1973, Vol. 34(8), pp. 350-359.

Tipton, I.-H., Cook, M.-J., 'Trace elements in human tissue. II. Adult subjects from the United States', Health Phys, 1963, Vol. 9, pp. 103-145.

Tipton, I.-H., Stewart, P.-L., Dickson, J., 'Patterns of elemental excretion in long term balance studies', Health Phys, 1969, Vol. 16(4), pp. 455-462.

UNEP United Nations Environment Programme, World Health Organization, International Labour Organisation, 'Barium – Environmental Health Criteria 107', 1990. Accessed November 2023, available from: https://wedocs.unep.org/20.500.11822/29454.

Widmer, W.-R., Blevins, W.-E., Jakovljevic, S., Levy, M., Teclaw, R.-F., Han, C.-M., Hurd, C.-D., 'A prospective clinical trial comparing metrizamide and iohexol for equine myelography', Vet Radiol Ultrasound, 1998, Vol. 39(2), pp. 106-109.

Williams, L.-B., Pinard, C.-L., 'Corneal ulcers in horses', Compend Contin Educ Vet, Jan 2013, Vol. 35(1), p. E4.

Wilson, K.-E., Wilcke, J.-R., Crisman, M.-V., Ward, D.-L., McKenzie, H.-C., Scarratt, W.-K, 'Comparison of serum iohexol clearance and plasma creatinine clearance in clinically normal horses', Am J Vet Res, 2009, Vol. 70(12), pp. 1545-1550.

Winter, M.-D., Berry, C.-R., Reese, D.-J., 'Nuclear scintigraphy in horses', Compend Contin Educ Vet, 2010, Vol. 32(12), p. E5.

Substances for gastrointestinal disorders

Agass, R.-F., Brennan, M., Rendle, D.-I., 'Extrapyramidal side effects following subcutaneous metoclopramide injection for the treatment of post operative ileus', Equine Veterinary Education, 2016, Vol. 29(10), pp. 564-568.

Alexander, F., 'The effect of some anti-diarrhoeal drugs on intestinal transit and faecal excretion of water and electrolytes in the horse', Equine Vet J, 1978, Vol. 10(4), pp. 229-234.

Allen, J.-C., Gralla, R., Reilly, L., Kellick, M., Young, C., 'Metoclopramide: dose-related toxicity and preliminary antiemetic studies in children receiving cancer chemotherapy', J Clin Oncol, 1985, Vol. 3(8), pp. 1136-1141.

Ammer, H., Potschka, H., 'Pharmakologie des zentralen Nervensystems (ZNS)', In Frey, H.-H., Löscher, W., (Eds.), 'Lehrbuch der Pharmakologie und Toxikologie für die Veterinärmedizin', Enke Verlag, Stuttgart, Edition 3, 2010, pp. 115-166.

Baker, W.-T., Frederick, J., Giguere, S., Lynch, T.-M., Lehmkuhl, H.-D., Slone, D.-E., 'Reevaluation of the effect of phenylephrine on resolution of nephrosplenic entrapment by the rolling procedure in 87 horses', Vet Surg, Oct 2011, Vol. 40(7), pp. 825-829.

Banse, H., Andrews, F.-M., 'Equine glandular gastric disease: prevalence, impact and management strategies', Vet Med (Auckl), 2019, Vol. 10, pp. 69-76.

Barr, B.-S., Wilkins, P.-A., Del Piero, F., Palmer, J.-E., 'Is prophylaxis for gastric ulcers necessary in critically ill equine neonates? A retrospective study of necropsy cases 1995-1999', Proceedings 18th Ann Meet Vet Med Forum, 2000, p. 705.

Barr, B., 'Gastric Ulcer Prophylaxis in the Critically Ill Equine Neonate', In Wilkins, P.-A., (Ed.), 'Recent Advances in Equine Neonatal Care', International Veterinary Information Service, 2001.

Bateman, D.-N., Kahn, C., Davies, D.-S., 'The pharmacokinetics of metoclopramide in man with observations in the dog', Br J Clin Pharmacol, 1980, Vol. 9(4), pp. 371-377.

Bauer, M., Barna, S., Blaickner, M., Prosenz, K., Bamminger, K., Pichler, V., Tournier, N., Hacker, M., Zeitlinger, M., Karanikas, G., Langer, O., 'Human Biodistribution and Radiation Dosimetry of the P-Glycoprotein Radiotracer [11C]Metoclopramide, Mol Imaging Biol, 2021, Vol. 23(2), pp. 180-185.

Bednarski, R.-M., 'Tracheal and Nasal Intubation', in Muir, W.-W., Hubbell, J.-A.-E., (Eds.), 'Equine Anesthesia', W.B. Saunders, 2009, Edition 2, pp. 277-287.

Bhandari, M., Bhandari, A., Bhandari, A., 'Recent updates on codeine', Pharm Methods, 2011, Vol. 2(1), pp. 3-8.

Bolser, D.-C., Hey, J.-A., Chapman, R.-W., 'Influence of central antitussive drugs on the cough motor pattern', J Appl Physiol (1985), 1999, Vol. 86(3), pp. 1017-1024.

Brown, J.-H., Taylor, P., 'Muscarinic Receptor Agonists and Antagonists', In Hardman, J.-G., Limbird, L.-E., Gilman, A.-G., (Eds.), 'Goodman and Gilman's the pharmacological basis of therapeutics', McGraw-Hill Professional, New York, Edition 10, 2001, pp. 155-173.

Burgess, E., 'Aluminium absorption following sucralfate therapy', Br J Clin Pharmacol, 1991, Vol. 32(5), pp. 642.

Candelli, M., Carloni, E., Armuzzi, A., Cammarota, G., Ojetti, V., Pignataro, G., Santoliquido, A., Pola, R., Pola, E., Gasbarrini, G., Gasbarrini, A., 'Role of sucralfate in gastrointestinal diseases', Panminerva Med, 2000, Vol. 42(1), pp. 55-9.

Dajani, E.-Z., Driskill, D.-R., 'Effect of oral and intravenous propantheline bromide on pentagastrin-stimulated canine gastric secretion', Arch Int Pharmacodyn Ther, 1978, Vol. 233(2), pp. 243-250.

Davis, J.-L. 'Nonsteroidal anti-inflammatory drug associated right dorsal colitis in the horse', Equine Veterinary Education, 2015, Vol. 29(2), pp. 104-113.

Davies, N.-M., Longstreth, J., Jamali, F., 'Misoprostol therapeutics revisited', Pharmacotherapy, 2001, Vol. 21(1), pp. 60-73.

ECHA, Registration Dossier – Metoclopramide. Accessed December 2023, available from:https://echa.europa.eu/registration-dossier/-/registered-dossier/21028/7/7/1

Eddershaw, P.-J., Chadwick, A.-P., Higton, D.-M., Fenwick, S.-H., Linacre, P., Jenner, W.-N., Bell, J.-A., Manchee, G.-R., 'Absorption and disposition of ranitidine hydrochloride in rat and dog', Xenobiotica, 1996, Vol. 26(9), pp. 947-956.

EMA/13239/2014 Corr.1, 'European Medicines Agency recommends changes to the use of metoclopramide', available from: Metoclopramide-containing medicines – referral | European Medicines Agency (europa.eu)

EMA/231394/2020. Rev.1, 'Suspension of ranitidine medicines in the EU', available from: Ranitidine-containing medicinal products – referral | European Medicines Agency (europa.eu)

EMA/235820/2015, 'Codeine containing medicinal products for the treatment of cough and/or cold in paediatric patients. Assessment Report', available from: Codeine-containing medicinal products for the treatment of cough or cold in paediatric patients – referral | European Medicines Agency (europa.eu)

Forth, W., Rummel, W., 'Pharmaka zur Beeinflussung der Funktionen von Magen, Dünn- und Dickdarm', In Forth, W., (Ed.) 'Allgemeine und spezielle Pharmakologie und Toxikologie', Spektrum akademischer Verlag, Heidelberg, 1998, Edition 7, pp. 513-539.

Fox, D.-A., Bauer, R., Bass. P., 'Use of gut cyclic motor activity to evaluate a stimulant (narcotic) and inhibitor (anticholinergic) or gastrointestinal-tract activity in the unanesthetized dog', J Pharmacol Methods, 1985, Vol. 13(2), pp. 147-155.

Frang, H., Cockcroft, V., Karskela, T., Scheinin, M., Marjamäki, A., 'Phenoxybenzamine binding reveals the helical orientation of the third transmembrane domain of adrenergic receptors', J Biol Chem, 2001, Vol. 276(33), pp. 31279-31284.

Frez, C., Awad, Y., Sánchez, N., Silva, A., Mercado, B., Marcotti, C., Arévalo, P., Apablaza, K., Fernández, P., Pérez, D., 'Farmacovigilancia activa de metoclopramida 10 mg oral en pacientes del Programa de Alivido del Dolor y Cuidados Paliativos o diabetes mellitus tipo 2 en cinco hospitales del Servicio de Salud Viña del Mar-Quillota', Rev Med Chile, 2018, Vol. 146, pp. 876-884.

Fultz, L.-E., Peloso, J.-G., Giguère, S., Adams, A.-R., 'Comparison of phenylephrine administration and exercise versus phenylephrine administration and a rolling procedure for the correction of nephrosplenic entrapment of the large colon in horses: 88 cases (2004-2010)', J Am Vet Med Assoc, Apr 2013, Vol. 242(8), pp. 1146-1151.

Garnett, W.-R., 'Sucralfate–alternative therapy for peptic-ulcer disease', Clin Pharm, 1982, Vol. 1(4), pp. 307-314.

Ge, S., Mendley, S.-R., Gerhart, J.-G., Melloni, C., Hornik, C.-P., Sullivan, J.-E., Atz, A., Delmore, P., Tremoulet, A., Harper, B., Payne, E., Lin, S., Erinjeri, J., Cohen-Wolkowiez, M., Gonzalez, D., Best Pharmaceuticals for Children Act – Pediatric Trials Network Steering Committee, 'Population Pharmacokinetics of Metoclopramide in Infants, Children, and Adolescents', Clin Transl Sci, 2020, Vol. 13(6), pp. 1189-1198.

Geyer, J., Herling, A.-W., 'Pharmakologie der Verdauung', In Löscher, W., Richter, A., (Eds.), 'Lehrbuch der Pharmakologie und Toxikologie für die Veterinärmedizin', Enke Verlag, Stuttgart, Edition 4, 2016, p. 656.

Haruta, S., Iwasaki, N., Ogawara. K., Higaki, K., Kimura, T., 'Absorption behavior of orally administered drugs in rats treated with propantheline', J Pharm Sci, 1998, Vol. 87(9), pp. 1081-1085.

Hinchcliff, K.-W., McKeever, K.-H., Muir, W.-W., 'Hemodynamic effects of atropine, dobutamine, nitroprusside, phenylephrine, and propranolol in conscious horses', J Vet Intern Med, 1991, Vol. 5(2), pp. 80-86.

Hjemdahl, P., Belfrage, E., Daleskog, M., 'Vascular and metabolic effects of circulating epinephrine and norepinephrine. Concentration-effect study in dogs', J Clin Invest, 1979, Vol. 64(5), pp. 1221-1228.

Holland, P.-S., Brumbaugh, G.-W., Ruoff, W.-W., Brown, S.-A., 'Plasma pharmacokinetics of ranitidine HCl in foals', J Vet Pharmacol Therap, 1997a, Vol. 20, pp. 447-452.

Holland, P.-S., Ruoff, W.-W., Brumbaugh, G.-W., Brown, S.-A., 'Plasma pharmacokinetics of ranitidine HCl in adult horses', J Vet Pharmacol Therap, 1997b, Vol. 20, pp. 145-152.

Huhn, J.-C., Koritz, G.-D., Nelson, D.-R., Brown, S.-A., 'Pharmacokinetics of metoclopramide in goats', J Vet Pharmacol Ther, 1992, Vol. 15(1), pp. 19-27.

Katori, N., Aoyagi, N., Kojima, S., 'Effects of codeine on the agitating force and gastrointestinal transit time in dogs, for use in drug absorption studies', Biol Pharm Bull, 1998, Vol. 21(4), pp. 418-420.

Koenig, J., Cote, N., 'Equine gastrointestinal motility—ileus and pharmacological modification', Can Vet J, 2006, Vol. 47(6), pp. 551-559.

Krugh, M., Maani, C.-V., 'Misoprostol', StatPearls. Treasure Island (FL), 2023. Accessed September 2023, available from: https://www.ncbi.nlm.nih.gov/books/NBK539873/

Kudaravalli, P., John, S., 'Sucralfate', StatPearls, Treasure Island (FL), 2022. Accessed November 2023, available from: https://www.ncbi.nlm.nih.gov/books/NBK551527/

Lau Moon Lin, M., Robinson, P.-D., Flank, J., Sung, L., Lee Dupuis, L., 'The Safety of Metoclopramide in Children: A Systematic Review and Meta-Analysis', Drug Saf, 2016, Vol. 39(7), pp. 675-687.

Lee, A., Kuo, B., 'Metoclopramide in the treatment of diabetic gastroparesis', Expert Rev Endocrinol Metab, 2010, Vol. 5(5), pp. 653-662.

Lopp, C.-T., McCoy, A.-M., Boothe, D., Schaeffer, D.-J., Lascola, K., 'Single-dose pharmacokinetics of orally and rectally administered misoprostol in adult horses', Am J Vet Res, 2019, Vol. 80(11), pp. 1026-1033.

Löscher, W., Bankstahl, M., 'Pharmakologie des vegetativen (autonomen) Nervensystems', In Löscher, W., Richter, A., (Eds.), 'Lehrbuch der Pharmakologie und Toxikologie für die Veterinärmedizin', Enke Verlag, Stuttgart, 2016, Edition 4, pp. 33-86.

Madani, S., Kauffman, R., Simpson, P., Lehr, V.-T., Lai, M.-L., Sarniak, A., Tolia, V., 'Pharmacokinetics and pharmacodynamics of famotidine and ranitidine in critically ill children', J Clin Pharmacol, 2014, Vol. 54(2), pp. 201-205.

Martin, E.-M., Till, R.-L., Sheats, M.-K., Jones, S.-L., 'Misoprostol Inhibits Equine Neutrophil Adhesion, Migration, and Respiratory Burst in an In Vitro Model of Inflammation', Front Vet Sci, 2017, Vol. 4, p. 159.

Martin, E.-M., Schirmer, J.-M., Jones, S.-L., Davis, J.-L., 'Pharmacokinetics and ex vivo anti-inflammatory effects of oral misoprostol in horses', Equine Vet J, 2019, Vol. 51(3), pp. 415-421.

McEvoy, G.-K., AHFS Drug Information. American Society of Hospital Pharmacists Inc, Bethesda (USA), 1992, p. 2363.

Nagashima, R., 'Development and characteristics of sucralfate', J Clin Gastroenterol, 1981, Vol. 3(Suppl 2), pp. 103-110.

National Specialized Commission on Maternal Mortality (Coordinator), National Specialized Commission on Fetal Medicine, National Specialized Commission on Childbirth Puerperium and Abortion Care, National Specialized Commission on Sexual Violence and Pregnancy Interruption Provided for by Law, National Specialized Commission on Antenatal Care, National Specialized Commission on High-Risk Pregnancy, National Specialized Commission on Urgency and Emergency, National Specialized Commission on Professional Defense and Appreciation, 'Revision. Misoprostol use in obstetrics', Rev Bras Ginecol Obstet, Jun 2023, Vol. 45(6), pp. 356-368.

Nicolson, R., Craven-Thuss, B., Smith, J., McKinlay, B.-D., Castellanos, F.-X., 'A randomized, double-blind, placebo-controlled trial of metoclopramide for the treatment of Tourette's disorder', J Am Acad Child Adolesc Psychiatry, 2005, Vol. 44(7), pp. 640-646.

Padda, I.-S., Derian, A., 'Bethanechol', StatPearls, Treasure Island (FL), 2023. Accessed September 2023, available from: https://www.ncbi.nlm.nih.gov/books/NBK560587/

Pannemans, J., Corsetti, M., 'Opioid receptors in the GI tract: targets for treatment of both diarrhea and constipation in functional bowel disorders?', Curr Opin Pharmacol, 2018, Vol. 43, pp. 53-58.

Pastuszak, A.-L., Schüler, L., Speck-Martins, C.-E., Coelho, K.-E., Cordello, S.-M., Vargas, F., Brunoni, D., Schwarz, I.-V., Larrandaburu, M., Safattle, H., Meloni, V.-F., Koren, G., 'Use of misoprostol during pregnancy and Möbius' syndrome in infants', N Engl J Med, 1998, Vol. 338(26), pp. 1881-1885.

Plumb D.-C., 'Plumb's Veterinary Drug Handbook', Wiley-Blackwell, Edition 8, 2015, p. 929.

Plumb, D.-C., 'Plumb's Veterinary Drug Handbook, PharmaVet Publishing, White Bear Lake (USA), 2023, p. 1568.

Ramsey, I., 'BSAVA small animal formulary', British Small Animal Veterinary Association, Quedgeley, Gloucester (UK), Edition 6, 2008, p. 404.

Roberts, C.-J.-C., 'Clinical Pharmacokinetics of ranitidine', Clin Pharmacokinet, 1984, Vol. 9(3), pp. 211-221.

Sahi, N., Nguyen, R., Santos, C., 'Loperamide', StatPearls, Treasure Island (FL), 2023. Accessed September 2023, available from: https://www.ncbi.nlm.nih.gov/books/NBK557885/

Sanchez, L.-C., Lester, G.-D., Merritt, A.-M., 'Intragastric pH in critically ill neonatal foals and the effect of ranitidine', J Am Vet Med Assoc, 2001, Vol. 218, pp. 907-911.

Schaefer, U., Höchner, P., Karrer, C., 'Arzneimittelkompendium der Schweiz', Documed AG, Basel (CH), Edition 34, 2013, p. 4992.

Scriba, G.-K.-E., In Sweetman C., (Eds.), 'Martindale: The Complete Drug Reference', Pharmaceutical Press, London, 2009, Edition 36.

Tang, O.-S., Gemzell-Danielsson, K., Ho, P.-C., 'Misoprostol: pharmacokinetic profiles, effects on the uterus and side-effects', Int J Gynaecol Obstet, 2007, Vol. 99(2), pp. S160-S167.

Takayasu, M., Hamamoto, K., Satoh, H., Ichijo, T., Takahashi, T., Furuhama, K., 'Pharmacokinetics of metoclopramide in calves with renal dysfunction', J Vet Med Sci, 2015, Vol. 77(2), pp. 261-263.

Van den Boom, R., 'Equine gastric ulcer syndrome in adult horses', Vet J, 2022, Vol. 283-284.

Van Hecken, A.-M., Tjandramaga, T.-B., Mullie, A., Verbesselt, R., de Schepper, P.-J., 'Ranitidine: single dose pharmacokinetics and absolute bioavailability in man', Br J Clin Pharmacol, 1982, Vol. 14(2), pp. 195-200.

Varley, G., Bowen, I.-M., Habershon-Butcher, J.-L., Nicholls, V., Hallowell, G.-D., 'Misoprostol is superior to combined omeprazole-sucralfate for the treatment of equine gastric glandular disease', Equine Vet J, 2019, Vol. 51(5), pp. 575-580.

Wang, S., Wu, F., Han, Y., Ni, S., Guo, S., Dai, Y., Xia, Q., Chang, D., Zhang, J., Wei, H., Zhao, X., 'Pharmacokinetics and Bioequivalence of Misoprostol Tablets: An Open-Label, Randomized, Single-dose, Crossover Study With Healthy Chinese Volunteers', Clin Pharmacol Drug Dev, 2022, Vol. 11(8), pp. 949-956.

Watcha, M.-F., White, P.-F., 'Postoperative nausea and vomiting. Its etiology, treatment, and prevention', Anesthesiology, 1992, Vol. 77(1), pp. 162-184.

Wilkins, P., Palmer, J., Rood, 'Gastric Ulcer Prophylaxis in the Critically Ill Equine Neonate', Recent Advances in Equine Neonatal Care, 2001.

Yoham, A.-L., Casadesus, D., 'Phenoxybenzamine', StatPearls, Treasure Island (FL), 2022. Accessed August 2023, available from: https://www.ncbi.nlm.nih.gov/books/NBK560667/

Substances for metabolic disorders

Aleman, M., Watson, J.-L., Williams, D.-C., LeCouteur, R.-A., Nieto, J.-E., Shelton, G.-D., 'Myopathy in horses with pituitary pars intermedia dysfunction (Cushing's disease)', Neuromuscul Disord, 2006, Vol. 16, pp. 737-744.

Allen, W.-R., Sanderson, M.-W., Greenwood, R.-E., Ellis, D.-R., Crowhurst, J.-S., Simpson, D.-J., Rossdale, P.-D., 'Induction of ovulation in anoestrous mares with a slow-release implant of a GnRH analogue (ICI 118 630)', J Reprod Fertil Suppl, 1987, Vol. 35, pp. 469-478.

ANSES. Opinion of the French Agency for Food, Environmental and Occupational Health & Safety on the "Request concerning extension of the list of substances with additional clinical benefit to other substances of interest in equine medicine", 2016, available from: https://www.anses.fr/en/system/files/MV2015SA0104EN.pdf

Asplin, K., Sillence, M., Pollitt, C., McGowan, K., 'Induction of laminitis by prolonged hyperinsulinaemia in clinically normal ponies', The Veterinary Journal, 2007, Vol. 174, pp. 530–535.

Blin, O., 'The pharmacokinetics of pergolide in Parkinson's disease', Curr Opin Neurol, 2003, Vol. 16(1), p. S9-12.

Buelke-Sam, J., Byrd, R.-A., Johnson, J.-A., Tizzano, J.-P., Owen, N.-V., 'Developmental toxicity of the dopamine agonist pergolide mesylate in CD-1 mice. I: Gestational exposure', Neurotoxicol Teratol, 1991a, Vol. 13(3), pp. 283-295.

Buelke-Sam, J., Cohen, I.-R., Tizzano, J.-P., Owen, N.-V., 'Developmental toxicity of the dopamine agonist pergolide mesylate in CD-1 mice. II: Perinatal and postnatal exposure', Neurotoxicol Teratol, 1991b, Vol. 13(3), pp. 297-306.

Chao, E.-C., Henry, R.-R., 'SGLT2 inhibition – a novel strategy for diabetes treatment', Nature reviews drug discovery, 2010, Vol. 9(7), pp. 551-559.

Chen, M., Suzuki, A., Thakkar, S., Yu, K., Hu, C., Tong, W., 'DILIrank: the largest reference drug list ranked by the risk for developing drug-induced liver injury in humans', Drug Discov Today, 2016, Vol. 21(4), pp. 648-653.

Clemens, J.-A., Okimura, T., Smalstig, E.-B., 'Dopamine agonist activities of pergolide, its metabolites, and bromocriptine as measured by prolactin inhibition, compulsive turning, and stereotypic behavior', Arzneimittelforschung, 1993, Vol. 43(3), pp. 281-286.

De Laat, M., McGree, J., Sillence, M., 'Equine hyperinsulinemia: investigation of the enteroinsular axis during insulin dysregulation', American Journal of Physiology, Endocrinology and Metabolism, 2015, Vol. 310, pp. E61–E71.

Donaldson M, LaMonte B, Morresey P, Smith G, Beech J., 'Treatment with pergolide or cyproheptadine of pituitary pars intermedia dysfunction (equine cushing's disease)', Journal of Veterinary Internal Medicine, 2002, Vol. 16, pp. 742–746.

Dunbar, L.-K., Mielnicki, K.-A., Dembek, K.-A., Toribio, R.-E., Burns, T.-A., 'Evaluation of four diagnostic tests for insulin dysregulation in adult light-breed horses', Journal of veterinary internal medicine, 2016, Vol. 30(3), pp. 885-891.

Durham, A.-E., Frank, N., McGowan, C.-M., Menzies-Gow, N.-J., Roelfsema, E., Vervuert, I, Feige, K., Fey, K., 'ECEIM consensus statement on equine metabolic syndrome', J Vet Intern Med, 2019, Vol. 33(2), pp. 335-349.

Durham, A.-E., Thiemann, A.-K., 'Nutritional management of hyperlipaemia', Equine Vet Educ, 2015, Vol. 27(9), pp. 482-488.

ECHA, Substance Info Card – Pergolide. Accessed February 2024, available from: https://echa.europa.eu/de/substance-information/-/substanceinfo/100.241.322

Elangbam, C.-S., 'Drug-induced valvulopathy: an update', Toxicol Pathol, 2010, Vol. 38(6), pp. 837-848.

EMEA/CHMP/322395/2008, 'EMEA recommends new warnings and contraindications for ergot-derived dopamine agonists'.

FDA Food and Drug Administration. Drug Induced Liver Injury Rank (DILIrank) Dataset. Accessed March 2024a, available from: https://www.fda.gov/science-research/liver-toxicity-knowledge-base-ltkb/drug-induced-liver-injury-rank-dilirank-dataset

FDA Food and Drug Administration. Postmarket Drug Safety Information for Patients and Providers – Pergolide Information. Accessed March 2024b, available from: https://www.fda.gov/drugs/postmarket-drug-safety-information-patients-and-providers/index-drug-specific-information

Fitzgerald, B.-P., Peterson, K.-D., Silvia, P.-J., 'Effect of constant administration of a gonadotropin-releasing hormone agonist on reproductive activity in mares: preliminary evidence on suppression of ovulation during the breeding season', Am J Vet Res, 1993, Vol. 54(10), pp. 1746-1751.

Francis, P.-C., Carlson, K.-H., Owen, N.-V., Adams, E.-R., 'Preclinical toxicology studies with the new dopamine agonist pergolide. Acute, subchronic, and chronic evaluations', Arzneimittelforschung, 1994, Vol. 44(3), pp. 278-284.

Frank, N., Tadros, E., 'Insulin dysregulation', Equine Veterinary Journal, 2014, Vol. 46, pp. 103–112.

Frank, N., Bailey, S., Bertin, F.-R., Burns, T., de Laat, M., Durham, A., Kritchevsky, J., Menzies-Gow, N., 'Recommendations for the Diagnosis and Management of Equine Metabolic Syndrome (EMS)', Equine Endocrinology Group, 2022. Accessed April 2024, available from: https://sites.tufts.edu/equineendogroup/files/2022/10/EMS-EEG-Recommendations-2022.pdf

Frank, N., Thane, K., Voth, R., Klee, R., Warnken, T., 'Effects of the sodium-glucose cotransporter-2 inhibitor velagliflozin on insulin concentrations in horses with insulin dysregulation', ACVIM Forum Research Report Program, J Vet Intern Med, 2023, Vol. 37, pp. 2638-2660.

Freestone, J., Beadle, R., Shoemaker, K., Bessin, R., Wolfsheimer, K., Church, C., 'Improved insulin sensitivity in hyperinsulinaemic ponies through physical conditioning and controlled feed intake', Equine Veterinary Journal, 1992, Vol. 24, pp. 187–190.

Gehring, R., Beard, L., Wright, A., Coetzee, J., Havel, J., Apley, M., 'Single-dose oral pharmacokinetics of pergolide mesylate in healthy adult mares', Vet Ther, 2010, Vol. 11(1), pp. E1-8.

Hastwell, P.-W., Webster, T.-W., Tate, M., Billinton, N., Lynch, A.-M., Harvey, J.-S., Rees, R.-W., Walmsley, R.-M., 'Analysis of 75 marketed pharmaceuticals using the GADD45a-GFP 'GreenScreen HC' genotoxicity assay', Mutagenesis, 2009, Vol. 24(5), pp. 455-463.

Hofmann, C., Penner, U., Dorow, R., Pertz, H.-H., Jähnichen, S., Horowski, R., Latté, K.-P., Palla, D., Schurad, B., 'Lisuride, a dopamine receptor agonist with 5-HT2B receptor antagonist properties: absence of cardiac valvulopathy adverse drug reaction reports supports the concept of a crucial role for 5-HT2B receptor agonism in cardiac valvular fibrosis', Clin Neuropharmacol, 2006, Vol. 29(2), pp. 80-86.

Hoyt, J.-A., Byrd, R.-A., Owen, N.-V., 'Reproductive and developmental toxicity of the dopamine agonist pergolide mesylate in mice', Arzneimittelforschung, 1994, Vol. 44(11), pp. 1177-1783.

Hughes, K.-J., Hodgson, D.-R, Dart, A.-J., 'Equine Hyperlipaemia: A review', Aus Vet J, 2004, Vol. 82, pp. 136-142.

Jeanty, P., Van den Kerchove, M., Lowenthal, A., De Bruyne, H., 'Pergolide therapy in Parkinson's disease', J Neurol, 1984, Vol. 231(3), pp. 148-152.

Kirkwood, N.-C., Kristopher, J.-H., Stewart, A.-J., 'Pituitary Pars Intermedia Dysfunction (PPID) in Horses', Vet Sci, 2022, Vol. 9, p. 556.

LiverTox, Clinical and Research Information on Drug-Induced Liver Injury – Pergolide, July 2017, available from: https://www.ncbi.nlm.nih.gov/books/NBK548593/

Lund, S.-S., Tarnow, L., Stehouwer, C.-D.-A., Schalkwijk, C.-G., Frandsen, M., Smidt, U.-M., Pedersen, O., Parving, H.-H., Vaag, A., 'Targeting hyperglycaemia with either metformin or repaglinide in non-obese patients with type 2 diabetes: results from a randomized crossover trial', Diabetes Obes Metab, 2007, Vol. 9(3), pp. 394-407.

McFarlane, D., Banse, H., Knych, H.-K., Maxwell, L.-K., 'Pharmacokinetic and pharmacodynamic properties of pergolide mesylate following long-term administration to horses with pituitary pars intermedia dysfunction', J Vet Pharmacol Ther, 2017, Vol. 40(2), pp. 158-164.

McGowan, C., Frost, R., Pfeiffer, D., Neiger, R., 'Serum insulin concentrations in horses with equine Cushing's syndrome: response to a cortisol inhibitor and prognostic value', Equine Veterinary Journal, 2004, Vol. 36, pp. 295–298.

Meier, A., Reiche, D., de Laat, M., Pollitt, C., Walsh, D., Sillence, M., 'The sodium-glucose co-transporter 2 inhibitor velagliflozin reduces hyperinsulinemia and prevents laminitis in insulin-dysregulated ponies', PLOS ONE, 2018, Vol. 13(9), p. e0203655.

Meier, A., de Laat, M., Reiche, D., Fitzgerald, D., Sillence, M. 'The efficacy and safety of velagliflozin over 16 weeks as a treatment for insulin dysregulation in ponies', BMC Vet Res, 2019, Vol. 15, p. 65.

Mendoza, F.-J., Serrano-Rodriguez, J.-M., Buzon-Cuevas, A., Perez-Ecija, A., 'Pharmacokinetics of the anti-androgenic drug flutamide in healthy stallions', Vet J, 2017, Vol. 224, pp. 50-54.

Menzies-Gow, N., 'Endocrinopathic laminitis: reducing the risk through diet and exercise', Veterinary Clinics of North America: Equine Practice, 2010, Vol. 26, pp. 371–378.

Mumford, E.-L., Squires, E.-L., Peterson, K.-D., Nett, T.-M., Jasko, D.-J., 'Effect of various doses of a gonadotropin-releasing hormone analogue on induction of ovulation in anestrous mares', J Anim Sci, 1994, Vol. 72(1), pp. 178-183.

Papich, M.-G., (Ed.), 'Papich Handbook of Veterinary medicine', Elsevier, Edition 5 2021, pp. 458-462.

Plumb, D.-C., (Ed.), 'Plumb's Veterinary Drug Handbook, Wiley-Blackwell, Edition 7, 2011.

Plumb, D.-C., (Ed.), 'Plumb's Veterinary Drug Handbook', Wiley-Blackwell, Edition 8, 2015, pp. 552-558.

Powell, D., Reedy, S., Sessions, D., Fitzgerald, B., 'Effect of short-term exercise training on insulin sensitivity in obese and lean mares', Equine Veterinary Journal, 2010, Vol. 34, pp. 81–84.

Rendle, D.-I., Hughes, K.-J., Doran, G.-S., Edwards, S.-H., 'Pharmacokinetics of pergolide after intravenous administration to horses', Am J Vet Res, 2015, Vol. 76(2), pp. 155-160.

Rendle, D.-I., Rutledge, F., Hughes, K.-J., Heller, J., Durham, A.-E., 'Effects of metformin hydrochloride on blood glucose and insulin responses to oral dextrose in horses', Equine Vet J, 2013, Vol. 45(6), pp. 751-754.

Rendle, D., McGregor Argo, C., Bowen, M., Carslake, H., German, A., Harris, P., Knowles, E., Menzies-Gow, N., Morgan, R., 'Equine obesity: current perspectives', UK-Vet Equine, 2018, Vol. 2(5), pp. 1-19.

Robin, D.-W., 'Pergolide in the treatment of Parkinson's disease', Am J Med Sci, 1991, Vol. 301(4), pp. 277-280.

Rubin, A., Lemberger, L., Dhahir, P., 'Physiologic disposition of pergolide', Clin Pharmacol Ther, 1981, Vol. 30(2), pp. 258-265.

Schönert, S., Reher, M., Gruber, A.-G., Carstanjen, B., 'Use of a deslorelin implant for influencing sex hormones and male behaviour in a stallion – Case report', Acta Vet Hung, 2012, Vol. 60(4), pp. 511-519.

Sundra, T., Kelty, E., Rendle, D., 'Preliminary observations on the use of ertugliflozin in the management of hyperinsulinaemia and laminitis in 51 horses: A case series', Equine Veterinary Education, 2023a, Vol. 35, pp. 311-320.

Sundra, T., Rossi, G., Kelty, E., Lester, G., Rendle, D., 'Oral sugar test responses to ertugliflozin in ten horses with insulin dysregulation', Equine Veterinary Education, 2023b, Vol. 36(6), pp. 317-324.

Tatum, R.-C., McGowan, C.-M., Ireland, J.-L., 'Efficacy of pergolide for the management of equine pituitary pars intermedia dysfunction: A systematic review', The Veterinary Journal, Dec 2020, Vol. 266, p. 10556.

Thalamas, C., Rajman, I., Kulisevsky, J., Lledó, A., Mackie, A.-E., Blin, O., Gillespie, T.-A., Seger, M., Rascol, O., 'Pergolide: multiple-dose pharmacokinetics in patients with mild to moderate Parkinson disease', Clin Neuropharmacol, 2005, Vol. 28(3), pp. 120-125.

Vallon, V., Thomson, S.-C., 'Targeting renal glucose reabsorption to treat hyperglycaemia: the pleiotropic effects of SGLT2 inhibition', Diabetologia, 2017, Vol. 60(2), pp. 215-225.

Viollet, B., Guigas, B., Sanz Garcia, N., Leclerc, J., Foretz, M., Andreelli, F., 'Cellular and molecular mechanisms of metformin: an overview', Clin Sci (Lond), 2012, Vol. 122(6), pp. 253-270.

Wong, C.-Y., Martinez, J., Dass, C.-R, 'Oral delivery of insulin for treatment of diabetes: status quo, challenges and opportunities', J Pharm Pharmacol, Sep 2016, Vol. 68(9), pp. 1093-1108.

Wright, A.-M., 'Pharmacokinetics of pergolide in normal mares', MSc Thesis, Kansas State University, 2009.

Substances for musculoskeletal disorders

Adams, W.-A., Robinson, K.-J., Mark Senior, J., Jones, R.-S., 'The use of the nondepolarizing neuromuscular blocking drug cis-atracurium in dogs', Vet Anaesth Analg, 2001, Vol. 28(3), pp. 156-160.

Aleman, M., Riehl, J., Aldridge, B.-M., Lecouteur, R.-A., Stott, J.-L., Pessah, I.-N., 'Association of a mutation in the ryanodine receptor 1 gene with equine malignant hyperthermia', Muscle Nerve, Sep 2004, Vol. 30(3), pp. 356-365.

Aleman, M., 'A review of equine muscle disorders', Neuromuscul Disord, Apr 2008, Vol. 18(4), pp. 277-87.

Aleman, M., Nieto, J.-E., Magdesian, K.-G., 'Malignant hyperthermia associated with ryanodine receptor 1 (C7360G) mutation in Quarter Horses', J Vet Intern Med, 2009, Vol. 23(2), pp. 329-334.

Allen, G.-C., Cattran, C.-B., Peterson, R.-G., Lalande, M., 'Plasma levels of dantrolene following oral administration in malignant hyperthermia-susceptible patients', Anesthesiology, Dec 1988, Vol. 69(6), pp. 900-904.

Alves, J.-C., Dos Santos, A.-M., Fernandes, Â.-D., 'Evaluation of the effect of mesotherapy in the management of back pain in police working dog', Vet Anaesth Analg, 2018, Vol. 45(1), pp. 123-128.

Alves, J.-C., Santos, A.-M., 'Evaluation of the Effect of Mesotherapy in the Management of Osteoarthritis-Related Pain in a Police Working Dog Using the Canine Brief Pain Inventory', Top Companion Anim Med, 2017, Vol. 32(1), pp. 41-43.

Auer, U., Moens, Y., 'Neuromuscular blockade with rocuronium bromide for ophthalmic surgery in horses', Vet Ophthalmol, 2011, Vol. 14(4), pp. 244-247.

Auer, U., Uray, C., Mosing, M., 'Observations on the muscle relaxant rocuronium bromide in the horse–a dose–response study', Vet Anaesth Analg, 2007, Vol. 34(2), pp. 75-81.

Boyd, A.-H., Eastwood, N.-B., Parker, C.-J., Hunter, J.-M., 'Comparison of the pharmacodynamics and pharmacokinetics of an infusion of cis-atracurium (51W89) or atracurium in critically ill patients undergoing mechanical ventilation in an intensive therapy unit', Br J Anaesth, 1996, Vol. 76(3), pp. 382-388.

Brito, R., Costa, D., Dias, C., Cruz, P., Barros, P., 'Chondroitin Sulfate Supplements for Osteoarthritis: A Critical Review', Cureus, Jun 2023, Vol. 15(6), p. e40192.

Buck, M.-L., Reed, M.-D., 'Use of nondepolarizing neuromuscular blocking agents in mechanically ventilated patients', Clin Pharm, 1991, Vol. 10(1), pp. 32-48.

Chapple, D.-J., Miller, A.-A., Ward, J.-B., Wheatley, P.-L., 'Cardiovascular and neurological effects of laudanosine. Studies in mice and rats, and in conscious and anaesthetized dogs', Br J Anaesth, 1987, Vol. 59(2), pp. 218-225.

Chen, C., Yamaguchi, N., Varin, F., 'Studies on the pharmacokinetics of cisatracurium in anesthetized dogs: in vitro-in vivo correlations', J Vet Pharmacol Ther, 2009, Vol. 32(6), pp. 571-576.

Chow, B., Bowden, M.-I., Ho, E., Weatherley, B.-C., Bion, J.-F., 'Pharmacokinetics and dynamics of atracurium infusions after paediatric orthotopic liver transplantation', Br J Anaesth, 2000, Vol. 85(6), pp. 850-855.

Clar, D.-T., Liu, M., 'Nondepolarizing Neuromuscular Blockers', StatPearls, Treasure Island (FL), July 2023, available from: https://www.ncbi.nlm.nih.gov/books/NBK534828/

Court, M.-H., Engelking, L.-R., Dodman, N.-H., Answer, M.-S., Seeler, D.-C., Clark, M., 'Pharmacokinetics of dantrolene sodium in horses', J Vet Pharmacol Ther, Sep 1987, Vol. 10(3), pp. 218-226.

De Amorim, M.-F.-C., Vago, P.-B., Gomes, M.-C., Silva, A.-M., 'Deformidade flexural em potro' [Flexural deformity in foal], Ciência Animal, 2020, Vol. 30(Suppl. 1), pp. 9-15.

Dear, G.-J., Harrelson, J.-C., Jones, A.-E., Johnson, T.-E., Pleasance, S., 'Identification of urinary and biliary conjugated metabolites of the neuromuscular blocker 51W89 by liquid chromatography/mass spectrometry', Rapid Commun Mass Spectrom, 1995, Vol. 9(14), pp. 1457-1464.

Dhonneur, G., Cerf, C., Lagneau, F., Mantz, J., Gillotin, C., Duvaldestin, P., 'The pharmacokinetics of cisatracurium in patients with acute respiratory distress syndrome', Anesth Analg, 2001, Vol. 93(2), pp. 400-404.

DiMaio-Knych, H.-K., Arthur, R.-M., Taylor, A., Moeller, B.-C., Stanley, S.-D., 'Pharmacokinetics and metabolism of dantrolene in horses', J Vet Pharmacol Ther, Jun 2011, Vol. 34(3), pp. 238-246.

Edwards, J.-G., Newtont, J.-R., Ramzan, P.-H., Pilsworth, R.-C., Shepherd, M.-C., 'The efficacy of dantrolene sodium in controlling exertional rhabdomyolysis in the Thoroughbred racehorse', Equine Vet J, Nov 2003, Vol. 35(7), pp. 707-711.

ECHA, Substance Info Card – Cisatracurium besylate. Accessed March 2024, available from https://echa.europa.eu/de/substance-information/-/substanceinfo/100.149.509

Fahey, M.-R., Rupp, S.-M., Fisher, D.-M., Miller, R.-D., Sharma, M., Canfell, C., Castagnoli, K., Hennis, P.-J., 'The pharmacokinetics and pharmacodynamics of atracurium in patients with and without renal failure', Anesthesiology, 1984, Vol. 61(6), pp. 699-702.

Fisher, D.-M., Canfell, P.-C., Spellman, M.-J., Miller, R.-D., 'Pharmacokinetics and pharmacodynamics of atracurium in infants and children', Anesthesiology, 1990, Vol. 73(1), pp. 33-37.

Fisher, D.-M., Cronnelly, R., Sharma, M., Miller, R.-D., 'Clinical pharmacology of edrophonium in infants and children', Anesthesiology, 1984, Vol. 61(4), pp. 428-433.

Fodale, V., Santamaria, L.-B., 'Laudanosine, an atracurium and cisatracurium metabolite', Eur J Anaesthesiol, 2002, Vol. 19(7), pp. 466-473.

Grigori, A.-M., Brusco Jr, L., Kuroda, M., Koorn, R., 'Laudanosine and atracurium concentrations in a patient receiving long-term atracurium infusion', Crit Care Med, 1998, Vol. 26(1), pp. 180-183.

Herrero, L.-J., Foo, S.-S., Sheng, K.-C., Chen, W., Forwood, M.-R., Bucala, R., Mahalingam, S., 'Pentosan Polysulfate: a Novel Glycosaminoglycan-Like Molecule for Effective Treatment of Alphavirus-Induced Cartilage Destruction and Inflammatory Disease', J Virol, Aug 2015, Vol. 89(15), pp. 8063-8076.

Hildebrand, S.-V., Holland, M., Copland, V.-S., Daunt, D., Brock, N., 'Clinical use of the neuromuscular blocking agents atracurium and pancuronium for equine anesthesia', J Am Vet Med Assoc, 1989, Vol. 195(2), pp. 212-219.

Hildebrand, S.-V., Howitt, G.-A., Arpin, D., 'Neuromuscular and cardiovascular effects of atracurium in ponies anesthetized with halothane', Am J Vet Res, 1986, Vol. 47(5), pp. 1096-1000.

Holmes, M.-A., 'Does dantrolene sodium prevent recurrent exertional rhabdomyolysis in horses?', Equine Veterinary Education A.E., Mar 2007, pp. 97-99.

Hunt, L.-M., Valberg, S.-J., Steffenhagen, K., McCue, M.-E., 'An epidemiological study of myopathies in Warmblood horses', Equine Vet J, Mar 2008, Vol. 40(2), pp. 171-177.

IARC International Agency for Research on Cancer. List of Classifications. Accessed January 2024: https://monographs.iarc.who.int/list-of-classifications/

Kampa, N., Kaenkangploo, D., Jitpean, S., Srithunyarat, T., Seesupa, S., Hoisang, S., Yongvanit, K., Kamlangchai, P., Tuchpramuk, P., Lascelles, B.-D.-X., 'Study of the effectiveness of glucosamine and chondroitin sulfate, marine based fatty acid compounds (PCSO-524 and EAB-277), and carprofen for the treatment of dogs with hip osteoarthritis: A prospective, block-randomized, double-blinded, placebo-controlled clinical trial', Front Vet Sci, Feb 2023, Vol. 10, p. 1033188.

Kitts, J.-B., Fisher, D.-M., Canfell, P.-C., Spellman, M.-J., Caldwell, J.-E., Heier, T., Fahey, M.-R., Miller, R.-D., 'Pharmacokinetics and pharmacodynamics of atracurium in the elderly', Anesthesiology, 1990, Vol. 72(2), pp. 272-275.

Kisor, D.-F., Schmith, V.-D., 'Clinical pharmacokinetics of cisatracurium besilate', Clin Pharmacokinet, 1999, Vol. 36(1), pp. 27-40.

Kisor, D.-F., Schmith, V.-D., Wargin, W.-A., Lien, C.-A., Ornstein, E., Cook, D.-R., 'Importance of the organ-independent elimination of cisatracurium', Anesth Analg, 1996, Vol. 83(5), pp. 1065-1071.

Kleinz, M.-J., Spence, I., 'The pharmacology of the autonomic nervous system', In Maddison J.-E., Page, S.-W., Church D.-B., (Eds.), 'Small Animal Clinical Pharmacology', Saunders Ltd, 2008, Edition 2.

Knych, H.-K., Stanley, S.-D., Benson, D., Arthur, R.-M., 'Pharmacokinetics of guaifenesin following administration of multiple doses to exercised Thoroughbred horses', J Vet Pharmacol Ther, 2016, Vol. 39(4), pp. 416-419.

Larsen, R., 'Muskelrelaxanzien', In Larsen, R., Fink, T., Müller-Wolff, T., (Eds.), 'Anästhesie und Intensivmedizin für die Fachpflege', Springer Berlin, Heidelberg, 2016, Edition 9, pp. 170–185.

Lerche, P., 'Total intravenous anesthesia in horses', Vet Clin North Am Equine Pract, 2013, Vol. 29(1), pp. 123-129.

Liu, X., Kruger, P.-S., Weiss, M., Roberts, M.-S., 'The pharmacokinetics and pharmacodynamics of cisatracurium in critically ill patients with severe sepsis', Br J Clin Pharmacol, 2012, Vol. 73(5), pp. 741-749.

Martin-Flores, M., 'Neuromuscular blocking agents and monitoring in the equine patient', Vet Clin North Am Equine Pract, 2013, Vol. 29(1), pp. 131-154.

Martin-Flores, M., Sakai, D.-M., 'Neuromuscular Blocking Agents in Horses', In Doherty, T., Valverde, A., Reed, R.-A., (Eds.), 'Manual of Equine Anesthesia and Analgesia', John Wiley and Sons, 2022, Edition 2, pp. 244-249.

Matthews, N.-S., Peck, K.-E., Mealey, K.-L., Taylor, T.-S., Ray, A.-C., 'Pharmacokinetics and cardiopulmonary effects of guaifenesin in donkeys', J Vet Pharmacol Ther, 1997, Vol. 20(6), pp. 442-446.

McIlwraith, C.-W., Frisbie, D.-D., Kawcak, C.-E., Van Weeren, R., (Eds.), 'Joint disease in the horse', Elsevier Health Sciences, 2015, Edition 2.

McKenzie, E.-C., Valberg, S.-J., Godden, S.-M., Finno, C.-J., Murphy, M.-J., 'Effect of oral administration of dantrolene sodium on serum creatinine kinase activity after exercise in horses with recurrent exertional rhabdomyolysis', Am J Vet Res, 2004, Vol. 65(1), pp. 74–79.

McKenzie, E.-C., Firshman, A.-M., 'Optimal diet of horses with chronic exertional myopathies', Vet Clin North Am Equine Pract, Apr 2009, Vol. 25(1), pp. 121-35, vii.

McLeay, J.-M., 'Drugs affecting skeletal muscle' In Bertone, J.-J., Horspool, L.-J.-I., (Eds.), 'Equine Clinical Pharmacology', Saunders Ltd, London, 2004.

Morris, R.-B., Cronnelly, R., Miller, R.-D., Stanski, D.-R., Fahey, M.-R., 'Pharmacokinetics of edrophonium in anephric and renal transplant patients', Br J Anaesth, 1981, Vol 53(12), pp. 1311-1314.

Neil, K.-M., Caron, J.-P., Orth, M.-W., 'The role of glucosamine and chondroitin sulfate in treatment for and prevention of osteoarthritis in animals', J Am Vet Med Assoc, Apr 2005, Vol. 226(7), pp. 1079-1088.

Nigrovic, V., Fox, J.-L., 'Atracurium decay and the formation of laudanosine in humans', Anesthesiology, 1991, Vol. 74(3), pp. 446-454.

Pereira, A.-D.-A., Gonçalves, T.-F., Dantas, J.-B.-G., de Oliveira, M.-P.-M., Gomes, J.-B., de Lima Tolentino, M.-L.-D., Pereira, E.-A., de Freitas Martins e Silva, K., de Sousa, D.-C., da Silva Filho, M.-L., 'Equine tetanus: case report', PUBVET, 2020., Vol. 13(6).

Pope, C.-N., 'Edrophonium' In 'Reference Module in Biomedical Sciences', Elsevier, 2017.

Scherrer, N.-M., Hopster, K., 'Neuromuscular blockade with atracurium for ophthalmic surgery in horses-Effects on surgical and anesthetic characteristics and recovery quality', Vet Ophthalmol, 2021, Vol. 24(5), pp. 442-446.

Schmith, V.-D, Phillips, L, Kisor, D.-F., Fiedler-Kelly, J., Weatherley, B.-C., Barry, C., 'Pharmacokinetics/pharmacodynamics of cisatracurium in healthy adult patients', Current Opinion in Anaesthesiology, 1996, Vol. 9, pp. 9-15.

Sun, W.-Q., Zhou, A.-G., Mo, H., Liu, M.-J., 'Determination of Cisatracurium Concentrations in Rabbit Plasma and Muscles With Neostigmine Antagonism', Lat Am J Pharm, 2019, Vol. 38(3), pp. 629-635.

Scriba, G.-K.-E., In Sweetman C., (Eds.), 'Martindale: The Complete Drug Reference', Pharmaceutical Press, London, Edition 36, 2009.

Shabbir, A., Shamsi, S., Shahzad, M., Butt, H.-I., Aamir, K., Iqbal, J., 'Evaluation of developmental toxicity of guaifenesin using pregnant female rats', Indian J Pharmacol, 2016, Vol. 48(3), pp. 264-269.

Szakmany, T., Woodhouse, T., 'Use of cisatracurium in critical care: a review of the literature', Minerva Anestesiol, 2015, Vol. 81(4), pp. 450-460.

Taylor, P.-M., Kirby, J.-J., Shrimpton, D.-J., Johnson, C.-B., 'Cardiovascular effects of surgical castration during anaesthesia maintained with halothane or infusion of detomidine, ketamine and guaifenesin in ponies', Equine veterinary journal, 1998, Vol. 30(4), pp. 304-309.

Thompson, G.-A., Solomon, G., Albrecht, H.-H., Reitberg, D.-P., Guenin, E., 'Guaifenesin Pharmacokinetics Following Single-Dose Oral Administration in Children Aged 2 to 17 Years', J Clin Pharmacol, 2016, Vol. 56(7), pp. 894-901.

Tutunaru, A., Dupont, J., Gougnard, A., Ida, K., Serteyn, D., Sandersen, C., 'Retrospective evaluation of clinical use of cis-atracurium in horses', PloS one, 2019, Vol. 14(8), e0221196.

Tutunaru, A., Dupont, J., Huberty, V., Ibrahim, M., Serteyn, D., Sandersen, C., 'Use of cis-atracurium to maintain moderate neuromuscular blockade in experimental pigs', Vet Anaesth Analg, 2017, Vol. 44(4), pp. 910-914.

Valverde, A., 'Balanced anesthesia and constant-rate infusions in horses', Veterinary Clinics: Equine Practice, 2013, Vol .29(1), pp. 89-122.

Ward, A., Chaffman, M.-O., Sorkin, E.-M., 'Dantrolene. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic use in malignant hyperthermia, the neuroleptic malignant syndrome and an update of its use in muscle spasticity', Drugs, Aug 1986, Vol. 32(2), pp. 130-168.

Ward, S., Weatherley, B.-C., 'Pharmacokinetics of atracurium and its metabolites', Br J Anaesth, 1986, Vol. 58(Suppl 1), pp. 6S-10S.

Welch, R.-M., Brown, A., Ravitch, J., Dahl, R., 'The in vitro degradation of cisatracurium, the R, cis-R'-isomer of atracurium, in human and rat plasma', Clin Pharmacol Ther, 1995, Vol. 58(2), pp. 132-142.

Yamada, A.-L.-M., do Prado Vendruscolo, C., Marsiglia, M.-F., 'Effects of oral treatment with chondroitin sulfate and glucosamine in an experimental model of metacarpophalangeal osteoarthritis in horses', BMC Vet Res, 2022, Vol. 18, p. 215.

Substances for nervous system disorders

Adams, H.-R., (Ed.) 'Drugs Acting on the Cardiovascular System', In 'Veterinary Pharmacology and Therapeutics', Iowa State University Press, 1995, Edition 7, pp. 451-500.

Adams, J., Vorhees, C.-V., Middaugh, L.-D., 'Developmental neurotoxicity of anticonvulsants: human and animal evidence on phenytoin', Neurotoxicol Teratol, 1990, Vol. 12(3), pp. 203-214.

Aleman, M., Gray, L.-C., Williams, D.-C., Holliday, T.-A., Madigan, J.-E., LeCouteur, R.-A., Magdesian, K.-G., 'Juvenile idiopathic epilepsy in Egyptian Arabian foals: 22 cases (1985-2005), J Vet Intern Med, 2006, Vol. 20(6), pp. 1443–1449.

Allen, D.-G., Dowling, P.-M., Smith, D.-A., (Eds.), 'Handbook of Veterinary Drugs', Lippincott Williams and Wilkins, Philadelphia, 2005, Edition 3.

Al-Tahan, F., Frey, H.-H., 'Absorption kinetics and bioavailability of phenobarbital after oral administration to dogs', J Vet Pharmacol Ther, 1985, Vol. 8(2), pp. 205-207.

Ambrosio, A.-F., Soares-Da-Silva, P., Carvalho, C.-M., Carvalho, A.-P., 'Mechanisms of action of carbamazepine and its derivatives, oxcarbazepine, BIA 2-093, and BIA 2-024', Neurochem Res, 2002, Vol. 27(1-2), pp. 121-130.

Anderson, G.-D., 'Phenobarbital and other barbiturates. Chemistry, biotransformation, and pharmacokinetics', In Levy, R.-H., Mattson, R.-H., Meldrum, B.-S., Perucca, E., (Eds.), 'Antiepileptic Drugs', Lippincott Williams and Wilkins, Philadelphia, 2002, Edition 5, pp. 496-503.

Bertone, J.-J., Horspool, L.-J., (Eds.), 'Equine Clinical Pharmacology', Elsevier limited, 2004, Edition 1, pp. 142-143.

Boothe, M.-D., 'Drugs Acting on the Nervous System', In Boothe, D.-M., (Ed.) 'Small Animal Clinical Pharmacology and Therapeutics', Elsevier Saunders, St. Louis, 2011, Edition 2, pp. 932-993.

Bowen, I.-M., Marr, C.-M., Elliott, J., 'Drugs acting on the cardiovascular system', In Bertone, J.-J., Horspool, L.-J.-I., (Eds.), 'Equine Clinical Pharmacology', WB Saunders, London, 2004, Edition 1, pp. 193-215.

Braddock, S.-W., Harrington, D., Vose, J., 'Generalized nodular cutaneous pseudolymphoma associated with phenytoin therapy. Use of T-cell receptor gene rearrangement in diagnosis and clinical review of cutaneous reactions to phenytoin', J Am Acad Dermatol, 1992, Vol. 27(2 Pt 2), pp. 337-340.

Brewer, J.-M., Waltman, P.-A., 'Epilepsy and pregnancy: maternal and fetal effects of phenytoin', Crit Care Nurse, 2003, Vol. 23(2), pp. 93–98.

Brown, S.-A., 'Anticonvulsant therapy in small animals', Vet Clin North Am Small Anim Pract, 1988, Vol. 18(6), pp. 1197-1216.

Browne, T.-R., LeDuc, B., 'Phenytoin: chemistry and biotransformation', In Levy, R.-H., Mattson, R.-H., Meldrum, B.-S., (Eds.), 'Antiepileptic Drugs', Raven Press, New York, 1995, Edition 4, pp. 283-300.

Davis, A.-L., Begg, E.-J., Kennedy, M.-C., Graham, G.-G., 'Application of a simplified method to determine bioavailability of an oral dose of phenytoin', J Pharmacokinet Biopharm, 1993, Vol. 21(2), pp. 195–208.

Delattre, J.-Y., Safai, B., Posner, J.-B., 'Erythema multiforme and Stevens-Johnson syndrome in patients receiving cranial irradiation and phenytoin', Neurology, 1988, Vol. 38(2), pp. 194-198.

Duran, S.-H., Ravis, W.-M., Pedersoli, W.-M., Schumacher, J., 'Pharmacokinetics of phenobarbital in the horse', Am J Vet Res, 1987, Vol. 48(5), pp. 807-810.

Eze, A.-A., Nwaohukwu, D.-C., Achukwu, P., Okwuosa, C.-N., 'Effects of Phenobarbital administration on the histology of the liver and brain, and the activities of some biochemical parameters of the liver of Wister rats', Bio-Research, 2009, Vol. 7(1), pp. 446-450.

Fontenot, R.-L., Mochal-King, C.-A., Sprinkle, S.-B., Wills, R.-W., Calder, C.-D. 'Retrospective Evaluation of Fluoxetine Hydrochloride Use in Horses: 95 Cases (2010-2019)', J Equine Vet Sci, 2021, Vol. 97.

Gupta, C., Shapiro, B.-H., Yaffe, S.-J., 'Reproductive dysfunction in male rats following prenatal exposure to phenobarbital', Pediatr Pharmacol (New York), 1980, Vol. 1(1), pp. 55-62.

Gupta, C., Yaffe, S.-J., 'Reproductive dysfunction in female offspring after prenatal exposure to phenobarbital: critical period of action', Pediatr Res, 1981, Vol. 15(12), pp. 1488-1491.

Habedank, M., Esser, K.-J., Brüll, D., Kotlarek, F., Stumpf, C., 'Increased sister chromatid exchanges in epileptic children during long-term therapy with phenytoin', Hum Genet, 1982, Vol. 61(1), pp. 71-72.

Harbison, R.-D., Becker, B.-A., 'Diphenylhydantoin teratogenicity in rats', Toxicol Appl Pharmacol, 1972, Vol. 22(2), pp. 193-200.

Harbison, R.-D., Becker, B.-A., 'Relation of dosage and time of administration of diphenylhydantoin to its teratogenic effect in mice', Teratology, 1969, Vol. 2(4), pp. 305-311.

Hobbs, K., Luethy, D., Davis, J., Mallicote, M., Torcivia, C., Kulp, J., Stefanovski, D., Futterman, C., Cooper, F., van Eps, A., 'The effects of orally administered trazodone on ambulation and recumbency in healthy horses', J Vet Intern Med, 2023, Vol. 37(5), pp. 1899–1906.

Hunke, M.-H., Carpenter, N.-J., 'Effects of diphenylhydantoin on the frequency of sister chromatid exchanges in human lymphocytes', Am J Hum Genet, 1978, Vol. 30, Abstr. 83A.

IARC International Agency for Research on Cancer, 'Phenobarbital and its Sodium Salt', In 'Some Thyrotropic Agents. IARC Monographs on the evaluation of carcinogenic risks to humans', 2001, Vol. 79, pp. 161-288.

IARC International Agency for Research on Cancer, 'Phenytoin', In 'Some Pharmaceutical Drugs. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans', 1996, Vol. 66, pp. 175-237.

IARC International Agency for Research on Cancer, 'Phenytoin and phenytoin sodium', In 'Some Miscellaneous Pharmaceutical Substances. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans', 1977, Vol. 13, pp. 201-226.

Inselman, A.-L., Hansen, D.-K., 'Phenytoin', In Wexler, P., (Ed.), 'Encyclopedia of Toxicology', Academic Press, London, 2014, Edition 3.

Jang, J.-J., Takahashi, M., Furukawa, F., Toyoda, K., Hasegawa, R., Sato, H., Hayashi, Y., 'Long-term in vivo carcinogenicity study of phenytoin (5,5-diphenylhydantoin) in F344 rats', Food Chem Toxicol, 1987, Vol. 25(9), pp. 697-702.

Johannessen, S.-I., 'Therapeutic drug monitoring of antiepileptic drugs', In 'Handbook of Analytical Separations', Vol. 5, 2004, pp. 221-253.

Kaul, A., Goyle, S., 'Genotoxicity of the anticonvulsant drug phenytoin (PHT): A follow-up study of PHT-untreated epileptic patients. I. Sister chromatid exchange (SCE) analysis', Teratog Carcinog Mutagen, 1999, Vol. 19(1), pp. 61-72.

Knox, D.-A., Ravis, W.-R., Pedersoli, W.-M., Spano, J.-S., Nostrandt, A.-C., Krista, L.-M., Schumacher, J., 'Pharmacokinetics of Phenobarbital in horses after single and repeated oral administration of the drug', Am J Vet Res, 1992, Vol. 53(5), pp. 706-710.

Kokwaro, G.-O., Ogutu, B.-R., Muchohi, S.-N., Otieno, G.-O., Newton, C.-R.-J.-C, 'Pharmacokinetics and clinical effect of phenobarbital in children with severe falciparum malaria and convulsions', Br J Clin Pharmacol, 2003, Vol. 56(4), pp. 453-457.

Kowalczyk, D.-F., Beech, J., 'Pharmacokinetics of phenytoin (diphenylhydantoin) in horses', J Vet Pharmacol Ther, 1983, Vol. 6(2), pp. 133-140.

Krueger, G.-R., Bedoya, V.-A., 'Hydantoin-induced lymphadenopathies and lymphomas: experimental studies in mice', Recent Results Cancer Res, 1978, Vol. 64, pp. 256-270.

Lacombe, V.-A., 'Seizures in horses: diagnosis and classification', Vet Med (Auckl), 2015, Vol. 6, pp. 301-308.

Lenkapothula, N., Cascella, M., 'Primidone', StatPearls, Treasure Island (FL), July 2023, available from: https://www.ncbi.nlm.nih.gov/books/NBK562297/.

Löscher, W., Klein, P., 'The Pharmacology and Clinical Efficacy of Antiseizure Medications: From Bromide Salts to Cenobamate and Beyond', CNS Drugs, Sep 2021, Vol. 35(9), pp. 935-963.

Maeda, T., Sano, N., Togei, K., Shibata, M., Izumi, K., Otsuka, H., 'Lack of carcinogenicity of phenytoin in (C57BL/6 x C3H)F1 mice', J Toxicol Environ Health, 1988, Vol. 24(1), pp. 111-119.

McClain, R.-M., Langhoff, L., 'Teratogenicity of diphenylhydantoin in the New Zealand white rabbit', Teratology, 1980, Vol. 21(3), pp. 371-379.

Messina, S., Battino, D., Croci, D., Mamoli, D., Ratti, S., Perucca, E., 'Phenobarbital pharmacokinetics in old age: a case-matched evaluation based on therapeutic drug monitoring data', Epilepsia, 2005, Vol. 46(3), pp. 372-377.

Mirkin, B.-L., 'Diphenylhydantoin: placental transport, fetal localization, neonatal metabolism, and possible teratogenic effects', J Pediatr, 1971, Vol. 78(2), pp. 329–337.

Mykkänen, A.-K., Hyytiäinen, H.-K., McGowan, C.-M. 'Generalised tetanus in a 2-week-old foal: use of physiotherapy to aid recovery', Aust Vet J, 2011, Vol. 89(11), pp. 447-451.

Nelson, E., Powell, J.-R., Conrad, K., Likes, K., Byers, J., Baker, S., Perrier, D., 'Phenobarbital pharmacokinetics and bioavailability in adults', J Clin Pharmacol, 1982, Vol. 22(2-3), pp. 141-148.

Newton, S.-A., Knottenbelt, D.-C., Eldridge, P.-R., 'Headshaking in horses: possible aetiopathogenesis suggested by the results of diagnostic tests and several treatment regimes used in 20 cases', Equine Vet J, 2000, Vol. 32(3), pp. 208-216.

NTP National Toxicology Program, 'Phenytoin and Phenytoin Sodium: CAS Nos. 57-41-0 and 630-93-3', In 'National Toxicology Program 15th Report on Carcinogens', 2021, available from: https://www.ncbi.nlm.nih.gov/books/NBK590894/

NTP National Toxicology Program, 'Toxicology and Carcinogenesis Studies of 5,5-Diphenylhydantoin (CAS No. 57-41-0) (Phenytoin) in F344/N Rats and B6C3F1 Mice (Feed Studies)', Natl Toxicol Program Tech Rep Ser, 1993, Vol. 404, pp. 1-303.

Okuda, H., Nagao, T., 'Cardiovascular malformations induced by prenatal exposure to phenobarbital in rats', Congenit Anom (Kyoto), 2006, Vol, 46(2), pp. 97-104.

Onishi, S., Ohki, Y., Nishimura, Y., Itoh, S., Isobe, K., Hosoe, A., Yamamoto, T., Yamakawa, T., 'Distribution of Phenobarbital in serum, brain and other organs from pediatric patients', Dev Pharmacol Ther, 1984, Vol. 7(3), pp. 153-159.

Patocka, J., Wu, Q., Nepovimova, E., Kuca, K., 'Phenytoin – An anti-seizure drug: Overview of its chemistry, pharmacology and toxicology', Food Chem Toxicol, 2020, Vol. 142.

Plumb, D.-C., (Ed.), 'Plumb's Veterinary Drug Handbook', PharmaVet Publishing, White Bear Lake, 1999, Edition 3, pp. 853.

Plumb, D.-C., (Ed.), 'Plumb's Veterinary Drug Handbook', Wiley-Blackwell, Ames, 2011, Edition 7, pp. 1187.

Powell, C.-M., Castillo, P.-E., 'Presynaptic Mechanisms in Plasticity and Memory' In Byrne, J.-H., (Ed.), 'Learning and Memory: A Comprehensive Reference', Academic Press Inc, 2008, pp. 741-769.

Rao, Y., Kuradi, L.-B., Paul, V., 'Effect of phenytoin sodium on reproductive parameters in adult male Wistar rats', Kuwait Medical Journal, 2009, Vol. 41(1), pp. 43-51.

Ravis, W.-R., Duran, S.-H., Pedersoli, W.-M., Schumacher, J., 'A pharmacokinetic study of Phenobarbital in mature horses after oral dosing', J Vet Pharmacol Ther, 1987, Vol. 10(4), pp. 283-289.

Reynolds, J.-E.-F., 'Martindale: The Extra Pharmacopoeia', Pharmaceutical Press, London, 1993, Edition 30, pp. 304-309.

Rho, J.-M., White, H.-S., 'Brief history of anti-seizure drug development', Epilepsia Open, October 2018, Vol. 3(2), pp. 114-119.

Riedel, L., Obe, G., 'Mutagenicity of antiepileptic drugs. II Phenytoin, primidone and phenobarbital', Mutat Res, 1984, Vol. 138(1), pp. 71-74.

Roberts, V., 'Trigeminal-mediated headshaking in horses: prevalence, impact, and management strategies', Vet Med (Auckl), 2019, Vol. 10, pp. 1-8.

Rogel-Fuchs, Y., Newman, M.-E., Trombka, D., Zahalka, E.-A., Yanai, J., 'Hippocampal cholinergic alterations and related behavioral deficits after early exposure to phenobarbital', Brain Res Bull, 1992, Vol. 29(1), pp. 1-6.

Sakurai, M., Isshiki, S., Sasaki, N., Sakai, Y., Yamada, Y., Morimoto, M., 'Leucoencephalopathy with Pigmented Glia in a Horse with Recurrent Convulsive Seizures', J Comp Pathol, 2021, Vol. 189, pp. 120-124.

Schaefer, U., Höchner, P., Karrer, C., 'Arzneimittelkompendium der Schweiz', Documed AG, Basel, 2013, Edition 34, pp. 4992.

Shaniko Shini, S., 'A review of diazepam and its use in the horse', Journal of Equine Veterinary Science, 2000, Vol. 20(7), pp. 443-449.

Soma, L.-R., Uboh, C.-E., Guan, F., Birks, E.-K., Teleis, D.-C., Rudy, J.-A., Tsang, D.-S., Watson, A.-O., 'Disposition, elimination, and bioavailability of phenytoin and its major metabolite in horses', Am J Vet Res, 2001, Vol. 62(4), pp. 483–489.

Sullivan, F.-M., McElhatton, P.-R., 'Teratogenic activity of the antiepileptic drugs phenobarbital, phenytoin and primidone in mice', Toxicol Appl Pharmacol, 1975, Vol. 34(2), pp. 271-282.

Sweetman, S.-C., 'Martindale: The Complete Drug Reference', Pharmaceutical Press, London, 2009, Edition 36.

Thomason, J.-M., Seymour, R.-A., Rawlins, M.-D., 'Incidence and severity of phenytoin-induced gingival overgrowth in epileptic patients in general medical practice', Community Dent Oral Epidemiol, 1992, Vol. 20(5), pp. 288-291.

Thorn, C.-F., Whirl-Carrillo, M., Leeder, J.-S., Klein, T.-E., Altman, R.-B., 'PharmGKB summary: phenytoin pathway', Pharmacogenet Genomics, 2012, Vol. 22(6), pp. 466-470.

Tilley, L.-P., 'EKG bei Hund und Katze: Grundlagen, Auswertung und Therapie', Schlütersche GmbH and Co., Hannover, 1997, Edition 2, pp. 483.

Trinka, E., 'Phenobarbital in Status epilepticus – Rediscovery of an effective drug', Epilepsy Behav, 2023, Vol. 141.

Vernaud, K.-M., Le Coteur, R.-A., 'Anticonvulsant drugs', In Maddison, J., Page, S.-W., Church, D., (Eds.), 'Small Animal Clinical Pharmacology', Saunders, Edinburgh, 2008, Edition 2, pp. 367-379.

Verster, J.-C., Volkerts, E.-R., 'Clinical pharmacology, clinical efficacy, and behavioral toxicity of alprazolam: a review of the literature', CNS Drug Rev, 2004, Vol. 10(1), pp. 45-76.

Waters, C.-H., Belai, Y., Gott, P.-S., Shen, P., De Giorgio, C.-M., 'Outcomes of pregnancy associated with antiepileptic drugs', Arch Neurol, 1994, Vol. 51(3), pp. 250-253.

Windholz, M., In Windholz, M., Budavari, S., Blumetti, R.-F., Otterbein, E.-S., (Eds.), 'The Merck Index', Merck and Co Inc, Rahway, 1983, pp. 2194.

Wong, D.-M., Alcott, C.-J., Davis, J.-L., Hepworth, K.-L., Wulf, L., Coetzee, J.-H., 'Use of alprazolam to facilitate mare-foal bonding in an aggressive postparturient mare', J Vet Intern Med, 2015, Vol. 29(1), pp. 414-416.

Yanai, J., Rosselli-Austin, L., Tabakoff, B., 'Neuronal deficits in mice following prenatal exposure to phenobarbital', Exp Neurol, 1979, Vol. 64(2), pp. 237-244.

Yates, L.-M., Aleman, M., Knych, H.-K., Knipe, M.-F., Crowe, C.-M., Chigerwe, M., 'Pharmacokinetics of Intravenous and Oral Phenobarbital Sodium in Healthy Goats', Front Vet Sci, 2020, Vol. 21(7), pp. 86.

Substances for ophthalmology

Alberts, M.-K., Clarke, C.-R., MacAllister, C.-G., Homer, L.-M., 'Pharmacokinetics of acetazolamide after intravenous and oral administration in horses', Am J Vet Res, Aug 2000, Vol. 61(8), pp. 965-968.

Aslam, S., Gupta, V., 'Carbonic Anhydrase Inhibitors', StatPearls, Treasure Island (FL), Jan 2023, available from: https://www.ncbi.nlm.nih.gov/books/NBK557736/.

Balfour, J.-A., Wilde, M.-I., 'Dorzolamide. A review of its pharmacology and therapeutic potential in the management of glaucoma and ocular hypertension', Drugs Aging, May 1997, Vol. 10(5), pp. 384-403.

Beech, J., Lindborg, S., 'Prophylactic efficacy of phenytoin, acetazolamide and hydrochlorothiazide in horses with hyperkalaemic periodic paralysis', Res Vet Sci, Sep 1995, Vol. 59(2), pp. 95-101.

Begou, O., Drabert, K., Theodoridis, G., Tsikas, D., 'GC-NICI-MS analysis of acetazolamide and other sulfonamide (R-SO2- NH2) drugs as pentafluorobenzyl derivatives [R-SO2- N(PFB)2] and quantification of pharmacological AZM in human urine', J Pharm Anal, 2020, Vol. 10(1), pp. 49-59.

Bessonnat, A., Vanore, M., 'Effect of topical cyclopentolate alone or combined with phenylephrine in healthy horses', Vet Ophthalmol, Nov 2021, Vol. 24(6), pp. 582-590.

BfR Bundesinstitut fur Risikobewertung, 'Health assessment of sports and weight loss products containing synephrine and caffeine', Nov 2012, available from: https://www.bfr.bund.de/cm/349/health-assessment-of-sports-and-weight-loss-products-containing-synephrine-and-caffeine.pdf

Biollaz, J., Munafo, A., Buclin, T., Gervasoni, J.-R., Magnin, J.-L., Jaquet, F., Brunner-Ferber, F., 'Whole-blood pharmacokinetics and metabolic effects of the topical carbonic anhydrase inhibitor dorzolamide', Eur J Clin Pharmacol, 1995, Vol. 47, p. 453460.

Bizrah, M., Corbett, M.-C., 'Intracameral Phenylephrine to Arrest Intraoperative Intraocular Bleeding: A New Technique', Ophthalmol Ther, Mar 2019, Vol. 8(1), pp. 137-141.

Busardò, F.-P., Lo Faro, A.-F., Sirignano, A., Giorgetti, R., Carlier, J., 'In silico, in vitro, and in vivo human metabolism of acetazolamide, a carbonic anhydrase inhibitor and common "diuretic and masking agent" in doping, Arch Toxicol, 2022, Vol. 96(7), pp. 1989-2001.

Campíns-Falcó, P., Herráez-Hernández, R., Sevillano-Cabeza, A., 'Application of column-switching techniques to the determination of medium polarity drugs: determination of acetazolamide in urine', Journal of Chromatography B: Biomedical Sciences and Applications, Vol. 654(1), pp. 85-90.

Cappon, G.-D., Fleeman, T.-L., Cook, J.-C., Hurtt, M.-E., 'Combined treatment potentiates the developmental toxicity of ibuprofen and acetazolamide in rats', Drug and Chemical Toxicology, 2005, Vol. 28(4), pp. 409-421.

Carr, M.-E., Engebretsen, K.-M., Ho, B., Anderson, C.-P., 'Tetrahydrozoline (Visine) concentrations in serum and urine during therapeutic ocular dosing: a necessary first step in determining an overdose', Clin Toxicol (Phila), 2011, Vol. 49(9), pp. 810-814.

Chandra, M., Frith, C., 'Spontaneously Occurring Leiomyosarcomas of the Mouse Urinary Bladder', Toxicological Pathology, 1991, Vol. 19(2), pp. 164-167.

Chapron, D.-J., Sweeney, K.-R., Feig, P.-U., Kramer, P.-A., 'Influence of advanced age on the disposition of acetazolamide', Br J Clin Pharmacol, 1985, Vol. 19(3), pp. 363-371.

Chapron, D.-J., White, L.-B., 'Determination of acetazolamide in biological fluids by reverse-phase high-performance liquid chromatography', J Pharm Sci, 1984, Vol. 73(7), pp. 985-989.

Contreras-Salinas, H., Orozco-Ceja, V., Romero-López, M.-S., Barajas-Virgen, M.-Y., Baiza-Durán, L.-M., Rodríguez-Herrera, L.-Y., 'Ocular Cyclopentolate: A Mini Review Concerning Its Benefits and Risks', Clin Ophthalmol, Nov 2022, Vol. 16, pp. 3753-3762.

Curling, A., 'Equine recurrent uveitis: treatment', Compend Contin Educ Vet, Jun 2011a, Vol. 33(6), p. E1.

Curling, A., 'Equine recurrent uveitis: classification, etiology, and pathogenesis', Compend Contin Educ Vet, Jun 2011b, Vol. 33(6), p. E2.

Cvetkovic, R.-S., Perry, C.-M., 'Brinzolamide: a review of its use in the management of primary open-angle glaucoma and ocular hypertension', Drugs Aging, 2003, Vol. 20(12), pp. 919-947.

Daggy, A., Kaplan, R., Roberge, R., Akhtar, J., 'Pediatric Visine (tetrahydrozoline) ingestion: case report and review of imidazoline toxicity', Vet Hum Toxicol, 2003, Vol. 45(4), pp. 210-212.

Davidson, H.-J., Pinard, C.-L., Keil, S.-M., Brightman, A.-H., Sargeant, J.-M., 'Effect of topical ophthalmic latanoprost on intraocular pressure in normal horses', Vet Ther, 2002, Vol. 3(1), pp. 72-80.

Deeks, E.-D., 'Tropicamide/Phenylephrine/Lidocaine Intracameral Injection: A Review in Cataract Surgery', Clin Drug Investig, Nov 2019, Vol. 39(11), pp. 1133-1139.

Demartis, S., Obinu, A., Gavini, E., Giunchedi, P., Rassu, G. 'Nanotechnology-based rose Bengal: a broad-spectrum biomedical tool', Dyes and Pigments, 2021, Vol. 188, p. 109236.

DeSantis, L., 'Preclinical overview of brinzolamide', Surv Ophthalmol, Jan 2000, Vol. 44(2), pp. S119-29.

Dodo, T., Uchida, K., Hirose, T., Fukuta, T., Kojima, C., Shiraishi, I., Kato, E., Horiba, T., Mineshima, H., Okuda, Y., Maeda, M., Katsutani, N., Hirano, K., Aoki, T., 'Increases in discontinuous ribcartilage and fused carpal bone in rat fetuses exposed to the teratogens, busulfan, acetazolamide, vitamin A, and ketoconazole', Human and Experimental Toxicology, 2010, Vol. 29(6), pp. 439-450.

Drugbank, Cyclopentolate [Updated March 2024]. Accessed March 2024, available from: https://go.drugbank.com/drugs/DB00979

Drugbank, Syneprhine [Updated 6 Dec 2020]. Accessed January 2024, available from: https://go.drugbank.com/drugs/DB09203

ECHA C&L. Notified Classification and Labelling – Rose Bengal. Accessed December 2023, available from: https://echa.europa.eu/de/information-on-chemicals/cl-inventory-database/-/discli/details/27847

ECHA, Registration dossier – Cyclopentolate. Accessed March 2024, available from: https://echa.europa.eu/de/registration-dossier/-/registered-dossier/25957/2/1

ECHA, Substance Info Card – Brinzolamide. Accessed March 2024, available from: https://echa.europa.eu/de/substance-information/-/substanceinfo/100.149.376.

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Bampidis, V., Azimonti, G., Bastos, M.-L., Christensen, H., Fašmon-Durjava, M., Kouba, M., López-Alonso, M., López-Puente, S., Marcon, F., Mayo, B., Pechová, A., Petkova, M., Ramos, F., Sanz, Y., Villa, R.-E., Woutersen, R., Brantom, P., Chesson, A., Westendorf, J., Manini, P., Pizzo, F., Dusemund, B., 'Safety

and efficacy of a feed additive consisting of a flavonoid-rich dried extract of Citrus × aurantium L. fruit (bitter orange extract) for use in all animal species (FEFANA asbl)', EFSA J, Jul 2021, Vol. 19(7), p. e06709.

Eller, M.-G., Schoenwald, R.-D., Dixson, J.-A., Segarra, T., Barfknecht, C.-F., 'Topical carbonic anhydrase inhibitors. III: Optimization model for corneal penetration of ethoxzolamide analogues', J Pharm Sci, Feb 1985, Vol. 74(2), pp. 155-60.

Farzam, K., Abdullah, M., 'Acetazolamide', StatPearls, Treasure Island (FL), Jan 2023, available from: https://www.ncbi.nlm.nih.gov/books/NBK532282/.

Faulds, D., Goa, K.-L., Benfield, P., 'Cyclosporine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in immunoregulatory disorders', Drugs, 1993, Vol. 45(6), pp. 953-1040.

Forsythe, P., Paterson, S., 'Cyclosporine 10 years on: indications and efficacy', Vet Rec., 2014, Vol. 174(Suppl 2), pp. 13-21.

Freeman, D.-J., 'Pharmacology and pharmacokinetics of cyclosporine', Clin Biochem, 1991, Vol. 24(1), pp. 9-14.

Friedrich, A., Olejniczak, K., 'Evaluation of carcinogenicity studies of medicinal products for human use authorised via the European centralised procedure (1995–2009)', Regulatory Toxicology and Pharmacology, 2011, Vol. 60, pp. 225–248.

Gerding, J.-C., Gilger, B.-C., 'Prognosis and impact of equine recurrent uveitis', Equine Vet J, May 2016, Vol. 48(3), pp. 290-298.

Germann, S.-E., Matheis, F.-L., Rampazzo, A., Burger, D., Roos, M., Spiess, B.-M., 'Effects of topical administration of 1% brinzolamide on intraocular pressure in clinically normal horses', Equine Vet J, Nov 2008, Vol. 40(7), pp. 662-665.

Gilger, B.-C., Michau, T.-M., 'Equine recurrent uveitis: new methods of management', Vet Clin North Am Equine Pract, 2004, Vol. 20(2), pp. 417-427.

Gilger, B.-C., Michau, T.-M., Salmon, J.-H., 'Immune-mediated keratitis in horses: 19 cases (1998-2004)', Vet Ophthalmol, 2005, Vol. 8(4), pp.233-239.

González-Medina, S., 'Equine eosinophilic keratitis: An emergent ocular condition?', Equine Veterinary Education, 2018, Vol. 31, pp. 988-992.

Good, S.-S., Miranda, P., 'Metabolic disposition of acyclovir in the guinea pig, rabbit and monkey', Am J Med, 1982, Vol. 73(1A), pp. 91-95.

Haaga, M., Kaila, T., Salminen, L., Ylitalo, P., 'Systemic and Ocular Absorption and Antagonist Activity of Topically Applied Cyclopentolate in Man', Pharmacology & Toxicology, 1998, Vol. 82, pp. 19-22.

Hacker, D.-V., Buyukmihci, N.-C., Franti, C.-E., Bellhorn, R.-W., 'Effect of topical phenylephrine on the equine pupil', Am J Vet Res, Feb 1987, Vol. 48(2), pp. 320-322.

Hahn, C.-N., Mayhew, I.-G., 'Phenylephrine eyedrops as a diagnostic test in equine grass sickness', Vet Rec, Nov 2000, Vol. 147(21), pp. 603-606.

Hall, R., Havner, G., Baker, J., 'Brinzolamide', In Brittain, H.-G., (Ed.), 'Analytical Profiles of Drug Substances and Excipients', Vol. 26, San Diego, Academic Press, 1999, pp. 47–96.

Hampson, A.-J., Babalonis, S., Lofwall, M.-R., Nuzzo, P.-A., Krieter, P., Walsh, S.-L., 'A pharmacokinetic study examining acetazolamide as a novel adherence marker for clinical trials', J Clin Psychopharmacol, 2016, Vol. 36(4), pp. 324-332.

Hardy, J., Bednarski, R.-M., Biller, D.-S., 'Effect of phenylephrine on hemodynamics and splenic dimensions in horses', Am J Vet Res, Nov 1994, Vol. 55(11), pp. 1570-1578.

Hilton, H., Affolter, V.-K., White, S.-D., 'Evaluation of four topical preparations for the treatment of cannon hyperkeratosis in a horse', Vet Dermatol, 2008, Vol. 19(6), pp. 385-390.

Hinchcliff, K.-W., McKeever, K.-H., Muir, W.-W., 'Hemodynamic effects of atropine, dobutamine, nitroprusside, phenylephrine, and propranolol in conscious horses', J Vet Intern Med, 1991, Vol. 5(2), pp. 80-68.

Holló, G., Bozkurt, B., Irkec, M., 'Brinzolamide/timolol fixed combination: a new ocular suspension for the treatment of open-angle glaucoma and ocular hypertension', Expert Opin Pharmacother, Aug 2009, Vol. 10(12), pp. 2015-2024.

Hossie, R.-D., Mousseau, N., Sved, S., Brien, R., 'Quantitation of acetazolamide in plasma by high-performance liquid chromatography', J Pharm Sci, 1980, Vol. 69(3), pp. 348-349.

Hosten, L.-O., Snyder, C., 'Over-the-Counter Ocular Decongestants in the United States - Mechanisms of Action and Clinical Utility for Management of Ocular Redness', Clin Optom (Auckl), 2020, Vol. 12, pp. 95-105.

IARC International Agency for Research on Cancer. List of Classifications. Accessed January 2024, available from: https://monographs.iarc.who.int/list-of-classifications/

Iester, M., 'Brinzolamide', Expert Opin Pharmacother, March 2008, Vol. 9(4), pp. 653-662.

Kaila, T., Huupponen, R., Salminen, L., Iisalo, E., 'Systemic absorption of ophthalmic cyclopentolate', Amer J Qphthalmol, 1989, Vol. 107, p. 562.

Kanfer, I., Dowse, R., Vuma, V., 'Pharmacokinetics of oral decongestants', Pharmacotherapy, 1993, Vol. 13(6 Pt2), pp. 116S-128S.

Kassamali, R., Sica, D.-A., 'Acetazolamide: a forgotten diuretic agent', Cardiology in review, 2011, Vol. 19(6), pp. 276-278.

Kazemi, A., McLaren, J.-W., Sit, A.-J., 'Effect of topical phenylephrine 2.5% on episcleral venous pressure in normal human eyes', Invest Ophthalmol Vis Sci, Oct 2021, Vol. 62(13), p. 4.

Khan-Lim, D., Berry, M., 'Still confused about rose bengal?' Curr Eye Res, 2004, Vol. 29(4-5), pp. 311-7.

Kilic, O., Ozturk, M., Gozcelioglu, E.-A., 'Tropicamide ophthalmic solution reduces clozapine-induced sialorrhea: a case report', Psychiatr Clin Psychopharmacol, 2017, Vol. 27(4), pp. 431-434.

King, T.-C., Gum, G.-G., Gelatt, K.-N., 'Evaluation of a topically administered carbonic anhydrase inhibitor (MK-927) in normotensive and glaucomatous beagles', Am J vet Res, 1991, Vol. 52, pp. 2067-2070.

Kintz, P., Gheddar, L., Raul, J.-S., 'Case reports: Adverse analytical finding due to red blood cells transfusion: A rare case involving the diuretic dorzolamide', Drug Test Anal, 2022, Vol. 14, pp. 1785–1790.

Klaassen, C.-D., 'Pharmacokinetics of rose bengal in the rat, rabbit, dog and guinea pig', Toxicol Appl Pharmacol, 1976, Vol. 38(1), pp. 85-100.

Konstas, A.-G., Schmetterer, L., Katsanos, A., Hutnik, C.-M.-L., Holló, G., Quaranta, L., Teus, M.-A., Uusitalo, H., Pfeiffer, N., Katz, L.-J., 'Dorzolamide/Timolol Fixed Combination: Learning from the Past and Looking Toward the Future', Adv Ther, Jan 2021, Vol. 38(1), pp. 24-51.

Korte, J.-M., Kaila, T., Saari, K.-M, 'Systemic bioavailability and cardiopulmonary effects of 0.5% timolol eyedrops', Graefes Arch Clin Exp Ophthalmol, 2002, Vol. 240(6), pp. 430-435.

Kumar, V., Schoenwald, R.-D., Barcellos, W.-A., Chien, D.-S., Folk, J.-C., Weingeist, T.-A., 'Aqueous vs viscous phenylephrine. I. Systemic absorption and cardiovascular effects', Arch Ophtalmol, 1986, Vol. 104(8), pp. 1189-1191.

Lahdes, K., Huupponen, R., Kaila, T., Ali-Melkkila, T., Salminen, L., Saari, M., 'Systemic absorption of ocular cyclopentolate in children', Ger J Ophthalmol, 1992, Vol. 1(1), pp. 16-18.

Lahdes, K., Huuponen, R., Kaila, T., Monti, D., Saeftone, M.-F., Salminen, L., 'Plasma concentrations and ocular effects of cyclopentolate after ocular application of three formulations', Br J clin Pharmac, 1993, Vol. 35, p. 479-483.

Layton, W.-M.-Jr., Hallesy, D.-W., 'Deformity of forelimb in rats: association with high doses of acetazolamide', Science, 1965, Vol. 149, pp. 306-308.

Lee, Y.-C., Park, C.-K., Kim, M.-S., Kim, J.-H., 'In vitro study for staining and toxicity of rose bengal on cultured bovine corneal endothelial cells', Cornea, 1996, Vol. 15(4), pp. 376-85.

Liu, Y., Zhao, J., Zhong, X., Wei, Q., Huang, Y., 'Efficacy and Safety of Brinzolamide as Add-On to Prostaglandin Analogues or β-Blocker for Glaucoma and Ocular Hypertension: A Systematic Review and Meta-Analysis', Front Pharmacol, Jun 2019, Vol. 10, p. 679.

Loftsson, T., Jansook, P., Stefánsson, E., 'Topical drug delivery to the eye: dorzolamide', Acta Ophthalmol, Nov 2012, Vol. 90(7), pp. 603-608.

Major, E., Dutson, T., Moshirfar, M., 'Cyclopegia in children: An Optometrist's Perspective', Clin Optom (Auckl.), 2020, Vol. 12, pp. 129-133.

March, W.-F., Ochsner, K.-I., 'The long-term safety and efficacy of brinzolamide 1.0% (azopt) in patients with primary open-angle glaucoma or ocular hypertension', The Brinzolamide Long-Term Therapy Study Group, Am J Ophthalmol, Feb 2000, Vol. 129(2), pp. 136-143.

Maren, T.-H., Conroy, C.-W., Wynns, G.-C., Levy, N.-S., 'Ocular Absorption, Blood Levels, and Excretion of Dorzolamide, a Topically Active Carbonic Anhydrase Inhibitor', Journal of Ocular Pharmacology and Therapeutics, 1997, Vol. 13(1), pp. 23-30.

Martens-Lobenhoffer, J., Banditt, P., 'Clinical pharmacokinetics of dorzolamide', Clin Pharmacokinet, 2002, Vol. 41(3), pp. 197-205.

Matuszewski, B.-K., Constanzer, M.-L., 'Indirect chiral separation and analyses in human biological fluids of the stereoisomers of a thienothiopyran-2-sulfonamide (TRUSOPT), a novel carbonic anhydrase inhibitor with two chiral centers in the molecule', Chirality, 1992, Vol. 4, pp. 515-519.

McMullen, R.-J.-Jr., Davidson, M.-G., Gilger, B.-C., 'The effect of 1% tropicamide-induced mydriasis and cycloplegia on spherical refraction of the adult horse', Vet Ophthalmol, Mar 2014, Vol. 17(2), pp. 120-125.

Meyer, S.-M., Fraunfelder, F.-T., 'Phenylephrine hydrochloride', Ophthalmology, Nov 1980, Vol. 87(11), pp. 1177-1180.

Mitchell, A., Hall, R.-W., Erickson, S.-W., Yates, C., Hendrickson, H., 'Systemic Absorption of Cyclopentolate and Adverse Events After Retinopathy of Prematurity Exams', Current Eye Research, 2016, Vol. 41(12), pp. 1601-1607.

Naageshwaran, V., Ranta, V.-P., Gum, G., Bhoopathy, S., Urtti, A., Del Amo, E.-M., 'Comprehensive Ocular and Systemic Pharmacokinetics of Brinzolamide in Rabbits After Intracameral, Topical, and Intravenous Administration', J Pharm Sci, Jan 2021, Vol. 110(1), pp. 529-535.

NCBI National Center for Biotechnology Information. PubChem Compound Summary for CID 6041, Phenylephrine. Accessed August 2023a, available from: https://pubchem.ncbi.nlm.nih.gov/compound/Phenylephrine

NCBI National Center for Biotechnology Information. PubChem Compound Summary for CID 5593, Tropicamide. Accessed August 2023b, available from: https://pubchem.ncbi.nlm.nih.gov/compound/Tropicamide

Negri, L., Ferreras, A., Iester, M., 'Timolol 0.1% in Glaucomatous Patients: Efficacy, Tolerance, and Quality of Life', J Ophthalmol, May 2019, pp. 4146124.

NTP National Toxicology Program, 'NTP Toxicology and Carcinogenesis Studies of Phenylephrine Hydrochloride (CAS No. 61-76-7) in F344/N Rats and B6C3F1 Mice (Feed Studies)', Natl Toxicol Program Tech Rep Ser, 1987 Vol 322, pp. 1-172.

Ormrod, D., McClellan, K., 'Topical dorzolamide 2%/timolol 0.5%: a review of its use in the treatment of open-angle glaucoma', Drugs Aging, Dec 2000, Vol. 17(6), pp. 477-496.

Padjasek, M., Qasem, B., Cislo-Pakuluk, A., Marycz, K., 'Cyclosporine A Delivery Platform for Veterinary Ophthalmology—A New Concept for Advanced Ophthalmology', Biomolecules, 2022, Vol. 12(10), p. 1525.

Panarese, V., Moshirfar, M., 'Pilocarpine', StatPearls, Treasure Island (FL), 2023. Accessed September 2023, available from: https://www.ncbi.nlm.nih.gov/books/NBK556128/

Pandit, R.-J., Taylor, R., 'Mydriasis and glaucoma: exploding the myth. A systematic review', Diabet Med, Oct 2000, Vol. 17(10), pp. 693-699.

Perova, I.-B., Eller, K.-I., Musatov, A.-V., Tymolskaya, E.-V., '[Synephrine in dietary supplements and specialized foodstuffs: biological activity, safety and methods of analysis]', Vopr Pitan, 2021, Vol. 90(6), pp. 101-113.

Plumb, D.-C., (Ed.), 'Plumb's Veterinary Drug Handbook', Wiley Blackwell, 2015, Edition 8, p. 1175.

Pokrywka, A., Skrzypiec-Spring, M., Krzywanski, J., Rynkowski, M., Saugy, M., Faiss, R., 'Case reports: Unintended anti-doping rule violation after dorzolamide use several months prior to a doping control', Drug Test Anal, 2021, Vol. 13, pp. 1803–1806.

Pumphrey, S.-A., Moon, Y., Francis, J.-M., Williams-Fritze, M.-J., Lee-Parritz, D., 'Duration of Mydriasis Produced by 0.5% and 1% Tropicamide in Sprague-Dawley Rats', J Am Assoc Lab Anim Sci, Sep 2021, Vol. 60(5), pp. 576-581.

Robertson, S.-A., Green, S.-L., Carter, S.-W., Bolon, B.-N., Brown, M.-P., Shields, R.-P., 'Postanesthetic recumbency associated with hyperkalemic periodic paralysis in a quarter horse', J Am Vet Med Assoc, Oct 1992, Vol. 201(8), pp. 1209-1212.

Ritschel, W.-A., Paulos, C., Arancibia, A., Agrawal, M.-A., Wetzelsberger, K.-M., Lücker, P.-W., 'Pharmacokinetics of acetazolamide in healthy volunteers after short- and long-term exposure to high altitude', J Clin Pharmacol, 1998a, Vol. 38(6), pp. 533-539; quoted from: Ritschel, W.-A., 'Pharmacokinetik parameters of important drugs', In 'Handbook of Basic Pharmacokinetics', Hamilton, IL, Drug Intelligence Publications, 1992, p. 533.

Ritschel, W.-A., Paulos, C., Arancibia, A., Agrawal, M.-A., Wetzelsberger, K.-M., Luecker, P.-W., 'Urinary excretion of acetazolamide in healthy volunteers after short- and long-term exposure to high altitude', Methods Find Exp Clin Pharmacol, 1998b, Vol. 20(2), pp. 133-137.

Roman, M.-C., Betz, J.-M., Hildreth, J., 'Determination of synephrine in bitter orange raw materials, extracts, and dietary supplements by liquid chromatography with ultraviolet detection: single-laboratory validation', J AOAC Int, 2007, Vol. 90(1), pp. 68-81.

Roy, L.-F., Dufresne, L.-R., Legault, L., Long, H., Morin, C., 'Acetazolamide in hemodialysis patients: a rational use after ocular surgery', Am J Kid Dis, 1992, Vol. 20, pp. 650-652.

Rozette, N.-A., Matragoon, S., Sethi, S., Goei, S., Manasco, K.-B., El-Remessy, A.-B., 'Systemic Effects of Ophthalmic Cyclopentolate on Body Weight in Neonatal Mice', Neonatology, 2014, Vol. 106, pp. 37-41.

Salminen, L., 'Review: Systemic Absorption of Topically Applied Ocular Drugs in Humans', Journal of Ocular Pharmacology, 1990, Vol. 6(3), pp. 243-249.

Scriba, G.-K.-E., In Sweetman, C., (Ed.), 'Martindale: The Complete Drug Reference', Pharmaceutical Press, London, 2009, Edition 36.

Selk Ghaffari, M., Sabzevari, A., Radmehr, B., 'Effect of topical 1% tropicamide on Schirmer tear test results in clinically normal horses', Vet Ophthalmol, 2009, Vol. 12(6), pp. 369-371.

Shoji, N., 'Brinzolamide: efficacy, safety and role in the management of glaucoma', Expert Review of Ophthalmology, 2007, Vol. 2(5), pp. 695-704.

Shukralla, A.-A., Dolan, E., Delanty, N., 'Acetazolamide: Old drug, new evidence?', Epilepsia Open, 2022, Vol. 7, pp. 378-392.

Silver, L.-H., 'Dose-response evaluation of the ocular hypotensive effect of brinzolamide ophthalmic suspension (Azopt)', Brinzolamide Dose-Response Study Group', Surv Ophthalmol, Jan 2000, Vol. 44(2), pp. S147-153.

Smith, P.-J., Samuelson, D.-A., Brooks, D.-E., Whitley, R.-D., 'Unconventional aqueous humor outflow of microspheres perfused into the equine eye', Am J Vet Res, Nov 1986, Vol. 47(11), pp. 2445-2453.

Spier, S.-J., Carlson, G.-P., Holliday, T.-A., Cardinet, G.-H., Pickar, J.-G., 'Hyperkalemic periodic paralysis in horses', J Am Vet Med Assoc, Oct 1990, Vol. 197(8), pp. 1009-1017.

Spiess, B.-M., 'Zur equinen rezidivierenden Uveitis (ERU) [Equine recurrent uveitis]', Schweiz Arch Tierheilkd, 1997, Vol. 139(3), pp. 126-133.

Stillwell, M.-E., Saady, J.-J., 'Use of tetrahydrozoline for chemical submission', Forensic Sci Int, 2012 Vol. 221(1-3), pp. 12-16.

Straughn, A.-B., Gollamudi, R., Meyer, M.-C., 'Relative bioavailability of acetazolamide tablets, Biopharm Drug Dispos, 1982, Vol. 3(1), pp. 75-82.

Strey, K., 'How Toxic indeed is Oxymetazoline? Collection and Comparison of Toxicological Data of Pharmacologically Active Imidazoline Derivatives', Preprints 2020.

Suzuki, G., Kunikane, E., Shigemi, W., Shinno, K., Kozai, S., Kurata, M., Kawamura, A., 'Ocular and systemic pharmacokinetics of brimonidine and brinzolamide after topical administration in rabbits: comparison between fixed-combination and single-drug formulations', Current Eye Research, 2021, Vol. 46(3), pp. 380-386.

Tabery, H.-M., 'Toxic effect of rose bengal dye on the living human corneal epithelium', Acta Ophthalmol Scand, 1998, Vol. 76(2), pp. 142-145.

Thomson, A.-W., Bonham, C.-A., Zeevi, A., 'Mode of action of tacrolimus (FK506): molecular and cellular mechanisms', Ther Drug Monit, 1995, Vol. 17(6), pp. 584-591.

Tocco, D.-J., Duncan, A.-E., Delauna, F.-A., Hucker, H.-B., Gruber, V.-F., Vandenheuvel, W.-J, 'Physiological disposition and metabolism of timolol in man and laboratory animals', Drug Metab Dispos, 1975, Vol. 3(5), pp. 361-370.

Tofflemire, K.-L., Whitley, E.-M., Flinn, A.-M., Dufour, V.-L., Ben-Shlomo, G., Allbaugh, R.-A., Griggs, A.-N., Peterson, C.-S., Whitley, D.-R., 'Effect of topical ophthalmic dorzolamide(2%)-timolol(0.5%) solution and ointment on intraocular pressure in normal horses', Vet Ophthalmol, Nov 2015, Vol. 18(6), pp. 457-461.

Tofflemire, K., Whitley, E.-M., Allbaugh, R., Ben-Shlomo, G., Griggs, A., Strong, T., Whitley, R.-D., 'Effect of topical ophthalmic latanoprost 0.005% solution alone and in combination with diclofenac 0.1% solution in healthy horses: a pilot study', Vet Ophthalmol, Sep 2017, Vol. 20(5), pp. 398-404.

Tolar, E.-L., Labelle, A.-L., 'How to Diagnose and Manage Horses With Glaucoma', AAEP Proceedings, 2013, Vol. 59, pp. 174-180.

Tsikas, D., 'Acetazolamide and human carbonic anhydrases: retrospect, review and discussion of an intimate relationship', Journal of Enzyme Inhibition and Medicinal Chemistry, 2024, Vol. 39(1), p. 2291336.

Vandewalle, E., Abegão Pinto, L., Olafsdottir, O.-B., Stalmans, I., 'Phenylephrine 5% added to tropicamide 0.5% eye drops does not influence retinal oxygen saturation values or retinal vessel diameter in glaucoma patients', Acta Ophthalmol, Dec 2013, Vol. 91(8), pp. 733-737.

Van der Woerdt, A., Gilger, B.-C., Wilkie, D.-A., Strauch, S.-M., Orczeck, S.-M., 'Normal variation in, and effect of 2% pilocarpine on, intraocular pressure and pupil size in female horses', Am J Vet Res, Nov 1998, Vol. 59(11), pp. 1459-1462.

Van Minderhout, H.-M., Joose, M.-V., Grootendorst, D.-C., Schalij-Delfos, N.-E., 'Adverse reactions following routine anticholinergic eye drops in a paedriatic population: an observational cohort study', BMJ Open, 2015, Vol. 5(12).

Vengust, M., Staempfli, H., Viel, L., Heigenhauser, G., 'Effects of chronic acetazolamide administration on fluid flux from the pulmonary vasculature at rest and during exercise in horses', Equine Vet J Suppl, Aug 2006, Vol. 36, pp. 508-515.

Vengust, M., Stämpfli, H., De Moraes, A.-N., Teixeiro-Neto, F., Viel, L., Heigenhauser, G., 'Effects of chronic acetazolamide administration on gas exchange and acid-base control in pulmonary circulation in exercising horses', Equine Vet J Suppl, Nov 2010, Vol. 38, pp. 40-50.

Vengust, M., Staempfli, H., Viel, L., Swenson, E.-R., Heigenhauser, G., 'Acetazolamide attenuates transvascular fluid flux in equine lungs during intense exercise', J Physiol, Sep 2013, Vol. 591(18), pp. 4499-4513.

Volotinen, M., Hakkola, J., Pelkonen, O., Vapaatalo, H., Mäenpää, J., 'Metabolism of ophthalmic timolol: new aspects of an old drug', Basic Clin Pharmacol Toxicol, 2011, Vol. 108(5), pp. 297-303.

Vuori, M-L., Kaila, T., Iisalo, E., Saari, K.-M. 'Systemic Absorption and anticholinergic activity of topically applied tropicamide', J Ocul Pharmacol, 1994, Vol. 10(2), pp. 431-437.

Willis, A.-M., Diehl, K.-A., Hoshaw-Woodard, S., Kobayashi, I., Vitucci, M.-P., Schmall, L.-M., 'Effects of topical administration of 0.005% latanoprost solution on eyes of clinically normal horses', Am J Vet Res, Dec 2001a, Vol. 62(12), pp. 1945-1951.

Willis, A.-M., Robbin, T.-E., Hoshaw-Woodard, S., Wilkie, D.-A., Schmall, M.-L., 'Effect of topical administration of 2% dorzlamide hydrochloride or 2% dorzlamide hydrochloride-0.5% timolol maleate on intraocular pressure in clinically normal horses', Am J Vet Res, May 2001b, Vol. 62(5), pp. 709-713.

Willis, A.-M., Diehl, K.-A., Robbin, T.-E., 'Advances in topical glaucoma therapy', Vet Ophthalmol, Mar 2002, Vol. 5(1), pp. 9-17.

Wilson, J.-G., Maren, T.-H., Takana, K., Ellison, A., 'Teratogenic action of carbonic anhydrase inhibitors in the rat', Teratology, 1968, Vol. 1, pp. 51-60.

Wong, B.-K., Bruhin, P.-J., Barrish, A., Lin, J.-H., 'Non-linear dorzolamide pharmacokinetics in rats: Concentration dependent erythrocyte distribution and drug-metabolite displacement interaction', Drug Metabolism and Disposition, 1996, Vol. 24(6), pp. 659-663.

Wong, J.-K.-Y., Choi, T.-L.-S., Kwok, K.-Y., Lei, E.-N.-Y., Wan, T.-S.-M., 'Doping control analysis of 121 prohibited substances in equine hair by liquid chromatography-tandem mass spectrometry', J Pharm Biomed Anal, Sep 2018, Vol. 158, pp. 189-203.

Yakatan, G.-J., Frome, E.-L., Leonard, R.-G., Shah, A.-C., Doluisio, J.-T., 'Bioavailability of acetazolamide tablets', J Pharm Sci, 1978, Vol. 67(2), pp. 252-256.

Yano, I., Takayama, A., Takano, M., Inatani, M., Tanihara, H., Ogura, Y., Honda, Y., Inui, K., 'Pharmacokinetics and pharmacodynamics of acetazolamide in patients with transient intraocular pressure elevation', Eur J Clin Pharmacol, 1998, Vol. 54(1), pp. 63-68.

Yazdani, N., Sadeghi, R., Momeni-Moghaddam, H., Zarifmahmoudi, L., Ehsaei, A., 'Comparison of cyclopentolate versus tropicamide cycloplegia: A systematic review and meta-analysis', J Optom, 2018, Vol. 11(3), pp. 135-143.

Substances for sedation and premedication (and antagonism)

Alef, M., Oechtering, G., 'Anästhesie', In Lutz, H., Kohn, B., Forterre, F., (Eds.), 'Krankheiten der Katze', Enke Verlag, Stuttgart (D), 2003, Edition 3, pp. 615-648.

Anttila, M., Penttilä, J., Helminen, A., Vuorilehto, L., Scheinin, H., 'Bioavailability of dexmedetomidine after extravascular doses in healthy subjects', Br J Clin Pharmacol, 2003, Vol. 56(6), pp. 691-693.

Ballard, S., Shults, T., Kownacki, A.-A., Blake, J.-W., Tobin, T., 'The pharmacokinetics, pharmacological responses and behavioral effects of acepromazine in the horse', J Vet Pharmacol Ther, 1982, Vol. 5(1), pp. 21-31.

Barr, C.-A., Haughan, J., Gianotti, G., Varner, K., Drobatz, K.-J., Stefanovski, D., Robinson, M., Pennigton, M., McGuire, A., M Otto, C., 'Pharmacokinetics and pharmacodynamics of intranasal and intramuscular administration of naloxone in working dogs administered fentanyl', J Intern Vet Med, 2023, Vol. 37(6), pp. 2422-2428.

Bettschart-Wolfensberger, R., Bettschart, R.-W., Vainio, O., Marlin, D., Clarke, K.-W., 'Cardiopulmonary effects of a two-hour medetomidine infusion and its antagonism by atipamezole in horses and ponies', Journal of Veterinary Anaesthesia, 1999, Vol. 26(1), pp. 8-12.

Bettschart-Wolfensberger, R., Kalchofner, K., Neges, K., Kästner, S., Fürst, A., 'Total intravenous anaesthesia in horses using medetomidine and propofol', Veterinary anaesthesia and analgesia, 2005a, Vol. 32(6), pp. 348-354.

Bettschart-Wolfensberger, R., Freeman, S.-L., Bowen, I.-M., Aliabadi, F.-S., Weller, R., Huhtinen, M., Clarke, K.-W., 'Cardiopulmonary effects and pharmacokinetics of IV dexmedetomidine in ponies', Equine Vet J, 2005b, Vol. 37(1), pp. 60-64.

Bidwell, L.-A., 'How to Use Propofol as an Adjunct to General Anesthesia in Foals', AAEP proceedings, 2012, pp. 236-237.

Bol, C., Danhof, M., Stanski, D.-R., Mandema, J.-W., 'Pharmacokinetic-pharmacodynamic characterization of the cardiovascular, hypnotic, EEG and ventilator responses to dexmedetomidine in the rat', J Pharm Exp, 1997, Vol. 283(3), pp. 1051-1058.

Boscan, P., Rezene, M.-L., Grimsrud, K., Stanley, S.-D., Mama, K.-R., Steffey, E.-P., 'Pharmacokinetic profile in relation to anaesthesia characteristics after a 5% micellar microemulsion of propofol in the horse', Br. J. Anaesth., 2010, Vol. 104(3), pp. 330-337.

Breimer, L.-T., Hennis, P.-J., Burm, A.-G., Danhof, M., Bovill, J.-G., Spierdijk, J., Vletter, A.-A., 'Pharmacokinetics and EEG effects of flumazenil in volunteers', Clin Pharmacokinet, 1991, Vol. 20(6), pp. 491-496.

Britch, S.-C., Walsh, S.-L., 'Treatment of opioid overdose: current approaches and recent advances', Psychopharmacology, 2022, Vol. 239(7), pp. 2063-2081.

Bueno, A.-C., Cornick-Seahorn, J., Seahorn, T.-L., Hosgood, G., Moore, R.-M., 'Cardiopulmonary and sedative effects of intravenous administration of low doses of medetomidine and xylazine to adult horses', American Journal of Veterinary Research, 1999, Vol. 60(11), pp. 1371-1376.

Carroll, G.-L., Hartsfield, S.-M., 'General anesthetic techniques in ruminants', Vet Clin North Am Food Anim Pract, 1996, Vol. 12(3), pp. 627-661.

Chamadia, S., Pedemonte, J.-C., Hobbs, L.-E., Deng, H., Nguyen, S., Cortinez, L.-I., Akeju, O., 'A pharmacokinetic and pharmacodynamic study of oral dexmedetomidine', Anesthesiology, Dec 2020, Vol. 133(6), pp. 1223-1233.

De Vries, A., Taylor, P.-M., Troughton, G., Liu, B., Fowden, A.-L., Sear, J.-W., 'Real time monitoring of propofol blood concentration in ponies anaesthetized with propofol and ketamine', J. Vet. Pharmacol. Ther., 2013, Vol. 36(3), pp. 258-266.

Freise, K.-J., Newbound, G.-C., Tudan, C., Clark, T.-P., 'Naloxone reversal of an overdose of a novel, long-acting transdermal fentanyl solution in laboratory Beagles', J Vet Pharmacol Ther, 2012, Vol. 35 (2), pp. 45-51.

Gozalo-Marcilla, M., Gasthuys, F., Luna, S.-P.-L., Schauvliege, S., 'Is there a place for dexmedetomidine in equine anaesthesia and analgesia? A systematic review (2005–2017)', Journal of veterinary pharmacology and therapeutics, 2018, Vol. 41(2), pp. 205-217.

Grimsrud, K.-N., Ait-Oudhia, S., Durbin-Johnson, B.-P., Rocke, D.-M., Mama, K.-R., Rezende, M.-L., Stanley, S.-D., Jusko, W.-J., 'Pharmacokinetic and pharmacodynamic analysis comparing diverse effects of detomidine, medetomidine, and dexmedetomidine in the horse: a population analysis', Journal of veterinary pharmacology and therapeutics, 2015, Vol. 38(1), pp. 24-34.

Hall, L.-W., Clarke, K.-W., Trim, C.-M., 'Principles of sedation, analgesia and premedication', In Hall, L.-W., Clarke, K.-W., Trim, C.-M., (Eds.), 'Veterinary Anaesthesia', WB Saunders, London (UK), 2001, Edition 10, pp. 75-112.

Hashem, A., Kietzmann, M., Scherkl, R., 'The pharmacokinetics and bioavailability of acepromazine in the plasma of dogs', Dtsch Tierarztl Wochenschr, 1992, Vol. 99(10), pp. 396-398.

Hashem, A., Keller, H. 'Disposition, bioavailability and clinical efficacy of orally administered acepromazine in the horse', J Vet Pharmacol Therap, 1993, Vol. 16(3), pp. 359-368.

Hollis, A.-R., Pascal, M., Van Dijk, J., Jolliffe, C., Kaartinen, J., 'Behavioural and cardiovascular effects of medetomidine constant rate infusion compared with detomidine for standing sedation in horses', Vet Anaesth Analg, Jan 2020, Vol. 47(1), pp. 76-81.

Huupponen, R., Karhuvaara, S., Anttila, M., Vuorilehto, L., Scheinin, H., 'Buccal delivery of an alpha 2-adrenergic receptor antagonist, atipamezole, in humans', Clin Pharmacol Ther, 1995, Vol. 58(5), pp. 506-511.

Inchem International Programme on Chemical Safety Poisons Information, 'Naloxone, flumazenil and dantrolene as antidotes', Vol. 1, 1993. Accessed August 2023, available from: https://inchem.org/documents/antidote/antidote/ant01.htm

Inchem International Programme on Chemical Safety Poisons Information, 'Diazepam Monograph 181', 1998. Accessed August 2023, available from: https://inchem.org/documents/pims/pharm/pim181.htm

Jarrett, M.-A., Bailey, K.-M., Messenger, K.-M., Prange, T., Gaines, B., Posner, L.-P., 'Recovery of horses from general anaesthesia after induction with propofol and ketamine versus midazolam and ketamine', Journal of the American Veterinary Medical Association, 2018, Vol. 253(1), pp. 101-107.

Jones, R.-D., Chan, K., Roulson, C.-J., Brown, A.-G., Smith, I.-D., Mya, G.-H., 'Pharmacokinetics of flumazenil and midazolam', Br J Anaesth, 1993, Vol. 70(3), pp. 286-292.

Kaegi, B., 'Anesthesia by injection of xylazine, ketamine and the benzodiazepine derivative climazolam and the use of the benzodiazepine antagonist Ro 15-3505', Schweiz Arch Tierheilkd, 1990, Vol. 132(5), pp. 251-257.

Karhuvaara, S., Kallio, A., Scheinin, M., Anttila, M., Salonen, J.-S., Scheinin, H., 'Pharmacological effects and pharmacokinetics of atipamezole, a novel α2-adrenoceptor antagonist: a randomized, double-blind cross-over study in healthy male volunteers', Br J Clin Pharmacol, 1990, Vol. 30, pp. 97-106.

Klotz, U., Kanto, J., 'Pharmacokinetics and clinical use of flumazenil (Ro 15-1788)', Clin Pharmacokinet, 1988, Vol. 14(1), pp. 1-12.

Knych, H.-K., Seminoff, K., McKemie, D.-S., Kass, P.-H. 'Pharmacokinetics, pharmacodynamics, and metabolism of acepromazine following intravenous, oral and sublingual administration to exercised Thoroughbred horses', J vet Pharmacol Therap, 2018, Vol. 41(4), pp. 522-535.

Kreeger, T.-J., Arnemo, J.-M., J. P. Raath, J.-P., 'Handbook of wildlife chemical immobilization', Wildlife Pharmaceuticals, Fort Collins, Colorado, 2002, International Edition, pp. 409.

López-Romero, B., Evrard, G., Durant, F., Sevrin, M., George, P., 'Molecular structure and stereoelectronic properties of sarmazenil - A weak inverse agonist at the omega modulatory sites (benzodiazepine receptors): Comparison with bretazenil and flumazenil', Bioorg Med Chem, 1998, Vol. 6(10), pp. 1745-1757.

Marland, A., Sarkar, P., Leavitt, R., 'The urinary elimination profiles of diazepam and its metabolites, nordiazepam, temazepam, and oxazepam, in the equine after a 10-mg intramuscular dose', J Anal Toxicol, 1999, Vol. 23(1), pp. 29-34.

Mason, D.-E., 'Anesthetics, tranquillizers and opioid analgesics', In Bertone, J.-J., Horspool, L.-J.-I., (Eds.), 'Equine Clinical Pharmacology', WB Saunders, London (UK), 2004, Edition 1, pp. 267-310.

Morant, J., 'Arzneimittelkompendium der Schweiz 2004', Documed AG, Basel (CH), 2004, Edition 25, pp. 3162.

Muir, W.-W., 'Standing Chemical Restraint in Horses: Tranquilizers, Sedatives and Analgesics', In Muir, W.-W., Hubbell, J.-A.-E., (Eds.), 'Equine Anaesthesia', Mosby - Year Book Inc, St. Louis (USA), 1991, Edition 1, pp. 247-280.

Neudeck, S., Twele, L., Kopp, V., Kästner, S., 'Pharmacodynamics and plasma concentrations of dexmedetomidine with or without vatinoxan as a constant-rate infusion in horses anaesthetized with isoflurane-A pilot study', J Vet Pharmacol Ther, 2021, Vol. 44(5), pp. 754-765.

Nordt, S.-P., Clark, R.-F., 'Midazolam: a review of therapeutic uses and toxicity', J Emerg Med, May-Jun 1997, Vol. 15(3), pp. 357-365.

Pablo, L.-S., Bailey, J.-E., 'Etomidate and Telazol', Vet Clin North Am Small Anim Pract, 1999, Vol. 29(3), pp. 779-792.

Pawson, P., Forsyth, S., 'Anesthetic Agents', In Maddison, J.-E., Page, S.-W., Church, D., (Eds.), 'Small Animal Clinical Pharmacology', WB Saunders, London (UK), 2002, Edition 1, pp. 97-100.

Pertovaara, A., Haapalinna, A., Sirviö, J., Virtanen, R., 'Pharmacological properties, central nervous system effects, and potential therapeutic applications of Atipamezole, a selective α2-adrenoceptor antagonist', CNS Drug Reviews, 2005, Vol. 11(3), pp. 273-288.

Plumb, D.-C., (Ed.), 'Plumb's Veterinary Drug Handbook', PharmaVet Publishing, White Bear Lake (USA), 2002, Edition 4, pp. 960.

Ranheim, B., Risberg, A.-I., Spadavecchia, C., Landsem, R., Haga, H.-A., 'The pharmacokinetics of dexmedetomidine administered as a constant rate infusion in horses', J Vet Pharmacol Ther, 2014, Vol. 38(1), pp. 93-96.

Reinhold, P., Kraus, G., Schluter, E., 'Propofol for anesthesia and short-term sedation. The final word on use in children under three years', Anaesthesist, 1998, Vol. 47(3), pp. 229-237.

Reves, G.-J., Glass, P.-S.-A., Lubarsky, D.-A., 'Nonbarbiturate intravenous anesthetics', In Miller, R.-D., (Eds.), 'Anesthesia', Churchill Livingstone, Philadelphia, USA, 2000, pp. 2228-2272.

Rezende, M.-L., Grimsrud, K.-N., Stanley, S.-D., Steffey, E.-P., Mama, K.-R., 'Pharmacokinetics and pharmacodynamics of intravenous dexmedetomidine in the horse', J Vet Pharmacol Ther, 2015, Vol. 38(1), pp. 15-23.

Riebold, T.-W., Geiser, D.-R., Goble, D.-O., 'Large Animal Anesthesia: principles and techniques', Iowa State University Press, Iowa (USA), 1995, Edition 2, pp. 296.

Rzasa Lynn, R., Galinkin, J.-L., 'Naloxone dosage for opioid reversal: current evidence and clinical implications', Ther Adv Drug Saf, 2018, Vol. 9(1), pp. 63-88.

Scheneiders, F.-I., Noble, G.-K., Boston, R.-C., Dunstan, A.-J., Sillence, M.-N., McKinney, A.-R., 'Acepromazine pharmacokinetics: a forensic perspective', Vet J, 2012, Vol. 194(1), pp. 48-54.

Schenk, I., Machnik, M., Broussou, D., Meuly, A., Roques, B.-B., Lallemand, E., Düe, M., Röttgen, H., Lagershausen, H., Toutain, P.-L., Thevis, M., 'Kinetic disposition of diazepam and its metabolites after intravenous administration of diazepam in the horse: relevance for doping control', J. Vet. Pharmacol. Ther., 2021, Vol. 44(5), pp. 733-744.

Scriba, G.-K.-E., In Sweetman, C., (Ed.) 'Martindale: The Complete Drug Reference', Pharmaceutical Press, London, 2009, Edition 36.

Shane, S.-E., Langston, V.-C., Wills, R.-W., Denney, W.-S., Knych, H., Fontenot, R.-L., Meyer, R.-E., Natalini, C.-C., 'Pharmacokinetics and pharmacodynamics of intravenous continuous rate infusion and repeated intramuscular administration of dexmedetomidine in standing horses', J Vet Pharmacol Ther, 2021, Vol. 44(4), pp. 533-543.

Shini, S., 'A review of diazepam and its use in the horse', Journal of Equine Veterinary Science, 2000, Vol. 20(7), pp. 443-449.

Smith, K., Hopp, M., Mundin, G., Bond, S., Bailey, P., Woodward, J., Bell, D., 'Low absolute bioavailability of oral naloxone in healthy subjects', Int. J. Clin. Pharmacol. Ther., 2012, Vol. 50(5), pp. 360-367.

Tariq, M., Cerny, V., Elfaki, I., Khan, H.-A., 'Effects of subchronic versus acute in utero exposure to dexmedetomidine on foetal developments in rats', Basic Clin Pharmacol Toxicol, 2008, Vol. 103(2), pp. 180-185.

Thurmon, J.-C., Tranquilli, W.-J., Benson, G.-J., 'Injectable Anesthetics', In Thurmon, C.-J., Tranquilli, W.-J., Benson, G.-J., (Eds.), 'Lumb & Jones Veterinary Anesthesia', Williams & Wilkins, Maryland (USA), 1996, Edition 3, pp. 210-240.

Vähä-Vahe, A.-T., 'The clinical effectiveness of atipamezole as a medetomidine antagonist in the dog', J Vet Pharmacol Ther, 1990, Vol. 13(2), pp. 198-205.

Van der Rijt, C.-C., Drost, R.-H., Schalm, S.-W., Schramel, M., 'Pharmacokinetics of flumazenil in fulminant hepatic failure', Eur J Clin Pharmacol, 1991, Vol. 41(5), pp. 501.

Verhaar, N., Kopp, V., Pfarrer, C., Neudeck, S., König, K., Rohn, K., Kästner, S., 'Alpha2 Antagonist Vatinoxan Does Not Abolish the Preconditioning Effect of Dexmedetomidine on Experimental Ischaemia-Reperfusion Injury in the Equine Small Intestine', Animals (Basel), 2023, Vol. 13(17), pp. 2755.

Wahler, B.-M., Lerche, P., Ricco Pereira, C.-H., Bednarski, R.-M., Kukanich, B., Lakritz, J., Aarnes, T.-K., 'Pharmacokinetics and pharmacodynamics of intranasal and intravenous naloxone hydrochloride administration in healthy dogs', Am. J. Vet. Res., 2019, Vol. 80(7), pp. 696-701.

Weerink, M.-A.-S., Struys, M.-M.-R., Hannivoort, L.-N., Barends, C.-R.-M., Absalom, A.-R., Colin, P., 'Clinical pharmacokinetics and pharmacodynamics of dexmedetomidine', Clin Pharmacokinet, 2017, Vol. 56(8), pp. 893-913.

Substances for systemic disorders

Ali-Melkkilä, T., Kanto, J., Iisalo, E., 'Pharmacokinetics and related pharmacodynamics of anticholinergic drugs', Acta Anaesthesiol Scand, 1993, Vol. 37(7), pp. 633-642.

Alwood, A.-J., Downend, A.-B., Brooks, M.-B., Slensky, K.-A., Fox, J.-A., Simpson, S.-A., Waddell, L. S., Baumgardner, J.-E., Otto, C.-M., 'Anticoagulant effects of low-molecular-weight heparins in healthy cats', Journal of veterinary internal medicine, 2007, Vol. 21(3), pp. 378-387.

Armengou, L., Monreal, L., Delgado, M.-Á., Ríos, J., Cesarini, C., Jose-Cunilleras, E., 'Low-molecular-weight heparin dosage in newborn foals', Journal of veterinary internal medicine, 2010, Vol. 24(5), pp. 1190-1195.

Ashkar, H., Adnan, G., Makaryus, A.-N., 'Dobutamine', StatPearls, Treasure Island (FL), Jan 2023, available from: https://www.ncbi.nlm.nih.gov/books/NBK470431/

Aviado, D.-M., Porter, J.-M., 'Pentoxifylline: a new drug for the treatment of intermittent claudication. Mechanism of action, pharmacokinetics, clinical efficacy and adverse effects', Pharmacotherapy, 1984, Vol. 4(6), pp. 297-307.

Aydin, A., Tugcu, G., 'Toxicological assessment of epinephrine and norepinephrine by analog approach', Food Chem Toxicol, 2018, Vol. 118, pp. 726-732.

Beloeil, H., Mazoit, J.-X., Benhamou, D., Duranteau, J., 'Norepinephrine kinetics and dynamics in septic shock and trauma patients', Br J Anaesth, 2005, Vol. 95(6), pp. 782-788.

Boyd, C.-J., Brainard, B.-M., Smart, L., 'Intravenous Fluid Administration and the Coagulation System', 2021, Front Vet Sci, Vol. 8., Article 662504.

Brainard, B.-M., Epstein, K.-L., LoBato, D.-N., Kwon, S., Darien, B.-J., Hurley, D.-J., Moore, J.-N., 'Treatment with aspirin or clopidogrel does not affect equine platelet expression of P selectin or platelet-neutrophil aggregates', Vet Immunol Immunopathol, 2012, Vol. 149(1-2), pp. 119-125.

Brooks, M.-B., Divers, T.-J., Watts, A.-E., Ness, S.-L., Frye, A.-H., Stokol, T., Fubini, S.-L., 'Effects of clopidogrel on the platelet activation response in horses', Am J Vet Res, 2013, Vol. 74(9), pp. 1212-1222.

Carr, E., 'Systemic Inflammatory Response Syndrome', In Sprayberry, K.-A., Robinson, N.-E., (Eds.), 'Robinson's Current Therapy in Equine Medicine', Saunders, St. Louis, Missouri, 2015, Edition 7, pp. 92-96.

Casoni, D., Spadavecchia, C., Adami, C., 'Cardiovascular changes after administration of aerosolized salbutamol in horses: five cases', Acta Veterinaria Scandinavica, 2014, Vol. 56(1), p. 49.

Chu, C.-A., Sindelar, D.-K., Neal, D.-W., Cherrington, A.-D., 'Hepatic and gut clearance of catecholamines in the conscious dog', Metabolism, 1999, Vol. 48(2), pp. 259-263.

Clark-Price, S.-C., Lascola, K.-M., Auckburally, A., Boone, L.-H., Caldwell, F.-J., Weatherall, K.-M., Hanson, R.-R., 'The Effect of Inhaled Albuterol on PaO2 in Anesthetized Horses Receiving a FiO2 of 0.5 or >0.95', J Equine Vet Sci, Jun 2022, Vol. 113, p. 103944.

Corley, K.-T.-T., 'Monitoring and treating haemodynamic disturbances in critically ill neonatal foals. Part 2: Assessment and treatment', Equine Veterinary Education, 2002, Vol. 14(6), pp. 328-336.

Csajka, C., Haller, C.-A., Benowitz, N.-L., Verotta, D., 'Mechanistic pharmacokinetic modelling of ephedrine, norephedrine and caffeine in healthy subjects', Br J Clin Pharmacol, 2005, Vol. 59(3), pp. 335-345.

Dancker, C., Hopster, K., Rohn, K., Kästner, S.-B. 'Effects of dobutamine, dopamine, phenylephrine and noradrenaline on systemic haemodynamics and intestinal perfusion in isoflurane anaesthetised horses', Equine Vet J, January 2018, Vol. 50(1), pp. 104-110.

De la Rebière de Pouyade, G., Grulke, S., Detilleux, J., Salciccia, A., Verwilghen, D.-R., Caudron, I., Gangl, M., Serteyn, D.-D., 'Evaluation of low-molecular-weight heparin for the prevention of equine laminitis after colic surgery', Journal of veterinary emergency and critical care (San Antonio, Tex.:2001), 2009, Vol. 19(1), pp. 113-119.

Deutsch, J., Taylor, P.-M., 'Mortality and morbidity in equine anaesthesia', Equine Veterinary Education, 2022, Vol. 34(3), pp. 152-168.

Divers, T.-J., 'Acute kidney injury and renal failure in horses', Veterinary Clinics: Equine Practice, 2022, Vol. 38(1), pp. 13-24.

Drucker, D.-J. 'Advances in oral peptide therapeutics', Nat Rev Drug Discov, 2020, Vol. 19(4), pp. 277–289.

Drugbank, Dopamine [Updated 12 Feb 2024]. Accessed December 2023, available from: https://go.drugbank.com/drugs/DB00988

Ducharme, N.-G., Fubini, S.-L., 'Gastrointestinal complications associated with the use of atropine in horses', Journal of the American Veterinary Medical Association, 1983, Vol. 182(3), pp. 229-231.

Eldadah, M.-K., Schwartz, P.-H., Harrison, R., Newth, C.-J., 'Pharmacokinetics of dopamine in infants and children', Crit Care Med, 1991, Vol. 19(8), pp. 1008-1011.

European Commission, 'Summary of European Union decisions on marketing authorisations in respect of medicinal products from 1 May 2022 to 31 May 2022', 2022, available from: EUR-Lex - 52022XC0630(10) - NL - EUR-Lex (europa.eu)

Feller, D.-R., Malspeis, L., 'Biotransformation of D(-)-ephedrine and L(+)-ephedrine in the rabbit, in vivo and in vitro', Drug Metab Dispos, 1977, Vol. 5(1), pp. 37-46.

Fletcher, D.-J., Brainard, B.-M., Epstein, K., Radcliffe, R. Divers, T., 'Therapeutic plasma concentrations of epsilon aminocaproic acid and tranexamic acid in horses', J Vet Intern Med, 2013, Vol. 27(6), pp. 1589–1595.

Gad, M.-Z., Azab, S.-S., Khattab, A.-R., Farag, M.-A., 'Over a century since ephedrine discovery: an updated revisit to its pharmacological aspects, functionality and toxicity in comparison to its herbal extracts', Food Funct, 2021, Vol. 12(20), pp. 9563-9582.

Gallanosa, A., Stevens, J.-B., Quick, J., 'Glycopyrrolate', StatPearls, Treasure Island (FL), 2023. Accessed September 2023, available from: https://www.ncbi.nlm.nih.gov/books/NBK526035/

Garcia-Filho, S.-G., de Andrade, F.-S.-R.-M., dos Santos, R.-S.-T., Gonçalves, L.-A. Pereira, M.-A.-A., de Souza, A.-F., Ambrósio, A.-M., Fantoni, D.-T., 'Comparison of Hemodynamic Effects of Dobutamine and Ephedrine Infusions in Isoflurane-Anesthetized Horses', Vet Sci, 2023, Vol. 10, p. 278.

Gratwick, Z., Viljoen, A., Page, P.-C., Goddard, A., Fosgate, G.-T., Lyle, C.-H., 'A comparison of the effects of a 4% modified fluid gelatin and a 6% hydroxyethyl starch on haemodilution, colloid osmotic pressure, haemostasis and renal parameters in healthy ponies', Equine Vet J, 2017, Vol. 49(3), pp. 363-368.

Hayes, G., Benedicenti, L., Mathews, K., 'Retrospective cohort study on the incidence of acute kidney injury and death following hydroxyethyl starch (HES 10% 250/0.5/5:1) administration in dogs (2007-2010)', J Vet Emerg Crit Care (San Antonio), 2016, Vol. 26(1), pp. 35-40.

Heidmann, P., Tornquist, S.-J., Qu, A., Cebra, C.-K., 'Laboratory measures of hemostasis and fibrinolysis after intravenous administration of epsilon-aminocaproic acid in clinically normal horses and ponies', Am J Vet Res, 2005, Vol. 66(2), pp. 313-318.

Hernandez, D., Yeo, W.-M., Brooks, M.-B., Ness, S.-L., Divers, T.-J., Stokol, T., 'Effects of various antiplatelet drugs on ex vivo platelet activation induced by equine herpesvirus type 1', Am J Vets Res, 2016, Vol. 77(12), pp. 1366–1373.

Holmes, C.-L., Landry, D.-W., Granton, J.-T., 'Science Review: Vasopressin and the cardiovascular system part 2 – clinical physiology', Crit Care, 2004, Vol. 8(1), pp. 15-23.

Hovda, T., Love, L., Chiavaccini, L., 'Risk factors associated with hypoxaemia in horses undergoing general anaesthesia: a retrospective study', Equine Veterinary Journal, 2022, Vol. 54(6), pp. 1055-1063.

Ingle-Fehr, J.-E., Baxter, G.-M., 'The effect of oral isoxsuprine and pentoxifylline on digital and laminar blood flow in healthy horses', Veterinary Surgery, 1999, Vol. 28(3), pp. 154-160.

Kashy, B.-K., Podolyak, A., Makarova, N., Dalto, J.-E., Sessler, D.-I., Kurz, A., 'Effect of Hydroxyethyl Starch on Postoperative Kidney Function in Patients Having Noncardiac Surgery', Anesthesiology, 2015, Vol. 121(4), pp. 730-739.

Le Corre, P., Malledant, Y., Tanguy, M., Le Verge, R., 'Steady-state pharmacokinetics of dopamine in adult patients', Crit Care Med, 1993, Vol. 21(11), pp. 1652-1657.

Lewis, S.-R., Pritchard, M.-W., Evans, D.-J., Butler, A.-R., Alderson, P., Smith, A.-F., Roberts, I., 'Colloids versus crystalloids for fluid resuscitation in critically ill people', 2018, Cochrane Database Syst Rev, Vol. 8(8), Article CD000567.

Liu, T., Zhanga, G., Li, S., Wang, Y., Ma, G., Su, Z., 'Analysis of gelatin plasma substitutes in blood based on detection of hydroxyproline derivatives', Artif Cells Blood Substit Immobil Biotechnol, Feb 2011, Vol. 39(1), pp. 19-25.

Loughran, C.-M., Raisis, A.-L., Hosgood, G., Secombe, C.-J., Lester, G.-D., 'The effect of dobutamine and bolus crystalloid fluids on the cardiovascular function of isoflurane-anaesthetised horses', Equine Vet J, May 2017, Vol. 49(3), pp. 369-374.

MacGregor, D.-A., Smith, T.-E., Prielipp, R.-C., Butterworth, J.-F., James, R.-L., Scuderi, P.-E., 'Pharmacokinetics of dopamine in healthy male subjects', Anesthesiology, 2000, Vol. 92(2), pp. 338-346.

Maglione, M., Miotto, K., Iguchi, M., Jungvig, L., Morton, S.-C., Shekelle, P.-G., 'Psychiatric effects of ephedra use: an analysis of food and drug administration reports of adverse events', Am J Psychiatry, 2005, Vol. 162(1), pp. 189-191.

Mahoney, L., Shah, G., Crook, D., Rojas-Anaya, H., Rabe, H., 'A literature review of the pharmacokinetics and pharmacodynamics of dobutamine in neonates', Pediatr Cardiol, 2016, Vol. 37(1), pp. 14-23.

Matsuda, H., Matsuda, K., Muko, R., Oikawa, M.A., Tanaka, A., 'Short term infusion of ultralow-dose dopamine in an adult horse with acute kidney injury: A case report', Veterinary and Animal Science, 2021, Vol. 12, p. 100176.

Maxwell, L., McCarrel, T.-M., Hay, S. Cole, C., 'Pharmacokinetics of aminocaproic acid in thoroughbred horses', J Vet Pharmacol Therap, 2023, Vol. 46(4), pp. 250–256.

Medling, B.-D., Bueno, R., Chambers, C., Neumeister, M.-W., 'The Effect of Vitamin E Succinate on Ischemia Reperfusion Injury', Hand (NY), Vol. 5(1), pp. 60-64.

Mills, P.-C., Smith, N.-C., Harris, R.-C., Harris, P., 'Effect of allopurinol on the formation of reactive oxygen species during intense exercise in the horse', Res Vet Sci, 1997, Vol. 62(1), pp. 11-16.

Mischke, R., Schmitt, J., Wolken, S., Böhm, C., Wolf, P., Kietzmann, M., 'Pharmacokinetics of the low molecular weight heparin dalteparin in cats', Veterinary journal (London, England: 1997), 2012, Vol. 192(3), pp. 299-303.

Mulloy, B., Hogwood, J., Gray, E., Lever, R., Page, C.-P., 'Pharmacology of Heparin and Related Drugs', Pharmacological reviews, 2016, Vol. 68(1), pp. 76-141.

Murata, K., Noda, K., Kohno, K., Samejima, M., 'Bioavailability and pharmacokinetics of oral dopamine in dogs', J Pharm Sci, 1988, Vol. 77(7), pp. 565-568.

Mutter, T.-C., Ruth, C.-A., Dart, A.-B., 'Hydroxyethyl starch (HES) versus other fluid therapies: effects on kidney function', Cochrane Database Syst Rev, 2013, Vol. 23(7).

Neto, F.-J.-T., McDonell, W.-N., Black, W.-D., Duronghphongtorn, S., 'Effects of glycopyrrolate on cardiorespiratory function in horses anesthetized with halothane and xylazine', American journal of veterinary research, 2004, Vol. 65(4), pp. 456-463.

Norris, J.-N., Watson, J.-L., Tablin, F., Kozikowski, T.-A., Knych, H.-K., 'Pharmacokinetics and competitive pharmacodynamics of ADP-induced platelet activation after oral administration of clopidogrel to horses', Am J Vet Res, 2019, Vol. 80(5), pp. 505–512.

NTP National Toxicology Program, 'Toxicology and Carcinogenesis Studies of Ephedrine Sulfate (CAS No. 134-72-5) in F344/N Rats and B6C3F1 Mice (Feed Studies)', Natl Toxicol Program Tech Rep Ser, May 1986, Vol. 307, pp. 1-186.

Onasch, A., Tanzeem, A., Isgro, F., Böninq, D., Strobel, G., 'Effect of intravenous dopamine infusion on plasma concentrations of dopamine and dopamine sulphate in men, during and up to 18 h after infusion', Eur J Clin Pharmacol, 2000, Vol. 55(10), pp. 755-759.

Oualha, M., Tréluyer, J.-M., Lesage, F., de Saint Blanquat, L., Dupic, L., Hubert, P., Spreux-Varoquaux, O., Urien, S., 'Population pharmacokinetics and haemodynamic effects of norepinephrine in hypotensive critically ill children', Br J Clin Pharmacol, 2014, Vol. 78(4), pp. 886-897.

Papich, M.-G., (Ed.), 'Papich Handbook of Veterinary Drugs', Elsevier, 2021, Edition 5.

Parkins, W.-M., Perlmutt, J.-H., Vars, H.-M., 'Dextran, oxypolygelatin and modified fluid gelatin as replacement fluids in experimental hemorrhage', Am J Physiol, 1953, Vol. 173(3), pp. 403–410.

Patel, N., Taki, M., Tunstell, P., Forsey, P., Forbes, B., 'Stability of dobutamine 500 mg in 50 ml syringes prepared using a Central Intravenous Additive Service', Eur J Hosp Pharm, 2012, Vol. 19(1), pp. 52-56.

Pelletier, J.-S., Dicken, B., Bigam, D., Cheung, P.-Y., 'Cardiac effects of vasopressin', J Cardiovasc Pharmacol, 2014, Vol. 64(1), pp. 100-107.

Pickup, M.-E., May, C.-S., Ssendagire, R., Paterson, J.-W., 'The pharmacokinetics of ephedrine after oral dosage in asthmatics receiving acute and chronic treatment', Br J Clin Pharmacol, Feb 1976, Vol. 3(1), pp. 123-134.

Plumb D.-C., (Ed.), Plumb's Veterinary Drug Handbook, Wiley-Blackwell, 2015, Edition 8.

PubChem. Glycopyrrolate. 2023a. Accessed June 2023, available from: https://pubchem.ncbi.nlm.nih.gov/compound/11693

PubChem. Noradrenaline/norepinephrine. 2023b. Accessed June 2023, available from: https://pubchem.ncbi.nlm.nih.gov/compound/439260

Rautakorpi, P., Manner, T., Ali-Melkkilä, T., Kaila, T., Olkkola, K., Kanto, J., 'Pharmacokinetics and oral bioavailability of glycopyrrolate in children', Pharmacol Toxicol, 1998, Vol. 83(3), pp. 132-134.

Reiter, S., Simmonds, H.-A., Webster, D.-R., Watson, A.-R., 'On the metabolism of allopurinol. Formation of allopurinol-1-riboside in purine nucleoside phosphorylase deficiency', Biochem Pharmacol, 1983, Vol. 32(14), pp. 2167-2174.

Rodríguez-Pozo, M.-L., Armengou, L., Monreal, L., Viu, J., Cesarini, C., Jose-Cunilleras, E., 'Evaluation of an oral direct factor Xa inhibitor anticoagulant in healthy adult horses', Journal of veterinary emergency and critical care (San Antonio, Tex.: 2001), 2017, Vol., 27(1), pp. 82-88.

Roscher, K.-A., Failing, K., Moritz, A., 'Inhibition of platelet function with clopidogrel, as measured with a novel whole blood impedance aggregometer in horses', Vet J, 2015, Vol. 203(3), pp. 332-336.

Ross, J., Dallap, B.-L., Dolente, B.-A., Sweeney, R.-W., 'Pharmacokinetics and pharmacodynamics of epsilon-aminocaproic acid in horses', Am J Vet Res, 2007, Vol. 68(9), pp. 1016–1021.

Rumpler, M.-J., Sams, R.-A., Colahan, P., 'Pharmacokinetics of glycopyrrolate following intravenous administration in the horse', J Vet Pharmacol Ther, 2011, Vol. 34(6), pp. 605-8.

Rumpler, M.-J., Kandala, B., Vickroy, T.-W., Hochnaus, G., Sams, R.-A., 'Pharmacokinetics and pharmacodynamics of glycopyrrolate following a continuous-rate infusion in the horse', J Vet Pharmacol Ther, 2014a, Vol. 37(2), pp. 133-44.

Rumpler, M.-J., Colahan, P., Sams, R.-A., 'Pharmacokinetics of glycopyrrolate in Standardbred horses', J Vet Pharmacol Ther, 2014b, Vol. 37(3), pp. 260-8.

Schauvliege, S., Gasthuys, F., 'Drugs for cardiovascular support in anesthetized horses', Vet Clin North Am Equine Pract, April 2013, Vol. 29(1), pp. 19-49.

Schier, M.-F., Raisis, A.-L., Secombe, C.-J., Hosgood, G., Musk, G.-C., Lester, G.-D., 'Effects of dobutamine hydrochloride on cardiovascular function in horses anesthetized with isoflurane with or without acepromazine maleate premedication', Am J Vet Res, December 2016, Vol. 77(12), pp. 1318-1324.

Schwarzwald, C.-C., Feige, K., Wunderli-Allenspach, H., Braun, U., 'Comparison of pharmacokinetic variables for two low-molecular-weight heparins after subcutaneous administration of a single dose to horses', American journal of veterinary research, 2002, Vol. 63(6), pp. 868–873.

Scriba, G.-K.-E., In Sweetman, C., (Ed.) 'Martindale: The Complete Drug Reference', Pharmaceutical Press, London, 2009, Edition 36.

Sever, P.-S., Dring, L.-G., Williams, R.-T., 'The metabolism of (-)-ephedrine in man', Eur J Clin Pharmacol, 1975, Vol. 9(2-3), pp. 193-198.

Singh, S., McDonell, W., Young, S., Dyson, D., 'The effect of glycopyrrolate on heart rate and intestinal motility in conscious horses', Veterinary Anaesthesia and Analgesia, 1997, Vol. 24(1), pp. 14-19.

Sigrist, N.-E., Kälin, N., Dreyfus, A., 'Effects of Hydroxyethyl Starch 130/0.4 on Serum Creatinine Concentration and Development of Acute Kidney Injury in Nonazotemic Cats', J Vet Intern Med, 2017a, Vol. 31(6), pp. 1749-1756.

Sigrist, N.-E., Kälin, N., Dreyfus, A., 'Changes in Serum Creatinine Concentration and Acute Kidney Injury (AKI) Grade in Dogs Treated with Hydroxyethyl Starch 130/0.4 From 2013 to 2015', J Vet Intern Med, 2017b, Vol. 31(2), pp. 434-441.

Tashkin, D.-P., Gross, N.-J., 'Inhaled glycopyrrolate for the treatment of chronic obstructive pulmonary disease', Int J Chron Obstruct Pulmon Dis, 2018, Vol. 12(13), pp. 1873-1888.

Trim, C.-M., Moore, J.-N., Clark, E.-S., 'Renal effects of dopamine infusion in conscious horses', Equine Vet J, 1989, Vol. 21, pp. 124–128.

Van Galen, G., Hallowell, G., 'Hydroxyethyl starches in equine medicine', J Vet Emerg Crit Care (San Antonio), 2019, Vol. 29(4), pp. 349-359.

Watts, A.-E., Ness, S.-L., Divers, T.-J., Fubini, S.-L., Frye, A.-H., Stokol, T., Cummings, K.-J., Brooks, M.-B., 'Effects of clopidogrel on horses with experimentally induced endotoxemia', Am J Vet Res, 2014, Vol. 75(8), pp. 760-769.

Welling, P.-G., Lee, K.-P., Patel, J.-A., Walker, J.-E., Wagner, J.-G., 'Urinary excretion of ephedrine in man without pH Control following oral administration of three commercial ephedrine sulphate preparations', J Pharm Sci, 1971, Vol. 60(11), pp. 1629-1634.

Whelchel, D.-D., Tennent-Brown, B.-S., Giguère, S., Epstein, K.-L., 'Pharmacodynamics of multi-dose low molecular weight heparin in healthy horses', Veterinary surgery, 2013, Vol. 42(4), pp. 448-454.

Yan, M., Webster, L.-T., Blumer, J.-L., '3-O-methyldobutamine, a major metabolite of dobutamine in humans', Drug Metab Dispos, 2002, Vol. 30(5), pp. 519-524.

Zarolisnki, J.-F., Possley, L.-H., Schwartz, R.-A., Morris, R.-N., Carone, F.-A., Browne, R.-K., 'The pharmacology and subacute toxicology of dopamine', Proc R Soc Med, 1977, Vol. 70(2), pp. 2-6.

Substances for tumours

Allen, J.-W., Poorman, P.-A., Backer, L.-C., Gibson, J.-B., Westbrook-Collins, B., Moses, M.-J., 'Synaptonemal complex damage as a measure of genotoxicity at meiosis', Cell biology and toxicology, 1988, Vol. 4(4), pp. 487-494.

Berruex, F., Gerber, V., Wohlfender, F.-D., Burger, D., Koch, C., 'Clinical course of sarcoids in 61 Franches-Montagnes horses over a 5–7 year period', Vet Q, 2016, Vol. 36, pp. 189-196.

Bose, A., Surugihalli, C., Pande, P., Champeil, E., Basu, A.-K., 'Comparative Error-Free and Error-Prone Translesion Synthesis of N(2)-2'-Deoxyguanosine Adducts Formed by Mitomycin C and Its Metabolite, 2,7-Diaminomitosene, in Human Cells', Chemical research in toxicology, 2016, Vol. 29(5), pp. 933-939.

Carr, E.-A., 'Skin Conditions Amenable to Surgery 28' In Equine Surgery-E-Book: Equine Surgery-E-Book, 2018, p. 425.

Clode, A.-B., Miller, C., McMullen, R.-J., Gilger, B.-C., 'A retrospective comparison of surgical removal and subsequent CO2 laser ablation versus topical administration of mitomycin C as therapy for equine corneolimbal squamous cell carcinoma', Veterinary ophthalmology, 2012, Vol. 15(4), pp. 254-262.

den Hartigh, J., McVie, J.-G., van Oort, W.-J., Pinedo, H.-M. 'Pharmacokinetics of mitomycin C in humans', Cancer research, 1983, Vol. 43(10), pp. 5017–5021.

Di Bucchianico, S., Gliga, A.-R., Åkerlund, E., Skoglund, S., Wallinder, I.O., Fadeel, B., Karlsson, H.-L., 'Calcium-dependent cyto- and genotoxicity of nickel metal and nickel oxide nanoparticles in human lung cells', Part Fibre Toxicol, 2018, Vol. 15(1), p. 32.

DocCheck Flexikon – Mitomycin C. Last updated 2024. DocCheck Community GmbH. Accessed May 2024, available from: https://flexikon.doccheck.com/de/Mitomycin_C

Drugbank, Mitomycin, [Updated May 2024]. Accessed May 2024, available from: https://go.drugbank.com/drugs/DB00305

ECHA, Substance Infocard – Mitomycin. Accessed May 2024, available from: https://echa.europa.eu/de/substance-information/-/substanceinfo/100.000.008

EPA Environmental Protection Agency, 'Evaluation of the potential carcinogenicity of mitomycin', 2002. Accessed May 2024, available from: https://cfpub.epa.gov/si/si_public_record_Report.cfm?Lab=ORD&dirEntryId=35230&CFID=18176928&CFTOKEN=55356861

Haspeslagh, M., Vlaminck, L.-E., Martens, A.-M., 'Treatment of sarcoids in equids: 230 cases (2008–2013)', Journal of the American Veterinary Medical Association, 2016, Vol. 249(3), pp. 311-318.

Hollis, A.-R., 'Management of equine sarcoids', The Veterinary Journal, 2023, Vol. 291, p. 105926.

Igarashi, M., Nagata, M., Itoh, S., Yamoto ,T., Tsuda, S., 'Relationship between DNA damage and micronucleus in mouse liver', J Toxicol Sci, 2010, Vol. 35(6), pp. 881-889.

Jindra, C., Hainisch, E.-K., Brandt, S., 'Immunotherapy of equine sarcoids—from early approaches to innovative vaccines', Vaccines, 2023, Vol. 11(4), p. 769.

Karalus, W., Subharat, S., Orbell, G., Vaatstra, B., Munday, J.-S., 'Equine sarcoids: A clinicopathologic study of 49 cases, with mitotic count and clinical type predictive of recurrence', Veterinary Pathology, 2024, Vol. 61(3), pp. 357-365.

King, M.-T., Wild, D., 'Transplacental mutagenesis: The micronucleus test on fetal mouse blood'. Hum Genet, 1979, Vol. 51, pp. 183-194.

Knottenbelt, D.-C., 'The Equine Sarcoid: Why Are There so Many Treatment Options?', Vet Clin North Am Equine Pract, 2019, Vol. 35(2), pp. 243–262.

Knox, C., Wilson, M., Klinger, C.-M., Franklin, M., Oler, E., Wilson, A., Pon, A., Cox, J., Chin, N.-E.-L., Strawbridge, S.-A., Garcia-Patino, M., Kruger, R., Sivakumaran, A., Sanford, S., Doshi, R., Khetarpal,

N., Fatokun, O., Doucet, D., Zubkowski, A., Rayat, D.-Y., […] Wishart, D.-S., 'DrugBank 6.0: the DrugBank Knowledgebase for 2024', Nucleic acids research, 2024, Vol. 52(D1), pp. D1265–D1275.

Luo, Q., Li, Y., Zhang, Z., 'A systematic assessment of genotoxicity on pivaloylacylation-7ADCA-a wide existing antibiotic impurity', Int J Clin Exp Med, 2014, Vol. 7(11), pp. 4260-4271.

Malalana, F., Knottenbelt, D.-C., McKane, S., 'Mitomycin C, with or without surgery, for the treatment of ocular squamous cell carcinoma in horses', Vet Rec, 2010, Vol. 167, pp. 373-376.

Malviya, V.-K., Young, J.-D., Boike, G., Gove, N., Deppe, G., 'Pharmacokinetics of mitomycin-C in plasma and tumor tissue of cervical cancer patients and in selected tissues of female rats', Gynecologic oncology, 1986, Vol. 25(2), pp. 160-170.

Munday, J.-S., Orbell, G., Fairley, R.-A., Hardcastle, M., Vaatstra, B., 'Evidence from a series of 104 equine sarcoids suggests that most sarcoids in New Zealand are caused by bovine papillomavirus type 2, although both BPV1 and BPV2 DNA are detectable in around 10% of sarcoids', Animals, 2021, Vol. 11(11), p. 3093.

Nogueira, S.-A., Torres, S.-M., Malone, E.-D., Diaz, S.-F., Jessen, C., Gilbert, S., 'Efficacy of imiquimod 5% cream in the treatment of equine sarcoids: a pilot study', Veterinary dermatology, 2006, Vol. 17(4), pp. 259-265.

NTP. Mitomycin-C (50-07-7). Chemical Effects in Biological Systems (CEBS). Research Triangle Park, NC (USA): National Toxicology Program (NTP). Genetic Toxicology. Accessed May 2024, available from: https://cebs.niehs.nih.gov/cebs/test_article/50-07-7

Ogłuszka, M., Starzyński, R.-R., Pierzchała, M., Otrocka-Domagała, I., Raś, A., 'Equine Sarcoids - Causes, Molecular Changes, and Clinicopathologic Features: A Review', Veterinary Pathology, 2021, Vol. 58(3), pp. 472-482.

Offer, K.-S., Dixon, C.-E., Sutton, D.-G.-M., 'Treatment of equine sarcoids: A systematic review', Equine Vet J, 2024, Vol. 56(1), pp. 12–25.

Oliveira, V.-C., Constante, S.-A.-R., Polloni, L., Capelari-Orsolin, P., Silva-Oliveira, R.-G., Moreira-Machado, N., José de Oliveira-Júnior, R., Nepomuceno, J.-C., 'Protective effect of aspirin against mitomycin C-induced carcinogenicity, assessed by the test for detection of epithelial tumor clones (warts) in Drosophila melanogaster', Drug and Chemical Toxicology, Jul 2018, Vol. 41(3), pp. 330-337.

Parton, J.-W., Garriott, M.-L., 'An evaluation of micronucleus induction in bone marrow and in hepatocytes isolated from collagenase perfused liver or from formalin-fixed liver using four-week-old rats treated with known clastogens', Environmental and molecular mutagenesis, 1997, Vol. 29(4), pp. 379-385.

Pettersson, C.-M., Broström, H., Humblot, P., Bergvall, K.-E., 'Topical treatment of equine sarcoids with imiquimod 5% cream or Sanguinaria canadensis and zinc chloride–an open prospective study', Veterinary dermatology, 2020, Vol. 31(6), pp. 471-e126.

PubChem – Mitomycin C (annotation). Data source: Hazardous Substances Data Bank (HSDB). Last updated 2002. Accessed May 2024, available from: https://pubchem.ncbi.nlm.nih.gov/source/hsdb/3239#section=Human-Toxicity-Excerpts-%28Complete%29

Rayner, S.-G., Van Zyl, N., 'The use of mitomycin C as an adjunctive treatment for equine ocular squamous cell carcinoma', Aust Vet J, 2006, Vol. 84(1-2), pp. 43-46.

Schilcher, R.-B., Young, J.-D., Ratanatharathorn, V., Karanes, C., Baker, L.-H. 'Clinical pharmacokinetics of high-dose mitomycin C', Cancer chemotherapy and pharmacology, 1984, Vol. 13(3), pp. 186-190.

Shimada, K., Yamamoto, M., Takashima, M., Wako, Y., Kawasako, K., Aoki, Y., Seki, J., Miyamae, Y., Wakat, A., 'Repeated-dose liver micronucleus assay of mitomycin C in young adult rats', Mutation Research, 2015, Vol. 780-781, pp. 85-89.

Stewart, A.-A., Rush, B., Davis, E., 'The efficacy of intratumoural 5-fluorouracil for the treatment of equine sarcoids', Aust Vet J, 2006, Vol. 84(3), pp. 101–106.

Suzuki, H., Takasawa, H., Kobayashi, K., Terashima, Y., Shimada, Y., Ogawa, I., Tanaka, J., Imamura, T., Miyazaki, A., Hayashi, M., 'Evaluation of a liver micronucleus assay with 12 chemicals using young rats (II): a study by the Collaborative Study Group for the Micronucleus Test/Japanese Environmental Mutagen Society–Mammalian Mutagenicity Study Group', Mutagenesis, 2009, Vol. 24(1), pp. 9-16.

Syed, T.-A., 'A review of the applications of imiquimod: a novel immune response modifier', Expert opinion on pharmacotherapy, 2001, Vol. 2(5), pp. 877-882.

Takasawa, H., Suzuki, H., Ogawa, I., Shimada, Y., Kobayashi, K., Terashima, Y., Matsumoto, H., Aruga, C., Oshida, K., Ohta, R., Imamura, T., Miyazaki, A., Kawabata, M., Minowa, S., Hayashi, M., 'Evaluation of a liver micronucleus assay in young rats (III): A study using nine hepatotoxicants by the Collaborative Study Group for the Micronucleus Test (CSGMT)/Japanese Environmental Mutagen Society (JEMS)–Mammalian Mutagenicity Study Group (MMS)', Mutation Research, 2010, Vol. 69, pp. 830-837.

Tanimura, T., 'Effects of mitomycin C administered at various stages of pregnancy upon mouse fetuses', Okajimas Folia Anat Jpn, 1968, Vol. 44(5), pp. 337-355.

Torres, S.-M., Malone, E.-D., White, S.-D., Koch, S.-N., Watson, J.-L., 'The efficacy of imiquimod 5% cream (Aldara) in the treatment of aural plaque in horses: a pilot open-label clinical trial', Veterinary dermatology, 2010, Vol. 21(5), pp. 503-509.

Witt, K.-L., Knapton, A., Wehr, C.-M., Hook, G.-J., Mirsalis, J., Shelby, M.-D., MacGregor, J.-T., 'Micronucleated erythrocyte frequency in peripheral blood of B6C3F1 mice from short-term, prechronic, and chronic studies of the NTP carcinogenesis bioassay program', Environ Molec Mutagen, 2000, Vol. 36, pp. 163-194.

Z. Miscellaneous

Badgujar, P.-C., Selkar, N.-A., Chandratre, G.-A., Pawar, N.-N., Dighe, V.-D., Bhagat, S.-T., Telang, A.-G., Vanage, G.-R., 'Fipronil-induced genotoxicity and DNA damage in vivo: Protective effect of vitamin E', Hum Exp Toxicol, 2017, Vol. 36(5), pp. 508-519.

Balbi, A., Muscettola, G., Staiano, N., Martire, G., De Lorenzo, F., 'Psychotropic drugs: evaluation of mutagenic effect', Pharmacol Res Commun, 1980, Vol. 12(5), pp. 423–431.

Besret, L., Debruyne, D., Rioux, P., Bonvalot, T., Moulin, M., Zarifian, E., Baron, J.-C., 'A comprehensive investigation of plasma and brain regional pharmacokinetics of imipramine and its metabolites during and after chronic administration in the rat', J Pharm Sci, 1996, Vol. 85(3), pp. 291-295.

Bishun, N., Smith, N., Williams, D.-C., 'Lack of in vivo cytogenetic effects of imipramine in rats', Teratology, 1974, Vol. 10(2), pp. 191–192.

Blodgett, D.-J., 'Fescue Toxicosis' Vet Clin North Am Equine Pract, 2001, Vol. 17(3), pp. 567-577.

Boothe, D.-M., 'Drugs Affecting Gastrointestinal Function' In Adams, H.-R., (Ed.), 'Veterinary Pharmacology and Therapeutics', Wiley-Blackwell, Ames, Iowa (USA), 2001, Edition 8, pp. 1041-1063.

Brès, J., Bressolle, F., 'Pharmacokinetics of sulpiride in humans after intravenous and intramuscular administrations', J Pharm Sci, 1991, Vol. 80(12), pp. 1119-1124.

Brogden, R.-N., Carmine, A.-A., Heel, R.-C., Speight, T.-M., Avery, G.-S., 'Domperidone. A review of its pharmacological activity, pharmacokinetics and therapeutic efficacy in the symptomatic treatment of chronic dyspepsia and as an antiemetic', Drugs, 1982, Vol. 24, pp. 360-400.

Brown, T.-C., 'Tricyclic antidepressant overdosage: experimental studies on the management of circulatory complications', Clin Toxicol, 1976, Vol. 9(2), pp. 255-272.

Caley, C.-F., Weber, S.-S., 'Sulpiride: an antipsychotic with selective dopaminergic antagonist properties', Ann Pharmacother, 1995, Vol. 29(2), pp. 152-160.

Cann, P.-A., Read, N.-W., Holdsworth, C.-D., 'Galactorrhoea as side effect of domperidone', Br Med J (Clin Res Ed), 1983, Vol. 286(6375), pp. 1395-1396.

Chang, Y.-N., Tsai, T.-H., 'Preclinical Transplacental Transfer and Pharmacokinetics of Fipronil in Rats', Drug Metab Dispos, 2020, Vol. 48(10), pp. 886–893.

Chavatte-Palmer, P., Arnaud, G., Duvaux-Ponter, C., Brosse, L., Bougel, S., Daels, P., Guillaume, D., Clément, F., Palmer, E., 'Quantitative and qualitative assessment of milk production after pharmaceutical induction of lactation in the mare', J Vet Intern Med, 2002, Vol. 16(4), pp. 472-477.

Cordel, C., Swan, G.-E., Mülders, M.-S., Bertschinger, H.-J., 'Pharmacokinetics of intravenous imipramine hydrochloride in cattle', J Vet Pharmacol Ther, 2001, Vol. 24(2), pp. 143-145.

Cordelli, E., Keller, J., Eleuteri, P., Villani, P., Ma-Hock, L., Schulz, M., Landsiedel, R., Pacchierotti, F., 'No genotoxicity in rat blood cells upon 3- or 6-month inhalation exposure to CeO2 or BaSO4 nanomaterials', Mutagenesis, 2017, Vol. 32(1), pp. 13–22.

Corrias, F., Atzei, A., Taddeo, R., Arru, N., Casula, M., Salghi, R., Russo, M., Angioni, A., 'Fipronil and Fipronil Sulfone Distribution in Chicken Feathers and Eggs after Oral and Dermal Exposure', Foods, 2021, Vol. 10(12), pp. 3077.

Cravedi, J.-P., Delous, G., Zalko, D., Viguié, C., Debrauwer, L., 'Disposition of fipronil in rats', Chemosphere, 2013, Vol. 93 (10), pp. 2276–2283.

Da Silva, A.-S., Tonin, A.-A., Lopes, L.-S., 'Outbreak of Lice in Horses: Epidemiology, Diagnosis, and Treatment', Journal of Equine Veterinary Science, 2013. Vol. 33(7), pp. 530-532.

Das, P.-C., Cao, Y., Cherrington, N., Hodgson, E., Rose, R.-L., 'Fipronil induces CYP isoforms and cytotoxicity in human hepatocytes', Chem Biol Interact, 2006, Vol. 164(3), pp. 200–214.

De Briyne, N., Holmes, D., Sandler, I., Stiles, E., Szymanski, D., Moody, S., Neumann, S., Anadón, A., 'Cannabis, Cannabidiol Oils and Tetrahydrocannabinol-What Do Veterinarians Need to Know?', Animals (Basel), Mar 2021, Vol. 11(3), p. 892.

Dos Santos, G.-C.-M., Scott, F.-B., Campos, D.-R., de Sousa Magalhães, V., Borges, D.-A., Miranda, F.-R., Alves, M.-C.-C., Pereira, G.-A., Moreira, L.-O., Lima, E.-A.-S., da Silva Rocha, M.-B., Cid, Y.-P., 'Oral pharmacokinetic profile of fipronil and efficacy against flea and tick in dogs', J Vet Pharmacol Ther, 2022, Vol. 45(1), pp. 23–33.

DPR California Department of Pesticide Regulation, 'Response to the Fipronil Task Force Request for Reconsideration of Findings in DPR's Draft Human Exposure Assessment for Fipronil', 2022, available from: https://www.cdpr.ca.gov/index.htm

Drugbank, Imipramine [Updated 21 March 2024]. Accessed March 2024, available from: https://go.drugbank.com/drugs/DB00458

Dündaröz, R., Türkbay, T., Sürer, I., Gök, F., Denli, M., Baltaci, V., 'DNA damage in children treated with imipramine for primary nocturnal enuresis', Pediatr Int, 2002, Vol. 44(6), pp. 617–621.

ECHA, 'Assessment Report Fipronil Product-type PT18 (insecticides, acaricides and products to control other arthropods)', 2011, available from: https://echa.europa.eu/information-on-chemicals/biocidal-active-substances/-/disas/factsheet/33/PT18

ECHA C&L. Notified Classification and Labelling – Fipronil. Accessed January 2024a, available from: https://echa.europa.eu/de/information-on-chemicals/cl-inventory-database/-/discli/details/38738

ECHA, Registration Dossier – Sulpiride. Accessed March 2024c available from: https://chem.echa.europa.eu/100.036.124/dossier-list/reach/dossiers/active?searchText=sulpiride

ECHA, Substance Info Card – Imipramine. Accessed January 2024b, available from: https://echa.europa.eu/de/substance-information/-/substanceinfo/100.000.039

EMA/465179/2014, 'Restrictions on the use of domperidone-containing medicines', available from: https://www.ema.europa.eu/en/medicines/human/referrals/domperidone-containing-medicines

EMA/CHMP/ICH/353369/2013, 'ICH guideline Q3D (R2) on elemental impurities' available from: https://www.ema.europa.eu/en/ich-q3d-elemental-impurities-scientific-guideline

European Commission, 'Addendum to the Review report for the active substance fipronil', 2013, available from: https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/active-substances/details/1002

European Commission. EU Pesticides database - Fipronil. Accessed January 2024, available from: https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/active-substances/details/1002

Fayez, R., Gupta, V., 'Imipramine', StatPearls, Treasure Island (FL), May 2023, available from: https://www.ncbi.nlm.nih.gov/books/NBK557656/

Fu, T.-K., Jarvik, L.-F., 'The in vitro effects of imipramine on human chromosomes', Mutat Res, 1977, Vol. 48(1), pp. 89–94.

Gillman, P.-K., 'Tricyclic antidepressant pharmacology and therapeutic drug interactions updated', Br J Pharmacol, 2007, Vol. 151(6), pp. 737-748.

Giorgi, M., Ozdemir, M., Camillo, F., Panzani, D., 'Pharmacokinetics of sulpiride after intravenous, intramuscular, and oral single-dose administration in nurse mares', Journal of Equine Veterinary Science, 2013, Vol. 33(7), pp. 533-538.

Guillaume, D., Chavatte-Palmer, P., Combarnous, Y., Duchamp, G., Martinat, N., Nagy, P., Daels, P.-F., 'Induced lactation with a dopamine antagonist in mares: different responses between ovariectomized and intact mares', Reprod Domest Anim, 2003, Vol. 38(5), pp. 394-400.

Harmon, J.-R., Webb, P.-J., Kimmel, G.-L., Delongchamp, R.-R., 'Effect of prenatal imipramine exposure on development of the postnatal rat heart and brain', Teratog Carginog Mutagen, 1986, Vol. 6(3), pp. 173-184.

Helmy, S.-A., El Bedaiwy, H.-M., 'Pharmacokinetics and comparative bioavailability of domperidone suspension and tablet formulations in healthy adult subjects', Clin Pharmacol Drug Dev, 2014, Vol. 3(2), pp. 126-131.

Heykants, J., Knaeps, A., Meuldermans, W., Michiels, M., 'On the pharmacokinetics of domperidone in animals and man. I. Plasma levels of domperidone in rats and dogs. Age related absorption and passage through the blood brain barrier in rats', Eur J Drug Metab Pharmacokinet, 1981, Vol. 6(1), pp. 27-36.

Hugnet, C., Cadore, J.-L., Bourdoiseau, G., 'Use of fipronil spray (0.25%) for the treatment of Damalinia equi [(Wereckiella equi) infestation]', Pratique Veterinaire Equine, 1999, Vol. 31, pp. 65-68.

IARC International Agency for Research on Cancer. List of Classifications. Accessed January 2024, available from: https://monographs.iarc.who.int/list-of-classifications/

IRIS Integrated Risk Information System, 'Toxicological Review of Barium Compounds', 2005, U.S. Environmental Protection Agency, Accessed January 2024, available from: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=10

Ishii, S., Ube, M., Okada, M., Adachi, T., Sugimoto, J., Inoue, Y., Uno, Y., Mutai, M. 'Collaborative work on evaluation of ovarian toxicity. 17) Two- or four-week repeated-dose studies and fertility study of sulpiride in female rats', J Toxicol Sci, 2009, Vol. 34 (Suppl 1), pp. SP175-SP188.

Johnson, L.-R., 'Tricyclic antidepressant toxicosis', Vet Clin North Am Small Anim Pract, 1990, Vol. 20(2), pp. 393-403.

Keating, J.-P., Rees, M., 'Gynaecomastia after long-term administration of domperidone', Postgrad Med J, 1991, Vol. 67(786), pp. 401-402.

Khan, S., Jan, M.-H., Kumar, D., Telang, A.-G., 'Fipronil induced spermotoxicity is associated with oxidative stress, DNA damage and apoptosis in male rats', Pestic Biochem Physiol, 2015, Vol. 124, pp. 8-14.

Kilbinger, H., Weihrauch, T.-R., 'Drugs increasing gastrointestinal motility', Pharmacology, 1982, Vol. 25(2), pp. 61-72.

Knych, H.-K., Weiner, D., Steinmetz, S., Flynn, K. McKemie, D.-S., 'Pharmacokinetics of hydroxyzine and cetirizine following oral administration of hydroxyzine to exercised Thoroughbred horses', J Vet Pharmacol Ther, 2019, Vol. 42(6), pp. 617- 623.

Kondratjeva, J., Combarros, D., Pressanti, C., Cadiergues, M.-C., 'What is the most effective treatment in horses with chorioptic mange?', Equine Veterinary Education, 2022, Vol. 35(3), pp. 163-168.

Kuroda, T., Nagata, S., Takizawa, Y., Tamura, N., Kusano, K., Mizobe, F., Hariu, K., 'Pharmacokinetics and pharmacodynamics of d-chlorpheniramine following intravenous and oral administration in healthy Thoroughbred horses', Vet J, Aug 2013, Vol. 197(2), pp. 433-437.

Larsen, V., 'The teratogenic effects of thalidomide, imipramine hc1 and imipramine-n-oxide hc1 on white danish rabbits', Acta Pharmacol Toxicol (Copenh), 1963, Vol. 20, pp. 186-200.

Madrigal-Bujaidar, E., Cardénas-García, Y., Álvarez-González, I., 'Chromosomal aberrations induced by imipramine and desipramine in mouse', Hum Exp Toxicol, 2010, Vol. 29(4), pp. 297–302.

Madrigal-Bujaidar, E., Madrigal-Santillán, E.-O., Alvarez-Gonzalez, I., Baez, R., Marquez, P., 'Micronuclei induced by imipramine and desipramine in mice: a subchronic study', Basic Clin Pharmacol Toxicol, 2008, Vol. 103(6), pp. 569–573.

Mansoor, A., Mahabadi, N., 'Volume of Distribution', StatPearls, Treasure Island (FL), Jul 2023, available from: https://www.ncbi.nlm.nih.gov/books/NBK545280/

Mauri, M.-C., Bravin, S., Bitetto, A., Rudelli, R., Invernizzi, G., 'A risk-benefit assessment of sulpiride in the treatment of schizophrenia', Drug Saf, 1996, Vol. 14(5), pp. 288-298.

McDonnel, S.-M., Kenney, R.-M., Meckley, P.-E., Garcia, M.-C., 'Conditioned suppression of sexual behavior in stallions and reversal with diazepam', Physiol Behav, 1985, Vol. 34(6), pp. 951-956.

McDonnell, S.-M., 'Stallion sexual behavior', In Juan, C.-S., (Ed.), 'Equine breeding management and artificial insemination', W B Saunders Co Ltd, 1999, pp. 53-61.

Meuldermans, W., Hurkmans, R., Swysen, E., Hendrickx, J., Michiels, M., Lauwers, W., Heykants, J., 'On the pharmacokinetics of domperidone in animals and man III. Comparative study on the excretion and metabolism of domperidone in rats, dogs and man', Eur J Drug Metab Pharmacokinet, 1981, Vol. 6(1), pp. 49-60.

Michiels, M., Hendriks, R., Heykants, J., 'On the pharmacokinetics of domperidone in animals and man II. Tissue distribution, placental and milk transfer of domperidone in the Wistar rat', Eur J Drug Pharmacokinet, 1981, Vol. 6(1), pp. 37-48.

Mohamed, F., Senarathna, L., Percy, A., Abeyewardene, M., Eaglesham, G., Cheng, R., Azher, S., Hittarage, A., Dissanayake, W., Sheriff, M.-H.-R., Davies, W., Buckley, N.-A., Eddleston, M., 'Acute human self-poisoning with the N-phenylpyrazole insecticide fipronil--a GABAA-gated chloride channel blocker', J Toxicol Clin Toxicol, 2004, Vol. 42(7), pp. 955–963.

Morresey, P.-R., Agalactia, 'Dysgalactia, and Nutrition of the Postpartum Mare', AAEP Proceedings, 2012, Vol. 58, pp. 370-374.

Newton, S.-A., Knottenbelt, D.-C., Eldridge, P.-R., 'Headshaking in horses: possible aetiopathogenesis suggested by the results of diagnostic tests and several treatment regimes used in 20 cases', Equine Vet J, 2000, Vol. 32(3), pp. 208-216.

Olsén, L., Bondesson, U., Broström, H., Tjälve, H., Ingvast-Larsson, C., 'Cetirizine in horses: pharmacokinetics and pharmacodynamics following repeated oral administration', Vet J, 2008, Vol. 177(2), pp. 242-249.

Paniagua-Pérez, R., Madrigal-Bujaidar, E., Reyes, C.-S., Pérez, G.-J., Velasco, M.-O., Molina, D., 'Sister chromatid exchanges produced by imipramine and desipramine in mouse bone marrow cells treated in vivo', Toxicol Lett, 2002, Vol. 132(2), pp. 123–129.

Panzani, D., Zicchino, I., Taras, A., Marmorini, P., Crisci, A., Rota, A., Camillo, F., 'Clinical use of dopamine antagonist sulpiride to advance first ovulation in transitional mares', Theriogenology, 2011, Vol. 75(1), pp. 138-143.

Papich, M.-G., (Ed.), 'Papich handbook of Veterinary drugs', Elsevier, 2021, Edition 5, pp. 167-168.

Peck, K.-E., Hines, M.-T., Mealey, K.-L., Mealey, R.-H., 'Pharmacokinetics of imipramine in narcoleptic horses', Am J Vet Res, 2001, Vol. 62(5), pp. 783-786.

Petersen, A., Schott 2nd, H.-C., 'Effects of dexamethasone and hydroxyzine treatment on intradermal testing and allergen-specific IgE serum testing results in horses', Vet Dermatol, 2009, Vol. 20(5-6), pp. 615-622.

Pharmawiki Medikamente und Gesundheit. Imipramin. [Updated May 2020]. Accessed February 2024, available from: https://www.pharmawiki.ch/wiki/index.php?wiki=imipramin

Plumb, D.-C., (Ed.), 'Plumb´s veterinary drug handbook', Willey-Blackwel, 2015, Edition 8, pp. 200-202.

Redmond, L.-M., Cross, D.-L., Strickland, J.-R., Kennedy, S.-W., 'Efficacy of domperidone and sulpiride as treatments for fescue toxicosis in horses', Am J Vet Res, 1994, Vol. 55(5), pp. 722-729.

Roberts, V., 'Trigeminal-mediated headshaking in horses: prevalence, impact, and management strategies', Vet Med (Auckl), 2019, Vol. 10, pp. 1-8.

Romero, A., Ramos, E., Ares, I., Castellano, V., Martínez, M., Martínez-Larrañaga, M.-R., Anadón, A., Martínez, M.-A., 'Fipronil sulfone induced higher cytotoxicity than fipronil in SH-SY5Y cells', Toxicol Lett, 2016, Vol. 252, pp. 42-49.

Sallee, F.-R., Pollock, B.-G., 'Clinical pharmacokinetics of imipramine and desipramine', Clin Pharmacokinet, 1990, Vol. 18(5), pp. 346–364.

Saxena, R., Ahuja, Y.-R., 'Genotoxicity evaluation of the tricyclic antidepressants amitriptyline and imipramine using human lymphocyte cultures', Environ Mol Mutagen, 1988, Vol. 12(4), pp. 421-430.

Sequeira, D.-J., Strobel, H.-W., 'High-performance liquid chromatographic method for the analysis of imipramine metabolism in vitro by liver and brain microsomes', J Chromatogr B Biomed Appl, 1995, Vol. 673(2), pp. 251-258.

Scriba, G.-K.-E., In Sweetman C., (Ed.) 'Martindale: The Complete Drug Reference', Pharmaceutical Press, London, 2009, Edition 36.

Sieme, H., Katila, T., Klug, E., 'Effect of semen collection practices on sperm characteristics before and after storage and on fertility of stallions', Theriogenology, 2004, Vol. 61(4), pp. 769-784.

Turner, S.-E., Knych, H.-K., Adams, A.-A., 'Pharmacokinetics of cannabidiol in a randomized crossover trial in senior horses', Am J Vet Res, Jul 2022, Vol. 83(9).

Sweetman, S.-C., (Ed.), 'Martindale: The Complete Drug Reference', Pharmaceutical Press, London, 2009, Edition 36.

Valencia, N.-A., Thompson Jr, D.-L., Southerland, C.-V., 'Effects of Various Methods of Sulpiride Administration on Prolactin Release in Horses', J Equine Vet Sci, 2018, Vol. 68, pp. 39-45.

Vanparys, P., Fabry, L., Leonard, A., Marsboom, R., 'Mutagenicity tests with domperidone in vitro and in vivo', Toxicol let, 1982, Vol. 12(2-3), pp. 215-222.

Vanparys, P., Gilot-Delhalle, J., Moutschen, J., Moutschen-Dahmen, M., Marsboom, R., 'In vivo mutagenicity evaluation of domperidone in Drosophila germ cells and rat bone marrow cells', Toxicology, 1985, Vol. 36(2-3), pp. 147-150.

Wang, C., Qin, Z., Ren, G., Zhai, Y., Fu, K., Lu, C., Yang, C., Chen, X., 'Development of a sensitive UPLC-MS/MS assay for domperidone and pharmacokinetics of domperidone tablet formulations in fasting and fed Chinese healthy subjects', Int J Clin Pharmacol Ther, 2020, Vol. 58(3), pp. 177-182.

Wiesel, F.-A., Alfredsson, G., Ehrnebo, M., Sedvall, G., 'The pharmacokinetics of intravenous and oral supiride in healthy human subjects', Eur J Clin Pharmacol, 1980, Vol. 17(5), pp. 385-391.

Williams, M.-R., Holbrook, T.-C., Maxwell, L., Croft, C.-H., Ientile, M.-M., Cliburn, K., 'Pharmacokinetic Evaluation of a Cannabidiol Supplement in Horses', J Equine Vet Sci, Mar 2022, Vol. 110, p. 103842.

Ye, L., Shen, M., Chen, Y., Li, X., Xu, M., Yao, C., Yang, S., 'Pharmacokinetics and Bioequivalence of 2 Oral Formulations of Domperidone After Single-Dose Administration in Healthy Chinese Subjects', Clin Pharmacol Drug Dev, 2022, Vol. 11(9), pp. 1082-1091.

Review of the entries in Table 2 of the Annex to Commission Regulation (EU) No 37/2010

AbdRabou, M.-A., Alrashdi, B.-M., Alruwaili, H.-K., Elmazoudy, R.-H., Alwaili, M.-A., Othman, S.-I., Alghamdi, F.-A., Fahmy, G.-H., 'Exploration of Maternal and Fetal Toxicity Risks for Metronidazole-Related Teratogenicity and Hepatotoxicity through an Assessment in Albino Rats', Toxics, 2023. Vol. 11, p. 303.

Akyol, D., Mungan, T., Baltaci, V., 'A comparative study of genotoxic effects in the treatment of trichomonas vaginalis infection: metronidazole or nalidixic acid', Arch Gynecol Obstet, 2000, Vol. 264, pp. 20-23.

Arnold, C., Pilla, R., Chaffin, K., Lidbury, J., Steiner, J., Suchodolski, J., 'Alterations in the Fecal Microbiome and Metabolome of Horses with Antimicrobial-Associated Diarrhea Compared to Antibiotic-Treated and Non-Treated Healthy Case Controls', Animals (Basel), 2021, Vol. 11(6), p. 1807.

Baggot, J.-D., Wilson, W.-D., Hietla, S., 'Clinical pharmacokinetics of metronidazole in horses', J Vet Pharmacol Ther, 1988, Vol. 11(4), pp. 417-20.

Baynes, R.-E., Dedonder, K., Kissell, L., Mzyk, D., Marmulak, T., Smith, G., Tell, L., Gehring, R., Davis, J., Riviere, J.E., 'Invited review. Health concerns and management of select veterinary drug residues', Food and Chemical Toxicology, 2016, Vol. 88, pp. 112-122.

Bendeskya, A., Menéndez, D., Ostrosky-Wegman, P., 'Review. Is metronidazole carcinogenic?', Mutation Research, 2002, Vol. 511, pp. 133-144.

Britzi, M., Gross, M., Lavy, E., Soback, S., Steinman, A., 'Bioavailability and pharmacokinetics of metronidazole in fed and fasted horses', J Vet Pharmacol Therap, 2010, Vol. 33, pp. 511-514.

Buschini, A., Ferrarini, L., Franzoni, S., Galati, S., Lazzaretti, M., Mussi, F., Northfleet de Albuquerque, C., Zucchi, T.M.A.D., Poli, P., 'Genotoxicity revaluation of three commercial nitroheterocyclic drugs: nifurtimox, benznidazole, and metronidazole', Journal of Parasitology Research, 2009, Article ID 463575, pp. 1-11.

Caro-Paton, T., Carvajal, A., Martin de Diego, I., Martin-Arias, L.-H., Alvarez Requejo, A., Rodriguez Pinilla, E., 'Is metronidazole teratogenic? A meta-analysis', Br J Clin Pharmacol, 1997, Vol. 44, pp. 179-182.

CFSPH (Center for Food Security and Public Health), 'Contagious Equine Metritis', September 2015. Accessed June 2023, available from: https://www.cfsph.iastate.edu/Factsheets/pdfs/contagious_equine_metritis.pdf

Chamberlain, R.-E., 'Chemotherapeutic properties of prominent nitrofurans', J Antimicrob Chemother, December 1976, Vol 2(4), pp. 325-36.

Církva, A., Málková, I., Rejtharová, M., Vernerová, E., Hera, A., Bureš, J., 'Residue study of nitroimidazoles depletion in chicken feathers in comparison with some other selected matrixes', Food Additives & Contaminants: Part A, 2019, Vol. 36(8), pp. 1206-1217.

Clark-Price, S.-C., Cox, J.-H., Bartoe, J.-T., Davis, E.-G., 'Use of dapsone in the treatment of Pneumocystis carinii pneumonia in a foal', J Am Vet Med Assoc, Feb 2004, Vol. 224(3), pp. 407-10, 371.

Cohen, N.-D., Woods, A.-M., 'Characteristics and risk factors for failure of horses with acute diarrhea to survive: 122 cases (1990-1996)', J Am Vet Med Assoc, 1999, Vol. 214, pp .382-390.

Constable, P.-D., Hinchcliff, K.-W., Done, S.-H., Gruenberg, W., 'Veterinary Medicine; A textbook of the diseases of cattle, horses, sheep, pigs and cats', Saunders Ltd., Edition 11., Oct 2016.

Cram, L.-F., Zapata, M., Toy, E., Baker, B., 'Genitourinary infections and their association with preterm labor', American Family Physician, 2015, Vol. 65(2), pp. 241-249.

Czeizel, A.-E., Rockenbauer, M., 'A population based case-control teratologic study of oral metronidazole treatment during pregnancy', British Journal of Obstetrics and Gynaecology, 1998, Vo. 105, pp. 322-327.

Davis, L.-E., Abbitt, B., 'Clinical pharmacology of antibacterial drugs in the uterus of the mare', J Am Vet Med Assoc, Jan 1977, Vol. 170(2), pp. 204-207.

Deftereos, S.G-., Beerkens, F.-J., Shah, B., Giannopoulos, G., Vrachatis, D.-A., Giotaki, S.-G., Siasos, G., Nicolas, J., Arnott, C., Patel, S., Parsons, M., Tardif, J.-C., Kovacic, J.-C., Dangas, G.-D., 'Colchicine in Cardiovascular Disease: In-Depth Review', Circulation, Jan 2022, Vol. 145(1), pp. 61-78.

Dingsdag, S.-A., Hunter, N., 'Metronidazole: an update on metabolism, structure–cytotoxicity and resistance mechanisms', J Antimicrob Chemother, 2018, Vol. 73, pp. 265-279.

Drugbank, Chloramphenicol [Updated 17 Aug 2023]. Accessed August 2023, available from: https://go.drugbank.com/drugs/DB00446

Eaglesome, M.-D., Garcia, M.-M., 'Contagious equine metritis: a review', Can Vet J, Aug 1979, Vol. 20(8), pp. 201-206.

EFSA, CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 'Update: methodological principles and scientific methods to be taken into account when establishing Reference Points for Action (RPAs) for non-allowed pharmacologically active substances present in food of animal origin', EFSA Journal, 2018, Vol. 16(7), p. 5332.

EFSA, CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 'Scientific Opinion on Chloramphenicol in food and feed', EFSA Journal, 2014, Vol. 12(11), p. 3907.

Eguchi, A., Mizukami, S., Nakamura, M., Masuda, S., Murayama, H., Kawashima, M., Inohana, M., Nagahara, R., Kobayashi, M., Yamashita, R., Uomoto, S., Makino, E., Ohtsuka, R., Takahashi, N., Hayashi, S.-M., Maronpot, R.-R., Shibutani, M., Yoshida, T., 'Metronidazole enhances steatosis-related early-stage hepatocarcinogenesis in high fat diet-fed rats through DNA double-strand breaks and modulation of autophagy', Environmental Science and Pollution Research, 2022, Vol. 29, pp. 779-789.

Ehrlich, J., Bartz, Q.-R., Smith, R.-M., Joslyn, D.-A., Burkholder, P.-R., 'Chloromycetin, a New Antibiotic From a Soil Actinomycete', Science, Oct 1947, Vol. 106(2757), p. 417.

El-Nahas, A.-F., El-Ashmawy, I.-M., 'Reproductive and Cytogenetic Toxicity of Metronidazole in Male Mice', Basic Clin Pharmacol Toxicol, 2004, Vol. 94, pp. 226-231.

EMEA/MRL/271/97-FINAL, 'Committee for Veterinary Medicinal Products. Aristolochia Summary Report'.

EMEA/MRL/111/96-FINAL, 'Committee for Veterinary Medicinal Products. Chlorpromazine Summary Report'

EMEA/MRL/044/95-FINAL, 'Committee for Veterinary Medicinal Products. Colchicine Summary Report'

EMA, European Medicines Agency. Dapsone Summary Report (EPMAR). 1996a,c. Link (1996a), (1996c). Accessed June 2023.

EMA, European Medicines Agency. Dimetridazole Summary Report (EPMAR). 1996d,e,f. Link (1996d), (1996e), (1996f). Accessed June 2023.

EMA, European Medicines Agency. Nitrofurans (including furazolidone) Summary Report (EPMAR). 1996g (nitrofurans), 1997a (furazolidone). Link (1996g), (1997a). Accessed June 2023.

EMA, European Medicines Agency. Ronidazole Summary Report (EPMAR). 1996h,i. Link (1996h), (1996i). Accessed June 2023.

EMEA/MRL/173/96-FINAL, 'Committee for Veterinary Medicinal Products. Metronidazole Summary Report'

EMA, European Medicines Agency. Chloramphenicol Summary Report (EPMAR). 1996b. Link. Accessed June 2023.

EquiMed, Chloramphenicol [Equine drugs and medications, 2014]. Accessed August 2023, available from: https://equimed.com/drugs-and-medications/reference/chloramphenicol

Erdman, M.-M., Creekmore, L.--H., Fox, P.-E., Pelzel, A.-M., Porter-Spalding, B.-A., Aalsburg, A.-M., Cox, L.-K., Morningstar-Shaw, B.-R., Crom, R.-L., 'Diagnostic and epidemiologic analysis of the 2008-2010 investigation of a multi-year outbreak of contagious equine metritis in the United States', Prev Vet Med, Sep 2011, Vol. 101(3-4), pp. 219-28.

Estell, K.-E., Knych, H.-K., Patel, T., Edman, J.-M., 'Pharmacokinetics of multiple doses of chloramphenicol in fed adult horses', The veterinary Journal, 2020, Vol. 257, p. 105446.

Fahrig, R., Engelke, M., 'Reinvestigation of in vivo genotoxicity studies in man. I. No induction of DNA strand breaks in peripheral lymphocytes after metronidazole therapy', Mutation Research, 1997, Vol. 395, pp. 215-221.

FAO, Food and Agricultural Organization of the United Nations. Maximum Residue limits (MRLs) and Risk Management Recommendations (RMRs) for Residues of Veterinary Drugs in Food, CX/MRL 2-2021. Accessed August 2023, available from: https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXM%2B2%252FMRL2e.pdf

Finkelstein, Y., Aks, S.-E., Hutson, J.-R., Juurlink, D.-N., Nguyen, P., Dubnov-Raz, G., Pollak, U., Koren, G., Bentur, Y., 'Colchicine poisoning: the dark side of an ancient drug', Clin Toxicol (Phila), Jun 2010, Vol. 48(5), pp. 407-414.

Foote, B.-C., Hendrix, D.-V.-H., Ward, D.-A., Rajeev, S., Smith, J.-S., Sun, Z., 'Monitoring trends in antimicrobial susceptibility patterns of bacterial isolates from horses with ulcerative keratitis', Equine Veterinary Education, 2023, Vol. 35, pp. 597-606.

Gomez-Arroyo, S., Melchor-Castro, S., Villalobos-Pietrini, R., Melendez Camargo, E., Salgado-Zamora, H., Campos Aldrete, E., 'Cytogenetic study of metronidazole and three metronidazole analogues in cultured human lymphocytes with and without metabolic activation', Toxicology in Vitro, 2004, Vol. 18, pp. 319-324.

Grollman, A.-P., Marcus, D.-M., 'Global hazards of herbal remedies: lessons from Aristolochia', EMBO Reports, 2016, Vol. 17(5), pp. 619-625.

Hollis, A.-R., Wilkins, P.-A., 'Current controversies in equine antimicrobial therapy', Equine Veterinary Education, 2009, Vol. 21, pp. 216-224.

IARC International Agency for Research on Cancer. Chloramphenicol Evaluation of Carcinogenic Risk to Humans. IARC Journal, Lyon, France, 1987, (Suppl. 7), pp. 250-251. Accessed November 2023, available from: https://publications.iarc.fr/139

Imazio, M., 'Colchicine for pericarditis', Trends Cardiovasc Med, Feb 2015, Vol. 25(2), pp. 129-136.

Jang, S.-S., Hansen, L.-M., Breher, J.-E., Riley, D.-A., Magdesian, K.-G., Madigan, J.-E., Tang, Y.-J., Silva Jr, J., Hirsh, D.-C., 'Antimicrobial susceptibilities of equine isolates of Clostridium difficile and molecular characterization of metronidazole-resistant strains', Clin Infect Dis, 1997, Vol. 25(Suppl 2), pp. S266-267.

JECFA, Joint FAO/WHO Expert Committee on Food Additives. 'Evaluation of certain food additives and contaminants: thirty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives', WHO technical report series, 1989, Vol.788. Accessed August 2023, available from: https://apps.who.int/iris/handle/10665/41543

JECFA, Joint FAO/WHO Expert Committee on Food Additives. 'Evaluation of certain food additives and contaminants: sixty-second report of the Joint FAO/WHO Expert Committee on Food Additives', WHO technical report series, 2004, Vol. 925. Accessed August 2023, available from: https://apps.who.int/food-additives-contaminants-jecfa-database/Home/Chemical/551

Kazy, Z., Puho, E., Czeizel, A.-E., 'Teratogenic potential of vaginal metronidazole treatment during pregnancy', Eur J Obstet Gynecol Reprod Biol, 2005, Vol. 123, pp. 174-178.

Kumari, M., Singh, P., 'Study on the reproductive organs and fertility of the male mice following administration of metronidazole', International Journal of Fertility and Sterility, 2013, Vol. 7(3), pp. 225-238.

Kuroda, T., Nagata, S., Tamura, N., Kinoshita, Y., Niwa, H., Mita, H., Minami, T., Fukuda, K., Hobo, S., Kuwano, A., 'Single-dose pharmacokinetics of orally administered metronidazole and intravenously administered imipenem in healthy horses and computer-based simulation of pleural fluid concentrations with multiple dosing', AJVR, 2020, Vol. 81(10), pp. 783-789.

Le, V.-V.-H., Rakonjac, J., 'Nitrofurans: Revival of an "old" drug class in the fight against antibiotic resistance', PLoS Pathog, Jul 2021, Vol. 17(7), e1009663.

Magdesian, K.-G., Hirsh, D.-C., Jang, S.-S., Hansen, L.-M., Madigan, J.-E., 'Characterization of Clostridium difficile isolates from foals with diarrhea: 28 cases (1993-1997)', J Am Vet Med Assoc, 2002, Vol. 220, pp. 67-73.

McElligott, E.-M., Sommardahl, C.-S., Cox, S.-K., 'Pharmacokinetics of chloramphenicol base after oral administration in adult horses', JAVMA, 2017, Vol. 251(1), pp. 90-94.

Menéndez, D., Rojas, E., Herrera, L.-A., López, M.-C., Sordo, M., Elizondo, G., Ostrosky-Wegman, P., 'DNA breakage due to metronidazole treatment', Mutation Research, 2001, Vol. 478, pp.153-158.

Menéndez, D., Bendesky, A., Rojas, E., Salamanca, F., Ostrosky-Wegman, P., 'Role of P53 functionality in the genotoxicity of metronidazole and its hydroxy metabolite', Mutation Research, 2002, Vol. 501, pp. 57-67.

Nath, S., Ghosh, N., Ansari, T.-A., Mundhra, A., Patil, M.-T., Mane, A., Gopalakrishnan, A.-V., Rahman, M.-H., Kumar, M., Radha, Ghorai, M., Paul, S., Dey, A., 'Genetic diversity assessment and biotechnological aspects in Aristolochia spp.', Appl Microbiol Biotechnol, Oct 2022, Vol. 106(19-20), pp. 6397-6412.

NTP National Toxicology Program. Report on Carcinogens, Edition 15. Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service. 2021. Accessed August 2023, available from: https://ntp.niehs.nih.gov/go/roc15

Nwosu, O.-C., Bloom, K., 'The safety of metronidazole in pregnancy', Health Care for Women International, 2021, Vol. 42(NOS.4–6), pp. 726-738.

Ornelas–Aguirre, J.-M., Gomez-Meda, B.-C., Zamora-Perez, A.-L., Ramos-Ibarra, M.-L., Batista-Gonzalez, C.-M., Zuniga-Gonzalez, G.-M., 'Micronucleus induction by metronidazole in rat vaginal mucosa', Environmental and Molecular Mutagenesis, 2006, Vol. 47, pp. 352-356.

Pan, Y., Yi, J., Zhou, B., Xie, S., Chen, D., Tao, Y., Qu, W., Liu, Z., Huang, L., Yuan, Z., 'Disposition and Residue Depletion of Metronidazole in Pigs and Broilers', Scientific Reports, 2017, Vol. 7, p. 7203.

Pascart, T., Richette, P., 'Colchicine in Gout: An Update', Curr Pharm Des, 2018, Vol. 24(6), pp. 684-689.

Patel, T., Magdesian, K.-G., Estell, K.-E., Edman, J.-M., Knych, H.-K., 'Pharmacokinetics of chloramphenicol base in horses and comparison to compounded formulations', J vet Pharmacol Therap, 2019, Vol. 42, pp. 609-616.

Peterson, H.-M., Manley, C.-I., Trepanier, L.-A., Pritchard, J.-C., 'Genotoxicity from metronidazole detected in vitro, but not in vivo, in healthy dogs in a randomized clinical trial', American Journal of Veterinary Research, 2022, Vol. 84(1).

PubChem. Clorpromazine. 2023. Accessed June 2023, available from: https://pubchem.ncbi.nlm.nih.gov/compound/2726

Re, J.-L., De Meo, M.-P., Laget, M., Guiraud, H., Castegnaro, M., Vanelle, P., Dumenil, G., 'Evaluation of the genotoxic activity of metronidazole and dimetridazole in human lymphocytes by the comet assay', Mutation Research, 1997, Vol. 375, pp. 147-155.

Rejtharová, M., Rejthar, L., Bureš, J., Vernerová, E., Hera, A., 'Persistence of chloramphenicol residues in chicken muscle tissue after a therapeutic dose administration', Food Addit Contam Part A Chem Anal Control Expo Risk Assess, 2017, Vol. 34(4), pp. 547-551.

Reuss, S.-M., 'Review of Equine Cutaneous Leishmaniasis: Not Just a Foreign Animal Disease', AAEP Proceedings, 2013, Vol. 59, pp. 256-260.

Robinson, K.-P., Chan, J.-J., 'Colchicine in dermatology: A review', Australis J Dermatol, Nov 2018, Vol. 59(4), pp. 278-285.

Schulman, M.-L., May, C.-E., Keys, B., Guthrie, A.-J., 'Contagious equine metritis: artificial reproduction changes the epidemiologic paradigm', Vet Microbiol, Nov 2013, Vol. 167(1-2), pp. 2-8.

Sekis, I., Ramstead, K., Rishniw, M., Schwark, W.-S., McDonough, S.-P., Goldstein, R.-E., Papich, M., Simpson, K.-W., 'Single-dose pharmacokinetics and genotoxicity of metronidazole in cats', Journal of Feline Medicine and Surgery, 2009, Vol. 11, pp. 60-68.

Sheehy, O., Santos, F., Ferreira, E., Berard, A., 'The use of metronidazole during pregnancy: A review of evidence', Current Drug Safety, 2015, Vol. 10(2), pp. 170-179.

Sisson, G., Jeong, J.-Y., Goodwin, A., Bryden, L., Rossler, N., Lim-Morrison, S., Raudonikiene, A., Berg, D.-E., Hoffman, P.-S., 'Metronidazole Activation Is Mutagenic and Causes DNA Fragmentation in Helicobacter pylori and in Escherichia coli Containing a Cloned H. pylori rdxA1 (Nitroreductase) Gene', Journal of Bacteriology, 2000, Vol. 182(18), pp. 5091-5096.

Specht, T.-E., Brown, M.-P., Gronwall, R.-R., Rib, W.-J., Houston, A.-E., 'Pharmacokinetics of metronidazole and its concentration in body fluids and endometrial tissues of mares', Am J Vet Res, 1992, Vol. 53(10), pp. 1807-12.

Stancil, S.-L., Van Haandel, L., Abdel-Rahmana, S., Pearce, R.-E., 'Development of a UPLC-MS/MS method for quantitation of metronidazole and 2-hydroxy metronidazole in human plasma and its application to a pharmacokinetic study', Journal of Chromatography B, 2018, Vol. 1092, pp. 272-278.

Steinman, A., Gips, M., lavy, E., Sinay, I., Soback, S., 'Pharmacokinetics of metronidazole in horses after intravenous, rectal and oral administration', J Vet Pharmacol Therap, 2000, Vol. 23, pp. 353-357.

Struthers, B.-J., 'Metronidazole appears not to be a human teratogen: review of literature', Infect Dis Obstet Gynecol, 1997, Vol. 5(5), pp. 326-35.

Suarez-Torres, J.-D., Orozco, C.-A., Ciangherotti, C.-E., 'The numerical probability of carcinogenicity to humans of some antimicrobials: nitro-monoaromatics (including 5-nitrofurans and 5-nitroimidazoles), quinoxaline-1,4-dioxides (including carbadox), and chloramphenicol', Toxicology in Vitro, 2021, Vol. 75, p. 105172.

Swain, E.-A., Magdesian, K.-G., Kass, P.-H., Edmann, J.-E., Knych, H.-K., 'Pharmacokinetics of metronidazole in foals: influence of age within the neonatal period', J Vet Pharmacol Therap, 2014, Vol. 38, pp. 227-234.

Sweeney, R.-W., Sweeney, C.-R., Soma, L.-R., Woodward, C.-B., Charlton, C.-A., 'Pharmacokinetics of metronidazole given to horses by intravenous and oral routes', American Journal of Veterinary Research, 1986, Vol. 47(8), pp. 1726-1729.

Timoney, P.-J., 'Contagious equine metritis', Comp Immunol Microbiol Infec Dis, Jun 1996, Vol. 19(3), pp. 199-204.