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SCIENCE MEDICINES HEALTH

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Committee for Veterinary Medicinal Products (CVMP)

Addendum to the scientific advice under Article 115(5) of Regulation (EU) 2019/6 on veterinary medicinal products

European Commission's request[1] for reviewing the additional scientific information provided for the substance midazolam 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

Review of the additional scientific information provided for the substance midazolam; request from the European Commission to the Agency on 28 November 2024

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

The assessment for midazolam in the Agency's July 2024 scientific advice[2] under Article 115(5) of Regulation (EU) 2019/6 is as follows:

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.

[1] For further details please refer to the background information to this addendum (EMA/112916/2025)
[2] With reference to the scientific advice provided to the European Commission on 19 July 2024, accessible via this link.

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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's 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.

As indicated, the stakeholders have a concern with the proposal for removing midazolam from the list and therefore provided additional information in pursuit of a re-evaluation of midazolam for possible inclusion in the list of essential substances for horses. Please refer to Ares(2024)6980209 and Ares(2024)8365149 for further details.

The stakeholders refer to the advice text where it is stated that midazolam and diazepam are benzodiazepines with very similar in terms of their effects and side effects; both produce unique tranquillisation without cardiorespiratory effects, which cannot be produced by the alpha-2 agonists or other substances with MRLs for *Equidae*. However, as detailed in the scientific advice, the request[3] from the European Commission included for the revision of the entries in the list established in Commission Regulation (EU) 1950/2006, and the referred text is a direct quote from that Regulation.

[3] 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.

While the scientific advice acknowledges the information in Commission Regulation (EU) 1950/2006, all these statements were scientifically discussed in accordance with the criteria given in the Commission's request and the agreed methodological approach, noting that no supporting documentation could be located for the former list.

As a reminder, the methodological approach for the assessment of all substances is detailed in section 2 of the Agency's advice. A standardised two-tiered assessment approach was established, consisting of (i) an assessment to determine the essentiality of the substance following the criteria given in the Commission's request, i.e. absence of satisfactory alternative treatment for an indication or added clinical benefit compared to other treatment options available, and (ii) a further assessment focusing on the consumer safety aspects of the proposed substance. For midazolam, the discussion focused on its added clinical benefit compared to existing alternatives detomidine and xylazine, which 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, noting also that diazepam was proposed to be retained.

The present review also focuses of any added clinical benefit of midazolam compared to the existing alternatives already identified.

In justifying that midazolam does not bring added clinical benefit when compared to the existing alternatives, including diazepam, the Agency's scientific advice primarily discussed the work by Jarret et al. (2018), which suggests a better recovery after propofol and ketamine compared to midazolam and ketamine for induction of anaesthesia, followed by maintenance with isoflurane for 60 minutes. However, the stakeholders consider that these findings of this study should be interpreted with extreme caution since (quoted text):

- the study was only performed on 6 horses,
- the anaesthetist was not blinded to the treatment,
- it was an experimental study and no procedures were carried out during anaesthesia, which makes it difficult to extrapolate the results to a clinical situation,
- all horses were anaesthetized twice and served as their own control, but 5 out of 6 horses received midazolam during the first anaesthetic episode and propofol during the second. This makes the comparison unfair, because there is scientific evidence which shows that recovery quality improves when horses are anaesthetized more than once, with improved balance and coordination and less knuckling (Platt et al., 2018).
- the dose of midazolam used in this study was 0.1 mg/kg IV, which is almost twice as high as the dose licensed for this drug (0.06 mg/kg).

The stakeholders have correctly indicated that study from Jarret et al. (2018) makes no comparison between diazepam and midazolam; the PK/PD similarities and differences between diazepam and midazolam are well known. The paragraph highlighted by the stakeholders indicates that combinations for short anaesthesia without midazolam exist, some suggesting better anaesthetic recovery quality. This is an important knowledge contribution of this study by identifying other substances and combinations that can achieve similar or better results, as alternatives.

The stakeholders also mention the doses used in the study from Jarret et al. (2018) for midazolam and ketamine were considerably higher than normally used in practice. Peer-reviewed scientific literature would suggest differently; similar or higher doses of both substances are published in various studies and considered safe and efficacious (Hubbell et al., 2013; Harðardóttir et al., 2019; Wise et al., 2021). Moreover, De Vries et al. (2015) did a direct comparison of diazepam and midazolam (both 0.06

mg/kg IV) as co-induction agents with ketamine (2.2 mg/kg IV) for anaesthesia in sedated ponies undergoing field castration (clinical prospective study, randomised, blinded) using approved SPC (lower) doses and found no differences in all measured parameters (cardiorespiratory function, quality of sedation, induction, endotracheal intubation, surgical conditions and recovery were scored by observers blinded to treatment). Also, Granados et al. (2004) compared IV diazepam and midazolam, both at 0.1mg/kg, as co-induction agents with ketamine (2.2 mg/kg) with an induction regime based on ketamine alone after romifidine sedation (80 µg/kg) in horses. There were no significantly different effects on cardiovascular function reported between the three groups.

Overall, while the limitations indicated by the stakeholder with regards the work by Jarret et al. (2018) are noted, the argument made in the Agency's advice is retained since it is supported by the additional peer-reviewed studies discussed.

To complement the discussion on any added clinical benefit for midazolam, the stakeholders provided information on other considerations (advantages) of midazolam over diazepam. Overall it is stated that any argument to retain diazepam on the list of essential substances is also valid for midazolam, whose effects are comparable to those of diazepam but the onset of action is faster, the duration of action is shorter due to a faster metabolization, it is water soluble and thus better suitable for intravenous injection and intravenous infusion in combination with other anaesthetics, it is more suitable for foals as it is shorter acting, and better for use over several days in adult horses with tetanus due to its water solubility. However, the request[4] from the European Commission provides that the 'essentiality' or 'added clinical benefit' for each substance is to be assessed based on its own clinical merits. The emphasis of the stakeholders in the letters relate to the differences between midazolam and diazepam, and as such it is unclear as to the similar arguments for inclusion on the list of essential substances. These points are discussed in more detail below.

As indicated by the stakeholders and captured in the Agency's advice, the duration of action of midazolam is shorter due to a faster metabolization. Although not clearly stated, based on the arguments and references submitted it is understood that the stakeholders wish to seek the reconsideration of midazolam for pre-medication prior to anaesthesia primarily. For management of equine seizures or tetanus no clinical trials or retrospective studies have been published with alpha-2 agonists or acepromazine, as well as midazolam and diazepam. Use of these substances is based on a combination of the pathophysiology of the diseases as well as the mechanism-of-action of the active substances. In these cases, a longer-acting agent (i.e. diazepam) is considered more clinically useful and an advantage based on clinical principles of treatment.

One of the advantages of midazolam over diazepam mentioned by the stakeholders is that diazepam is poorly water soluble and is therefore supplied in a solution of an organic solvent, including propylene glycol and ethanol. This means intramuscular administration of diazepam is painful and can cause irritation. As stated by the stakeholders, this is not the case for midazolam, which is highly water soluble thanks to its open diazepine ring and can be administered intramuscularly in animals which are uncooperative; it is rapidly absorbed after intramuscular administration.

Although, as noted by the stakeholder, one of the differences between midazolam and diazepam relates to water solubility, no studies are available as to whether this represents a clinically meaningful difference for the management of the relevant diseases. Also, the water solubility of midazolam is not fixed and appears to depend on whether the benzepine ring is open or closed (Kanto, 1985). In acidic conditions (pH less than 4), the benzepine ring of midazolam is open, resulting in increased water solubility; however, at physiological pH, the ring closes and midazolam becomes lipophilic (not water soluble), which accounts for its rapid onset of action. This fact is also captured in the product

[4] 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.

information of an approved midazolam-containing veterinary medicinal product. Therefore, for the common uses of midazolam (IV route-of-administration), water solubility is not likely. Thus, formulations of midazolam use excipients that lower the pH so that midazolam is water soluble for injections. Such an acidic formulation would also be irritating with an intramuscular injection. However, in vivo the pH quickly changes to physiologic pH changing the properties of midazolam.

Midazolam can be administered IV, IM, or continuous rate infusion (CRI). Depending on the formulation, diazepam can also be given IM. Based on clinical experiences, the IM route for midazolam is very rarely utilised, if ever. One of the advantages of the intramuscular route for midazolam is, according to the stakeholders, its use for fractious horses. However, regardless of the route of administration, a substance with sedative properties would be more advantageous for fractious horses. For immediate seizure control, the IV route is preferred. However, rapid IV administration of midazolam can cause hypotension and a period of apnoea. There are no published experiences with using IM midazolam for seizure control. Foals that have more persistent seizures and repetitive seizures that require frequent dosing may be maintained with a CRI. CRI is generally not needed for >24–72 h. However, any use of midazolam in very young foals should be carefully considered since midazolam has negative effects on the immune system. In horses, midazolam induces a dose-dependent reduction on peripheral blood neutrophil and peritoneal macrophage oxidative burst, as well as reduces the capacity of both peripheral blood neutrophils and peritoneal macrophages to phagocyte *S. aureus* (Massoco and Palermo-Neto, 2003). Also, repeated injections of midazolam have not been investigated, and care is recommended in foals <3 weeks of age, due to the possibility of drug accumulation, as stated in a midazolam-containing veterinary medicinal product for non-food-producing horses.

The stakeholders also argue that diazepam, because it is marketed in a complex solvent system, should not be mixed with other drug solutions as it is widely incompatible with other drugs (Murney, 2008). They also argue that in horses, one of the most often used combinations for maintenance of anaesthesia with intravenous drugs (e.g. for castration or other procedures under field conditions) is the so called 'triple drip' consisting of a combination of an alpha2-agonist, ketamine and midazolam; given the incompatibility of diazepam with other drugs, it is not safe to use it in such a combination protocol, although no evidence is provided. A separate injection of diazepam is given typically for premedication/anaesthetic protocols. However, the fact that a substance can (or cannot) be mixed with others is not considered as a clinical added benefit in itself; it is rather a technical advantage for the clinicians. The combination proposed by the stakeholders is indeed an alternative to the more traditional 'triple drip', which is generally regarded as the combination of xylazine, ketamine and guaifenesin used to maintain anaesthesia in horses, particularly in places where there are issues with access to guaifenesin (Aarnes et al., 2018). There is no evidence available as to justifying a clinical advantage of one combination over another one.

Another argument presented by the stakeholders is that midazolam is more suitable for use in neonates or young foals. Benzodiazepines are required for safe sedation because alpha-2 agonists cause bradycardia. However, in the stakeholders' opinion, diazepam is less suitable than midazolam since midazolam has a shorter half-life and faster hepatic metabolism compared to diazepam. This is particularly important in neonates or young foals whose liver enzymes may not be fully developed (e.g. <21 days of age). Also, diazepam has active metabolites (e.g. desmethyldiazepam) that can prolong its sedative effects, especially in young animals with immature liver function, although there are similar active metabolites for midazolam (alpha-1 hydroxymidazolam, 1-OH-midazolam-glucuronide). Norman et al. (1997) investigated the pharmacokinetic disposition of diazepam in foals and concluded that care should be taken when administering repeated doses to foals less than 21 days old. Hubbell et al. (2013) found a median (range) terminal half-life of 216 (120-248) minutes for midazolam, while Schenk et al. (2021) reported a terminal half-life of 19.9 hours for diazepam.

The stakeholders provide one reference that allegedly raises concerns about diazepam in young foals. However, the remaining references provided concerning terminal half-life investigations were conducted in adult horses. For adult horses, the reported diazepam terminal half-life is 19.9 hours (initial dose 0.2 mg/kg), in one study, whereas utilizing a different methodology, another study found a midazolam median terminal half-life of 216 minutes (range: 120–248 min) and 408 minutes (range: 192–924 min) following a dose of 0.05 mg/kg or 0.1 mg/kg body weight, respectively. In a midazolam-containing veterinary medicinal product for non-food-producing horses, the elimination half-life is approximately 3.48 hours in horses. The stakeholders suggest that these differences in terminal half-life could become a clinical issue in young foals with lesser developed hepatic metabolic enzymes. However, the terminal half-life of diazepam studied in young foals (4 to 84 days), given a slightly higher dose (0.25 mg/kg) was between 187 to 331 minutes, depending on the age of the foal (Norman et al., 1997) and thus is clearly of lesser concern. Thus, the terminal half-life of diazepam, in foals, appears lowers than the terminal half-life of midazolam for adult horses, and considerably lower than the terminal half-life of diazepam for adult horses. It appears that the PK/PD characteristics of midazolam in young foals has not been investigated, including possible accumulation. Norman et al. (1997) identified a slower clearance of diazepam in foals < 3 weeks of age. Given the lower potency of diazepam compared to midazolam provides an added margin-of-safety that can be further accounted for by an adjustment of the diazepam dosing regimen. Furthermore, the effects of clearance on changes in elimination half-life is counter-balanced by changes in drug volume-of-distribution that can occur with age. Diazepam is primarily metabolized via the cytochrome P450 enzyme system, specifically CYP2C19 and CYP3A4 to the major active metabolite (desmethyldiazepam) as well as others (nordiazepam, oxazepam, and temazepam). Midazolam metabolism also occurs via hepatic CYP450 enzymes and glucuronide conjugation. Midazolam converts into its active metabolite alpha-1 hydroxymidazolam and 1-OH-midazolam-glucuronide, which also contributes to the drug action. Other commonly used drugs (omeprazole, rifampicin, macrolides) in young foals are also metabolized by the CYP450 enzymes, without any reported problems. Given the difference in terminal half-life between diazepam and midazolam, then it is unclear as to how this constitutes a safety risk when the dosage regimen can be adjusted accordingly. Moreover, the neonatal foal's liver-associated enzyme activity is generally higher and has greater variability between individuals when compared to adult horses (Gold, 2024). Immature liver function during first 4 weeks of the foal’s life relates mostly to limited glycogen reserves and gluconeogenesis, higher metabolic rates, and a tendency for hypoglycaemia (Baggot, 1994; Dunlop, 1994).

Two additional arguments are put forward by the stakeholders but these are considered outside of the criteria stated in the EC’s request for consideration of the essentiality and the clinical added benefit of a substance.

In justifying its wide use, the stakeholders indicate that the most recent version of the confidential enquiry of perioperative equine fatalities (the largest epidemiological study in equine anaesthesia in the past 20 years), midazolam was part of the anaesthetic protocol in nearly 20,000 equine patients anaesthetized over a 3-year period. This confidential report was not provided.

In addition, the stakeholders continue to indicate that while there is no formulation with diazepam licensed for use in horses, midazolam is licensed for use in non-food-producing horses which, in the stakeholders’ opinion, confirms its safety profile in this species. Therefore, the stakeholders consider that with diazepam retained on the list of essential substances, equine veterinarians will be in a position that midazolam use will be for non-food-producing horses and diazepam for food-producing horses, which may lead to confusion. However, equine veterinarians are not under any obligation to use any particular pre-medication protocol prior to anaesthesia, either being non-food-producing or food-producing horses. Also, there are other VMPs approved with MRLs for pre-medication/anaesthesia protocols for horses with appropriate safety and efficacy, without the use of benzodiazepines, as

supported by the peer-review scientific literature. Some field surgeries can also be performed as standing procedures. The published scientific literature reviewed seems not to favour one specific protocol or combination over others (Gangl et al., 2001; Umar et al., 2006, 2007; Hopster et al., 2014; Steblaj et al., 2014; Allison et al., 2018; Sage et al., 2018).

As also pointed out by other authors (see Gozalo-Marcilla et al., 2021), midazolam has recently gained market authorisation for use in horses in Europe, which would account for its increased use. However, this does not necessarily provide evidence with regards the discussion on the essentiality or added clinical benefit of the substance within the context of the Agency's advice. Furthermore, Gozalo-Marcilla et al. (2021) presented a multicentre, cohort, longitudinal and prospective survey of anaesthesia and peri-operative equine mortality. Less than 10000 horses are represented in two tables that summarise drugs used for either general anaesthesia or standing sedation. It is noted that out of the drugs used in 6701 horses surveyed by Gozalo-Marcilla et al. (2021) for general anaesthesia, midazolam was used in <50% phases of anaesthesia, including 3049 occasions (87+2795+40+125+2), and less frequently than diazepam in 3424 occasions; out of drugs used for standing sedation in 1955 horses surveyed in this report, midazolam was used in <10% of cases, including 110 cases, and diazepam in 14 cases. In general, drugs with established MRL entries were used more frequently. The Agency's advice recognises the existence of an authorised midazolam-containing VMP for non-food producing horses. As indicated by the stakeholders, existence of an authorised VMP provides certainty that efficacy and safety have been tested in the target animal. However, the existence of a VMP would not be a deciding factor for including a substance on the list over another one. Also, there are VMPs approved containing substances with MRLs for pre-medication/anaesthesia protocols. Regarding the approved midazolam-containing veterinary medicinal product, as indicated in its product information, it is noted that all safety aspects have not been fully investigated e.g. the safety of repeated bolus dosing and the possibility of drug accumulation.

Based on the criteria provided in the Commission's request[5] and the available evidence, midazolam was not proposed to be qualified as bringing added clinical benefit in the Agency's advice. The concerns from the stakeholders with regards the exclusion of midazolam from the list and the additional references provided have been discussed at the request from the European Commission. Overall, it is considered that the reasons that lead to the exclusion of midazolam from the proposed list of the essential substances prevail, even when taking into account the additional references provided by the stakeholders.

The advice does not dispute that midazolam is an effective drug in horses but rather concludes that the threshold of scientific evidence on its added clinical benefit, over what existing alternatives provide, is not met in this case. The possibility for improved availability has also been discussed and concluded that a need has not been identified. It is also noted that some of the arguments provided by the stakeholders do not fall within the framework of the EMA scientific assessment given, according to the criteria provided in the Commission's request.

On a side note, the World Veterinary Association (WVA) recently developed an essential medicines list for selected food animal species, including horses (https://worldvet.org/evml/). Although based on different criteria, it is noted that midazolam was not included on the WVA essential medicine list.

Knowledge regarding midazolam in horses for this assessment was derived from peer-reviewed publications, textbooks, discussion with experts as well as established expert databases.

[5] 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.

B. Considerations regarding consumer safety

Midazolam is a short-acting benzodiazepine administered also as a water-soluble salt, and thus the main routes of administration are intravenous (IV) or intramuscular (IM) injection. It is available on the EU market as veterinary and human medicinal products.

Midazolam exhibits similar pharmacologic actions as other benzodiazepines; subcortical levels (primarily limbic, thalamic, and hypothalamic) of the CNS are depressed, which produces anxiolytic, sedative, skeletal muscle relaxant, and anticonvulsant effects. The exact mechanism of action is unknown, but postulated to include antagonism of serotonin, increased release of and/or facilitation of gamma-aminobutyric acid (GABA) activity, and diminished release or turnover of acetylcholine in the CNS (Budde and McCluskey, 2023). Benzodiazepine-specific receptors have been located within the mammalian brain, kidney, liver, lungs, and heart.

Pharmacokinetic data in food-producing species (horses, donkeys, alpacas, sheep) and humans are available. In five horses given midazolam 0.05 or 0.1 mg/kg IV, the total clearance was 10.5 mL/minute/kg (average of medians of both dose groups). The volume of distribution (Vd) at steady state was observed at approximately 2 to 3 L/kg, for which a very wide tissue distribution is not expected. The terminal half-life varied widely and was maximum at 15.4 hours, ranging from 120 to 924 minutes (medians being 216 minutes after 0.05 mg/kg, and 408 minutes after 0.1 mg/kg) (Hubbell et al., 2013).

In gelded donkeys, the pharmacokinetics of midazolam were investigated in eight healthy animals aged 1-3 years. Blood samples were collected after intravenous administration of midazolam at a dose of 0.1 mg/kg. Plasma midazolam and 1-hydroxymidazolam were measured using reversed-phase high-performance liquid chromatography; pharmacokinetic variables were calculated using non-compartmental analysis in seven donkeys. After administration, midazolam was observed for up to 60 minutes. The median total body clearance was 1210 mL/kg/h, the volume of distribution at steady state was 359 mL/kg (thus a wide tissue distribution is not expected), the elimination half-life was 0.27 hours, and the area under concentration-time profile was 82.7 h X ng/mL. In 4 donkeys, the metabolite 1-hydroxymidazolam was detected at low levels (29 to 105 ng/mL) between 5 and 15 minutes after treatment with some differences between the individuals, although it was concluded that the pharmacodynamic effects were probably mediated by the parent drug rather than the metabolite (O et al., 2022).

In alpacas given midazolam 0.05 mg/kg IM or IV, bioavailability after IM administration was 92%, and peak plasma concentrations after IM administration were three times lower than after IV administration. Mean elimination half-lives were 98 minutes (IV) and 234 minutes (IM) (maximum 6.2 hours). The steady-state volume of distribution observed was 525 mL/kg (thus a wide tissue distribution is not expected). The authors concluded that midazolam appears to provide a short duration of action with moderate levels of sedation and minimal cardiovascular adverse effects or behavioural changes in alpacas (Aarnes et al., 2013; Budde and McCluskey, 2023).

In eight healthy adult sheep receiving midazolam 0.5 mg IV, the volume of distribution was 838 ± 330 mL/kg and the elimination half-life was 0.79 ± 0.44 hours. After IM administration of 0.5 mg/kg, the total drug exposure as calculated by the area under the curve (AUC) was 3.3 times higher than with IV administration. Time to peak plasma concentration following IM injection was few minutes (Simon et al., 2017; Budde and McCluskey, 2023)

In humans, the onset of action following IV administration is rapid due to the high lipophilicity of the agent, and loss of the lash reflex occurs within 30 to 97 seconds after administration. Following IM injection, midazolam is rapidly and almost completely (91%) absorbed. The drug is well absorbed after oral administration; however bioavailability is limited (approximately 36%) because of a rapid first-

pass effect (Nordt and Clark, 1997). Midazolam is highly protein-bound (i.e. 94% to 97%) and rapidly-crosses the blood-brain barrier. Changes in plasma protein concentrations and resultant protein binding may significantly alter the response to a given dose because only unbound drugs cross into the CNS. The serum half-life and duration of activity of midazolam in humans are shorter than diazepam. Midazolam is eliminated almost entirely by renal mechanisms as conjugates of hydroxylated metabolites. Elimination half-life in humans is 2 hours (diazepam is 30 hours) and is almost doubled in patients with significant hepatic impairment. The main active metabolite is alpha-hydroxymidazolam which has a pharmacologic activity that contributes to midazolam clinical effects. Alpha-hydroxymidazolam has a half-life of 1 to 1.5 hours, which is prolonged in patients with renal impairment (Budde and McCluskey, 2023; Drugbank, 2025).

Adverse effects of benzodiazepines on the fetus during pregnancy or the infants when administrated during birth have been observed in humans and animals; however, midazolam has not been demonstrated to cause fetal abnormalities. In humans, other benzodiazepines have been implicated in causing congenital abnormalities when administered during the first trimester of pregnancy. Anesthetic agents administered to female animals during the third trimester of pregnancy (and during other periods of rapid brain growth) may adversely affect the developing brain. Human infants born to mothers given large doses of benzodiazepines shortly before delivery have been reported to suffer from apnea, impaired metabolic response to cold stress, difficulty feeding, hyperbilirubinemia, and hypotonia. Signs of withdrawal have occurred in infants whose mothers chronically took benzodiazepines during pregnancy (Budde and McCluskey, 2023).

In humans, midazolam and alpha-hydroxymidazolam are excreted in milk and may cause CNS effects in nursing neonates (Budde and McCluskey, 2023).

To determine repeat dose toxicity, 13-week oral studies were conducted in rats and dogs (Schläppi, 1983). In rats, no findings were found at 50 mg midazolam/kg bw day, whereas an increase of about 30% in absolute liver weights were observed at 100 mg/kg bw day, and a slight reduction in weight gain (10% in females, 3% in males), an increase in liver weight of about 50% and slightly fat-enriched hepatocytes at 200 mg/kg bw day. For the dogs that were investigated, rare clinical effects were reported (no dose groups are given). Also in rats, adverse effects on male or female fertility were not observed for midazolam given orally (0, 1, 4, or 16 mg/kg) to male and female individuals before and during mating, and also in female rats throughout gestation and lactation (Drugbank, 2025).

Benzodiazepine-associated teratogenicity and congenital malformations are known but no study with midazolam could be retrieved from the literature search. According to the classifications provided by companies to ECHA, midazolam is sometimes classified as suspected to be harmful for the unborn child (ECHA C&L, 2024).

There is no current information on genotoxicity/mutagenicity from the public literature. Summary of product characteristics (SPC) for human medicinal products containing midazolam contain information on non-genotoxicity (Drugbank, 2025), e.g. indicating that midazolam was not mutagenic in *Salmonella typhimurium* (5 bacterial strains), Chinese hamster lung cells (V79), human lymphocytes or in the micronucleus test in mice. The ECHA lead registration dossier also report the existence of negative tests and refers to tests in bacteria (*Salmonella*), in CHO cells (i.e. epithelial ovary cells from Chinese hamster); further, there was no chromosomal aberrations within peripheral human lymphocytes and that the conducted *in vivo* and *in vitro* micronucleus tests were negative (ECHA, 2024).

There is some evidence for tumour-promoting properties of midazolam, however, no studies could be reviewed. The carcinogenicity of midazolam has not been assessed by the IARC (IARC, 2024) and there are no carcinogenicity studies publicly available. From human-medicine approved SPCs, there is

information available that following chronic administration, at the highest dose used (80 mg/kg day) hepatic tumours in mice and benign thyroid follicular cell tumours in rats were observed. The effects were dose-dependent with an unknown pathogenesis. Dosages of 9 mg/kg/day of midazolam maleate and lower for two years did not increase the incidence of tumours in rats and mice. There were no indications from human studies. In the ECHA lead registration dossier it is summarised that, "on the basis of tests performed, it was concluded that Midazolam at high doses is a promoter of hepatic tumour formation in sensitive mouse strains (e.g. B6C3F1) but has no initiating-type carcinogenic activity" (ECHA, 2024).

Thus, it can be expected that midazolam is not genotoxic. Some evidence of carcinogenicity has been found in rodents at high chronic dosages and a threshold may be assumed. However, it is also noted that due to the short terminal half-life of midazolam, residues in horse meat after six-month withdrawal period are not to be expected (see also below).

No residue depletion study data for midazolam in horses or in other species could be retrieved from the published literature.

From a tissue disposition study, midazolam residues within skeletal muscle of 11 horses were investigated directly after euthanasia for medical reasons. The horses had been initially anaesthetised with a total intravenous dose of xylazine (means 2.5 mg/kg), midazolam (0.1 mg/kg) and ketamine hydrochloride (a mean 5.8 mg/kg) and then were euthanised with lidocaine (means 4 mg/kg). An atlanto-occipital cisterna centesis was performed for the collection of cerebrospinal fluid (CSF) and administer of lidocaine hydrochloride. After confirmation of death, blood samples (serum and plasma), skeletal muscle (*triceps brachii, gluteus medius*), and CSF were immediately collected and frozen in liquid nitrogen, stored at -80°C. Midazolam concentration were detected in plasma (mean 23.2 ng/ml), serum (mean 23.2 ng/ml), CSF (mean 1.2 ng/ml) and skeletal muscle (*m. gluteus* mean 36.6 ng/; triceps mean 50.2 ng/g) performing liquid chromatography tandem mass spectrometry (LC-MS/MS) with positive heated electrospray ionization (HESI[+]) at 300°C). Frozen skeletal muscle available from 5 additional horses euthanised by intravenous injection (anaesthetised with xylazine 1.1 mg/kg, midazolam 0.1 mg/kg, and ketamine 2.2 mg/kg; euthanised with lidocaine 4mg/kg) was also analysed; the mean concentration of midazolam detected for skeletal muscle (*gluteus medius*) was 32.4 ng/g. Overall, residues of midazolam in muscle were found in all horses, but at very low concentrations (Aleman et al., 2016).

Considering that the substance has a relatively short terminal half-life in horses, data available suggests that a wide tissue distribution is not to be expected in horses or other species, a very low skeletal muscle disposition was detected in euthanised horses and thus residues in horse meat are not to be expected, it can be accepted that midazolam will not pose an unacceptable risk for the consumers when used in food producing animals of the equine species and a six month withdrawal period is respected.

Conclusion

Based on the above assessment and justifications, the recommendation to remove midazolam from the list of essential substances is retained.

Overall, it is considered that the reasons that lead to the exclusion of midazolam from the proposed list of the essential substances prevail, even when taking into account the additional references provided by the stakeholders. The advice does not dispute that midazolam is an effective drug in horses but rather concludes that the threshold of scientific evidence on its added clinical benefit, over what existing alternatives provide, is not met in this case. The possibility for improved availability has also been discussed and concluded that a need has not been identified.

References

Substances for sedation and premedication (and antagonism) - midazolam

Aarnes, T.-K., Fry, P.-R., Hubbell, J.-A., Bednarski, R.-M., Lerche, P., Chen, W., Bei, D., Liu, Z., Lakritz, J., 'Pharmacokinetics and pharmacodynamics of midazolam after intravenous and intramuscular administration in alpacas', American journal of veterinary research, 2013, Vol. 74(2), pp. 294-299.

Aarnes, T.-K., Lerche, P., Bednarski, R.-M., Hubbell, J.-A.-E., 'Total intravenous anaesthesia using a midazolam-ketamine-xylazine infusion in horses: 46 cases (2011-2014)', Can Vet J, May 2018, Vol. 59(5), pp. 500-504.

Aleman, M., Davis, E., Knych, H., Guedes, A., Smith, F., Madigan, J.-E., 'Drug Residues after Intravenous Anesthesia and Intrathecal Lidocaine Hydrochloride Euthanasia in Horses', Journal of veterinary internal medicine, 2016, Vol.30(4), pp. 1322-1326.

Allison, A., Robinson, R., Jolliffe, C., Taylor, P.-M., 'Evaluation of the use of midazolam as a co-induction agent with ketamine for anaesthesia in sedated ponies undergoing field castration', Equine veterinary journal, 2018, Vo. 50(3), pp. 321-326.

Baggot, J.-D., 'Drug therapy in the neonatal foal. The Veterinary clinics of North America', Equine practice, 1994, Vol. 10(1), pp.87-107.

Budde, J.-A., McCluskey, D.-M., 'Plumb's Veterinary Drug Handbook', In Wiley-Blackwell (Ed.), 2023, Edition 10.

de Vries, A., Thomson, S., Taylor, P.-M., 'Comparison of midazolam and diazepam as co-induction agents with ketamine for anaesthesia in sedated ponies undergoing field castration', Veterinary anaesthesia and analgesia, 2015, Vol. 42(5), pp. 512–517.

Drugbank, Midazolam [Updated 14 Jan 2025]. Accessed January 2025, available from: https://go.drugbank.com/drugs/DB00683

Dunlop, C.-I., 'Anesthesia and Sedation of Foals', Veterinary Clinics of North America: Equine Practice, 1994, Vol. 10(1), pp. 67-85.

ECHA C&L. Notified Classification and Labelling – Midazolam. Accessed December 2024, available from: https://echa.europa.eu/de/information-on-chemicals/cl-inventory-database/-/discli/details/40054

ECHA, Registration Dossier – Modazolam. Accessed December 2024, available from: https://echa.europa.eu/de/registration-dossier/-/registered-dossier/32755

Gangl, M., Grulke, S., Detilleux, J., Caudron, I., Serteyn, D., 'Comparison of thiopentone/guaifenesin, ketamine/guaifenesin and ketamine/midazolam for the induction of horses to be anaesthetised with isoflurane', The Veterinary record, 2001, Vol. 149(5), pp. 147-151.

Gozalo-Marcilla, M., Bettschart-Wolfensberger, R., Johnston, M., Taylor, P.-M., Redondo, J.-I., 'Data Collection for the Fourth Multicentre Confidential Enquiry into Perioperative Equine Fatalities (CEPEF4) Study: New Technology and Preliminary Results', Animals : an open access journal from MDPI, 2021, Vol. 11(9), p. 2549.

Gold, J., 'Clinical Chemistry in the Foal', In Equine Neonatal Medicine (eds D.M. Wong and P.A. Wilkins), 2024. Available in: https://doi.org/10.1002/9781119617228.ch44

Granados, M.-M., Gómez-Villamandos, R.-J., Palacios, C., et al., 'Diazepam and midazolam as preanesthetics in horses before sevoflurane anesthesia', Abstract presented at the World Congress of Veterinary Anesthesia, Knoxville, USA, Vet Anaesth Analg, 2004, Vol. 31, p. 31.

Harðardóttir, H., Murison, P.-J., Blissitt, K., Olason, S., Clutton, R.-E., 'A comparison of two ketamine doses for field anaesthesia in horses undergoing castration', Equine veterinary journal, 2019, Vol. 51(4), pp.458–463.

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.

Hubbell, J.-A., Kelly, E.-M., Aarnes, T.-K., Bednarski, R.-M., Lerche, P., Liu, Z., Lakritz, J., 'Pharmacokinetics of midazolam after intravenous administration to horses', Equine veterinary journal, 2013, Vol. 45(6), pp. 721–725.

IARC International Agency for Research on Cancer. List of Classifications. Accessed January 2024, available from: https://monographs.iarc.who.int/list-of-classifications/

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.

Kanto, J.-H., 'Midazolam: the first water-soluble benzodiazepine. Pharmacology, pharmacokinetics and efficacy in insomnia and anesthesia', Pharmacotherapy, 1985, Vol. 5(3), pp. 138-155.

Massoco, C., Palermo-Neto, J., 'Effects of midazolam on equine innate immune response: a flow cytometric study', Veterinary immunology and immunopathology, 2003, Vol. 95(1-2), pp. 11-19.

Murney, P., 'To mix or not to mix – compatibilities of parenteral drug solutions', Australian Prescriber, 2008, Vol. 31(4), pp. 98-101.

Nordt, S.-P., Clark, R.-F., 'Midazolam: a review of therapeutic uses and toxicity', The Journal of emergency medicine, 1997, Vol. 15(3), pp. 357-365.

Norman, W.-M., Court, M.-H., Greenblatt, D.-J., 'Age-related changes in the pharmacokinetic disposition of diazepam in foals', American journal of veterinary research, 1997, Vol. 58(8), pp. 878-880.

O, O., Simon, B.-T., Ebner, L.-S., Lizarraga, I., Sun, X., Cox, S.-K., 'The pharmacokinetics and pharmacodynamics of midazolam after intravenous administration to donkeys (Equus africanus asinus)', Canadian journal of veterinary research (Revue canadienne de recherche veterinaire), 2022, Vol. 86(2), pp. 125-131.

Platt, J.-P., Simon, B.-T., Coleman, M., Martinez, E.-A., Lepiz, M.-A., Watts, A.-E., ‚The effects of multiple anaesthetic episodes on equine recovery quality', Equine Vet J, 2018, Vol. 50, pp. 111-116.

Sage, A.-M., Keating, S.-C., Lascola, K.-M., Schaeffer, D.-J., Clark-Price, S.-C., 'Cardiopulmonary effects and recovery characteristics of horses anesthetized with xylazine-ketamine with midazolam or propofol', Veterinary anaesthesia and analgesia, 20108, Vol. 45(6), pp. 772-781.

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', Journal of veterinary pharmacology and therapeutics, 2021, Vol. 44(5), pp. 733-744.

Schläppi, B., 'Safety aspects of midazolam', British journal of clinical pharmacology, 1983, Vol. 16(1), pp. 37S-41S.

Simon, B.-T., Scallan, E.-M., O, O., Ebner, L.-S., Cerullo, M.-N., Follette, C., Cox, S.-K., Doherty, T.-J., Lizarraga, I., 'Pharmacokinetics and pharmacodynamics of midazolam following intravenous and intramuscular administration to sheep', American journal of veterinary research, 2017, Vol. 78(5), pp. 539-549.

Steblaj, B., Schauvliege, S., Pavlidou, K., Gasthuys, F., Savvas, I., Duchateau, L., Kowalczyk, L., Moens, Y., 'Comparison of respiratory function during TIVA (romifidine, ketamine, midazolam) and isoflurane anaesthesia in spontaneously breathing ponies Part I: blood gas analysis and cardiorespiratory variables', Veterinary anaesthesia and analgesia, 2014, Vol. 41(6), pp. 583-591.

Umar, M.-A., Yamashita, K., Kushiro, T., Muir, W.-W., 'Evaluation of total intravenous anesthesia with propofol or ketamine-medetomidine-propofol combination in horses', Journal of the American Veterinary Medical Association, 2006, Vol. 228(8), pp. 1221-1227.

Umar, M.-A., Yamashita, K., Kushiro, T., Muir, W.-W., 'Evaluation of cardiovascular effects of total intravenous anesthesia with propofol or a combination of ketamine-medetomidine-propofol in horses', American journal of veterinary research, 2007, Vol 68(2), pp. 121-127.

Wise, I.-K., Klöppel, H., Leece, E.-A., 'Comparison of two doses of ketamine for induction of anaesthesia in ponies undergoing field castration', Open veterinary journal, 2021, Vol. 11(4), pp. 747–754.