14 May 2020
EMA/CVMP/211307/2019
Committee for Medicinal Products for Veterinary Use

European public MRL assessment report (EPMAR)

Bambermycin (rabbit)

On 17 January 2020 the European Commission adopted a Regulation[1] establishing maximum residue limits for bambermycin in rabbit, valid throughout the European Union. These maximum residue limits were based on the favourable opinion and the assessment report adopted by the Committee for Medicinal Products for Veterinary Use.

Bambermycin is intended for use in rabbits for the reduction of mortality in Enzootic Rabbit Enteropathy (ERE) at an oral dose of 1.8 mg/kg bw per day for 14 days, administered in feed.

Huvepharma N.V. submitted to the European Medicines Agency an application for the establishment of maximum residue limits for bambermycin in rabbit on 19 April 2017.

Based on the original and complementary data in the dossier, the Committee for Medicinal Products for Veterinary Use recommended on 16 April 2019 the establishment of maximum residue limits for bambermycin in rabbit.

Subsequently the Commission recommended on 24 October 2019 that maximum residue limits in rabbit are established. This recommendation was confirmed on 14 November 2019 by the Standing Committee on Veterinary Medicinal Products and adopted by the European Commission on 17 January 2020.

[1] Commission Implementing Regulation (EU) No 2020/42, O.J. L 15, of 20 January 2020

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Summary of the scientific discussion for the establishment of MRLs

Substance name:	Bambermycin
Therapeutic class:	Anti-infectious agents/Antibiotics
Procedure number:	EMEA/V/MRL/004828/FULL/0001
Applicant:	Huvepharma N.V.
Target species requested:	Rabbit
Intended therapeutic indication:	Reduction of mortality in Enzootic Rabbit Enteropathy (ERE)
Route(s) of administration:	Oral

1. Introduction

Bambermycin, also referred to as flavomycin, moenomycin and flavophospholipol (CAS number 11015-37-5), is an antibiotic obtained from cultures of *Streptomyces bambergiensis* and related strains. It is composed of at least five active components: moenomycins A, A1/2, C1, C3 and C4, with moenomycin A (flavomycin A) being the major component.

Bambermycin is intended for use in rabbits for the reduction of mortality in Enzootic Rabbit Enteropathy (ERE) at an oral dose of 1.8 mg/kg bw per day for 14 days, administered in feed.

Bambermycin has not previously been used in veterinary medicinal products.

Bambermycin is not used in human medicine.

2. Scientific risk assessment

2.1. Safety assessment

2.1.1. Overview of pharmacological properties

Pharmacodynamic properties including mode of action

Bambermycin is a phosphoglycolipid antimicrobial produced by various strains of Streptomyces. It is an antibiotic, primarily active against Gram-positive bacteria, exerting its antimicrobial action by the direct inhibition of peptidoglycan glycosyltransferases, bacterial enzymes which are involved in the penultimate step of bacterial cell wall biosynthesis.

The antimicrobial spectrum of bambermycin includes *Staphylococcus aureus*, coagulase-negative staphylococci, and most strains of *Enterococcus faecalis*. Literature data show that the minimum inhibitory concentration (MIC) of bambermycin for *S. aureus* range from 0.05 to 0.4 µg/ml, for coagulase-negative staphylococci from 0.25 to 2 µg/ml, for *E. faceium* from 0.5 to >128 µg/ml and for *E. faecalis* from 0.125 to 128 µg/ml. However, it has to be noted that most of the data originate from scientific papers published from 1983 to 2004.

Also, most strict and facultative anaerobes appear to be resistant to bambermycin. For *Clostridum perfringens*, other *Clostridium spp.*, *Lactobacillus spp.* (excluding *Lactobacillus acidophilus*), and *Bifidobacterium spp.,* elevated bambermycin MICs have been reported. Intrinsic resistance to bambermycin is reported for fermentative (e.g., Enterobacteriaceae) and non-fermentative Gram-negative bacilli (e.g., *Pseudomonas spp*.). Wild-type strains of *Escherichia coli*, *Salmonella spp*., *Klebsiella spp., Pseudomonas spp*., and *Campylobacter spp*. exhibit MIC values to bambermycin equal or higher than 128 µg/ml and are therefore considered to be resistant.

Bacteria of the *Enterococcus gallinarum* group (*E. gallinarum* and *E. casseliflavus*), and most species from the *E. faecium* group (*E. faecium, E. mundtii,* and *E. hirae*) show natural resistance to bambermycin.

Resistance to bambermycin seems to develop slowly amongst bacterial populations through mutations in the bacterial chromosome and is non-transferable.

Pharmacokinetic properties (mainly in laboratory animals)

Four GLP reports of radiolabelled studies were provided, in which ^{14}C-bambermycin was administered orally to rabbits, rats, chickens and pigs. These studies were conducted in order to provide information on the absorption and distribution of bambermycin in these species. The studies had similar experimental designs. After dosing groups of animals with unlabelled bambermycin for 10 days via the feed, animals were then given a single oral dose of ^{14}C-labeled bambermycin. At 4 and 48 hours after administration of the radiolabelled compound, a group of animals was killed and samples of organs (spleen, kidney and liver) and tissues (skin, fat and muscle) were taken for analyses. After dosing with the ^{14}C - labelled compound, the urine and faeces were collected quantitatively for the 48 hour group, and at various times blood samples were taken. The amount of radioactivity in the samples was measured using standard liquid scintillation counting methods. Since labelled bambermycin was synthesised via fermentation using (2-14C) acetate as a precursor, in theory any fraction corresponding to acetic acid in the molecule could have been labelled. Therefore information is lacking on the exact position of the ^{14}C-label in the labelled studies.

In order to provide information on the biotransformation of bambermycin, an *in vitro* study was provided that compares the metabolic pathways of bambermycin in suspensions of hepatocytes obtained from rats, rabbits, cows, chickens and pigs.

In the pivotal radiolabelled GLP study (reported in more detail in section 2.2.2 below), ^{14}C-bambermycin was administered orally for 14 days to 4 groups of rabbits (2 male and 2 female/ group). At time points 4 hours, 2, 5 and 10 days after the last dose a group was slaughtered, and radioactivity was measured in the edible tissues and in plasma. Of the group slaughtered at 10 days, amounts of radioactivity excreted in faeces and urine were measured in order to determine the mass balance of ^{14}C- bambermycin in rabbits. Extracts of pooled liver and kidney samples were analysed using liquid chromatography methods, in order to investigate the *in vivo* biotransformation pattern.

Absorption

Bambermycin was poorly absorbed in all species studied (rabbit, rat, chicken, pig). A final estimate on the percentage of absorption in rabbits could be derived from the pivotal radiolabelled study on the metabolism, mass balance and residues of ^{14}C-bambermycin. It could be calculated that approximately 0.2% of the daily dose was absorbed. Therefore absorption of bambermycin after oral administration can be considered to be negligible. It is noted that the radiolabelled studies provided no details on the actual amount of bambermycin in the excreta, possible degradation in the digestive tract, and the identity of the radioactive compounds in the urine. Plasma data after intravenous versus oral administration were not provided, so an accurate measure of bioavailability could not be done. The time dependent blood concentration curves of radioactivity showed a rapid onset of the absorption in all species studied. None of the presented studies allowed the reliable estimation of pharmacokinetic parameters (e.g. Cmax, Tmax, $T_{1/2}$) for bambermycin, since the data in blood were not specific and/or detailed enough. In all studies however blood levels showed a relatively small decline over time, suggesting a long half-life.

Considering the negligible absorption of bambermycin after oral administration, the influence of a possible enterohepatic recirculation on the pharmacokinetics of bambermycin can be excluded.

Biotransformation

Data provided supports that the rate of biotransformation in rabbits can be considered to be very low. No detailed *in vivo* information regarding biotransformation was provided. In the *in vivo* studies with radioactive bambermycin, radioactivity levels were too low to perform further research on the identity of possible metabolites. High Performance Liquid Chromatography chromatograms (radiochemical detection) from pooled kidney and liver extract, revealed the presence of a large number of compounds (up to 30), each of them representing an amount of radioactivity smaller than 10% of the total radioactivity. Their structure and origin could, however, not be elucidated.

The *in vitro* study in hepatocytes from rats, rabbits, cows, chickens and pigs generated comparative information on possible routes of degradation of the various components of bambermycin. In all the species tested these metabolic pathways (phase I and phase II) were generally comparable. No information on the actual molecular structure of the metabolites formed was provided. The rate of biotransformation of bambermycin compared to the reference compounds testosterone and ethoxy-coumarin seems very low, probably due to slow diffusion of bambermycin into the hepatocytes.

Distribution

An unlabelled residue study (reported in more detail in section 2.2.2 below) revealed that, after oral administration to rabbits, the highest concentrations of bambermycin can be found in liver and kidney. The measured levels in both organs are more or less comparable. In radio-kinetic studies performed in rabbits, rats, chickens and pigs, it was shown that the levels of radioactivity were the highest in kidney and liver. In the pivotal radiolabelled study on the metabolism/mass balance and residues of bambermycin in rabbits, levels of radioactivity were highest in kidney, fat and liver.

Excretion

Since the majority of an oral dose of bambermycin is not absorbed, most of it is excreted via the faeces, whilst the excretion of the absorbed fraction via the urine plays numerically a minor role.

2.1.2. Calculation of pharmacological ADI, if relevant

The pharmacological activity of bambermycin is limited to its antimicrobial action, therefore the establishment of a pharmacological NO(A)EL is not deemed necessary, in line with the CVMP Guideline on the approach to establish a pharmacological ADI (EMA/CVMP/SWP/355689/2006).

2.1.3. Overview of toxicology

Information provided regarding the precise identification of bambermycin reported that the total sum of impurities amount to less than 10%. However, the test substances used in the toxicity studies had very different compositions, with some studies using unpurified bambermycin in mycelium, while other studies did not state the purity of bambermycin used. In most studies, the dose in terms of mg bambermycin per kg bodyweight could be calculated. It should be noted that the interpretation of the results will become more difficult at lower purities. In some studies, the purity was below 1%. Studies with such low purity have been considered to be unsuitable for use in the risk assessment for bambermycin and were not considered further.

Single-dose toxicity

The acute oral toxicity of bambermycin was tested in mice and rats (GLP). No overt signs of toxicity and no mortality was observed up to the highest doses tested (843 and 2000 mg/kg bw in mice and 2000 mg/kg bw in rats).

Repeated dose toxicity

90-day oral repeated dose toxicity studies were performed in rats and dogs (no GLP).

In the 90-day study in rats, bambermycin was given via the feed at doses corresponding to 0, 100, and 1000 mg/kg bw per day, to groups of rats that were suffering from a bacterial infection that had to be treated. In this period, a temporary drop in weight gain was noted. No treatment related effects were reported. The NOAEL in this study was established at 1000 mg/kg bw per day, the highest dose tested. However, the results from this study have to be considered with caution due to, amongst other things, all treated rats having a bacterial infection. In addition, the study was quite old (1969, pre GLP) and did not follow any internationally accepted protocols, such as OECD test guidelines. There were only two dose levels, and the vehicle and the purity were different in these two dose groups. The level of purity was low (13.6% for the 100 mg/kg bw per day group and 50-60% for the 1000 mg/kg bw per day group).

In the 90-day study in dogs, the dose levels of bambermycin in feed corresponded to 0, 18, and 180 mg/kg bw per day. No treatment related effects were observed in any of the groups. The NOAEL in this study was established at 180 mg/kg bw per day, the highest dose tested. However, as for the study in dogs, the results from this study have to be considered with caution. The study was quite old (1969, pre GLP) and did not follow any internationally accepted protocols such as OECD test guidelines. There were only two dose levels, and the vehicle and the purity were different in the different dose groups. The level of purity was low (less than 1% for the 18 mg/kg bw per day group and 58-82% for the 180 mg/kg bw per day group).

Long term toxicity of bambermycin was addressed in a combined chronic toxicity and carcinogenicity study in rats (reported in the section below on carcinogenicity).

Reproductive toxicity, including developmental toxicity

A 2-generation reproductive toxicity study (GLP) was conducted in rats. The study also included a teratology phase. The animals were dosed via the feed at levels corresponding to approximately 0, 80, 400, and 800 mg/kg bw per day. Treatment started 70 days prior to mating for males and 28 days prior to mating for females. The purity of the test substance was approximately 10%.

At the two highest doses, reduced feed intake and reduced bodyweights and bodyweight gain were observed in the parents, as well as in the pups. The F0 males had increased liver weight at 400 mg/kg bw per day (relative) and 800 mg/kg bw per day (both absolute and relative), and in females at 400 and 800 mg/kg bw per day (both absolute and relative). The kidney weight of F0 parents increased at 400 and 800 mg/kg bw per day in males (both absolute and relative), and females (relative only). In males, the relative testes weight increased in the 400 and 800 mg/kg bw per day groups. The effects on liver and kidney weight were also apparent in the F2 generation (although these were not picked up in the F1). However, in these animals the effects were also noted at the lowest dose of 80 mg/kg bw per day and reached statistical significance: liver weight increased in males at 80 mg/kg bw per day (relative), 400 mg/kg bw per day (both absolute and relative), and 800 mg/kg bw per day (relative), and in females at 400 and 800 mg/kg bw per day (both absolute and relative). Kidney weight increased in males at 80 mg/kg bw per day (absolute), at 400 mg/kg bw per day (both absolute and relative), and at 800 mg/kg bw per day (both absolute and relative), and in females at 800 mg/kg bw per day (relative). In males, the relative testes weight was increased at 800 mg/kg bw per day. Slight hepatocyte hypertrophy was observed in males and females of the 800 mg/kg bw per day group. A minimal but dose-related increase in severity of chronic interstitial nephritis (a common spontaneous lesion in rats) was seen in a few animals of the 400 and 800 mg/kg bw per day dose groups. Treatment with bambermycin did not induce any reproductive toxicity or teratogenicity in rats.

No NOAEL could be established in view of the dose-related effects on absolute kidney weight at the lowest dose (8% increase, $P<0.05$), and increased relative liver weight at the lowest dose (also 8%, $P<0.05$), in male rats of the F2 generation. Given that these increases are dose-related, and accompanied with histopathological changes at higher doses, and because the pharmacokinetic studies showed that the highest bambermycin concentrations are found in liver and kidney, these changes at the lowest dose cannot be ignored. Therefore, the lowest dose of 1% in the diet, equivalent with 80 mg/kg bw per day, is considered to be the LOAEL in this study. Furthermore, the purity of the test substance was very low (approximately 10%), hence the results of this study have to be taken with caution.

In a developmental toxicity study (GLP), pregnant rabbits were given bambermycin by gavage at doses of 1.4, 14, and 140 mg/kg bw per day during 18 days. The purity of the test substance was approximately 5%. A lower body weight gain, consistent with a reduced feed intake, was noted in the dams at the highest dose. Treatment with bambermycin did not induce embryo/foetotoxicity or teratogenicity. The NOAEL for maternal toxicity was 14 mg/kg bw per day, and the NOAEL for both embryo/foetotoxicity and teratogenicity was 140 mg/kg bw per day, the highest dose tested.

Genotoxicity

Four *in vitro* studies were submitted, two Ames tests to detect gene mutations in prokaryotes and two mouse lymphoma tests to detect gene mutations in eukaryotes. In addition, an *in vivo* micronucleus test to detect chromosomal breaks and aneuploidy in mice was provided.

The two Ames tests were negative, the two mouse lymphoma tests were weakly positive, and the micronucleus test was negative.

The results of the older Ames test (1977) should be taken with caution, as this was a non-GLP study and the origin and purity of the test substance was not specified. A newer Ames test (2014), conducted under GLP and using *Salmonella typhimurium* strains and *E. coli* WP2 *uvrA*, with and without metabolic activation, indicates that bambermycin does not induce gene mutations in prokaryotic cells.

In the two mouse lymphoma tests (GLP), the purity of test substances was not determined, although it appeared that the test substances had a relatively high degree of impurity. These studies rendered weakly positive results, which were dose-related, and occurred only in the presence of metabolic activation and clearly in the cytotoxic range, and where precipitation of the test substance was observed. The authors analysed the mutant colony size distribution patterns and concluded that the responses obtained with bambermycin were likely to be associated primarily with chromosomal aberrations rather than small deletions or point mutations. Given these results, it is concluded that the positive results were likely to be the cause of cytotoxicity and associated dysfunction of the cells in the test system. Therefore, a direct (DNA-reactive) mutagenic effect in eukaryotic cells appears to be absent.

The *in vivo* micronucleus test (GLP) was clearly negative.

On the basis of the weight of evidence, it is concluded that bambermycin is not genotoxic.

Carcinogenicity

In a 2-year combined chronic toxicity and carcinogenicity study (GLP), rats received bambermycin in their diet at doses corresponding to 0, 23.5, 47, and 94 mg/kg bw per day in males and 0, 29.75, 59.5, and 119 mg/kg bw per day in females. The study also included a reproductive phase. No effects on reproductive parameters were noted. No neoplastic changes and no other signs of toxicity were observed up to the highest dose tested. Under the conditions of this study, the NOAEL appeared to be 94 mg/kg bw per day, the highest dose tested. This result has to be taken with great caution, as the

purity of the test substance was very low (approximately 5%). In addition, the study did not follow international recognised protocols, such as OECD.

In a 2-year carcinogenicity study (GLP), mice were given bambermycin in their feed at doses corresponding to 0, 75, 150, and 300 mg/kg bw per day in males, and 0, 97.5, 195, and 390 mg/kg bw per day in females. At the highest dose, an increased nuclear alteration (enlarged lobular nuclei) of hepatocytes was noted (25% versus 2.4% in controls). The significance of this finding is unknown. No other treatment related effects were noted at any of the dose levels. No (pre)neoplastic lesions were observed. Under the conditions of this study, the NOAEL appeared to be 300 mg/kg bw per day, the highest dose tested. This result has to be taken with great caution, as the purity of the test substance was very low (approximately 5%).

Despite the shortcomings of the two carcinogenicity studies and the repeated dose toxicity studies with bambermycin, (pre-)neoplastic lesions were not observed in any of these studies. Furthermore, studies on genotoxicity concluded that bambermycin is not genotoxic. Altogether, it can be concluded that there is no concern in relation to carcinogenicity.

Studies of other effects including immunotoxicity and neurotoxicity

Neurotoxicity studies and immunotoxicity studies have not been performed with bambermycin. However, these studies are not considered necessary because there is no initial concern of immune system damage or neuropathological effects after prolonged exposure to bambermycin.

2.1.4. Calculation of the toxicological ADI or alternative limit

Summary of NOELs and all relevant studies:

Species	Study type and duration	NOEL	Comments
Rat	Oral (feed) repeated dose study, 90 days	1000 mg/kg bw per day, the highest dose tested	Pre-GLP (1969). Two dose levels only. Vehicle and purity differed between dose groups (1.3% and 50-60%). Bacterial infection during the study. Results to be taken with caution.
Dog	Oral (feed) repeated dose study, 90 days	180 mg/kg bw per day, the highest dose tested	Pre-GLP (1969).Two dose levels only. Vehicle and purity differed between dose groups (<1% and 58-82%). Results to be taken with caution.

Species	Study type and duration	NOEL	Comments
Rat	Oral (feed) 2-generation reproductive toxicity study (including teratogenicity phase)	80 mg/kg bw per day is the LOAEL, based on increased liver and kidney weights in male F2 rats	GLP study. Treatment did not cause reproductive toxicity or teratogenicity. Purity approximately 10%. Results to be taken with caution.
Rabbit	Oral (gavage) teratogenicity study	Maternal toxicity: 14 mg/kg bw per day, based on decreased bodyweight gain and feed consumption at the highest dose. Foetal/embryo-toxicity: 140 mg/kg bw per day, the highest dose tested. Teratogenicity: 140 mg/kg bw per day, the highest dose tested.	GLP study. Purity approximately 5%. Results to be taken with caution.
Rat	Oral (feed) long-term toxicity and carcinogenicity study, including reproductive phase	94 mg/kg bw per day, the highest dose tested.	GLP study. Purity approximately 5%. Results to be taken with caution.
Mice	Carcinogenicity study	300 mg/kg bw per day, the highest dose tested.	GLP study. Purity approximately 5%. Results to be taken with caution.

A complete package of toxicity studies was available. However, all studies are more than 20 and up to nearly 50 years old (except for some of the genotoxicity studies). Various studies were not GLP and most of them did not follow international standards such as OECD. In addition to this, the purity of the test substance was in most cases extremely low (even below 1%). On the other hand, the (re-calculated) doses in terms of mg bambermycin/kg bw per day were still quite high and did – in most cases – not induce any toxic effects. The exception appears to be the two generation reproductive toxicity study in rats, where effects on liver and kidney were noted. The LOAEL in this study was 80 mg/kg bw per day.

This data package, with the shortcomings as mentioned above, would normally not be acceptable for the establishment of a toxicological ADI. However, the applicant demonstrated that the microbiological effects on the intestinal microbiota are more relevant for the risk assessment (occurring at far lower doses, see 2.1.5). Therefore no further toxicity studies are required. A toxicological ADI was not derived.

2.1.5. Overview of microbiological properties of residues

Disruption of the colonisation barrier

For the determination of the microbiological effects of bambermycin on the disruption of the colonisation barrier of the intestinal microbiota, three *in vitro* MIC studies were submitted. In the older study (1998, GLP), eight out of the nine bacterial species as required by VICH Guideline 36 (R2) were tested; the missing species was *Fusobacterium*. Moreover, the isolates were not obtained from faecal samples from multiple healthy humans (as required). Therefore, this study could not be used. A newer study (2017) could also not be used because of deviations from GLP and from the VICH Guideline 36 (R2). The most recent study (2018) was adequate and provided MIC50 values between 0.5 and >128 µg/ml for nine isolates from the human intestinal microbiota.

Increase of the population of resistant bacteria

An *in vivo* study was conducted to investigate bambermycin's potential to increase populations of resistant bacteria in the human intestinal microbiota. Groups of 5 male and 5 female rats received bambermycin in the diet at doses of 0, 0.5 or 5 mg/kg bw per day for 20 days. At termination, the intestinal contents were analysed. Viable counts of both Gram positive and negative bacteria in treated animals were not different from controls. The MICs of bambermycin in *Lactobacilli* were increased at the highest dose. The study was non GLP, which is considered a major shortcoming. Nevertheless, the dose levels in this study correspond to approximately 300 and 3000 times the microbiological ADI calculated for the disruption of the colonisation barrier (see 2.1.6). Therefore, it can be concluded that the disruption of the colonisation barrier is the endpoint on which the microbiological ADI should be calculated.

2.1.6. Calculation of microbiological ADI

Studies were provided to address the two endpoints "disruption of the colonisation barrier" and "increase in the population of resistant bacteria" of the human intestinal microbiota. The studies clearly indicated that the disruption of the colonisation barrier is the most sensitive endpoint, therefore these data should be the basis of the microbiological ADI.

The *in vitro* MIC50 data showed that most of the tested strains were insensitive to bambermycin (MIC of 128 µg/ml or higher). In accordance with VICH GL 36 (R2), insensitive strains are to be excluded from the calculation of the NOAEC. Because the MICs of all bacterioides and *Escherichia coli* strains were ≥128 µg/ml, these species were excluded altogether. From the results of the remaining eight bacterial species, the 10% lower confidence limit of the MIC50s was 2.9 µg/ml. This result is used as the NOAEC in the calculation of the microbiological ADI.

The calculation of the microbiological ADI is as follows:

$$ADI = \frac{NOAEC \times colonic\ volume\ (500\ ml)}{Fraction\ of\ an\ oral\ dose\ available\ to\ microorganisms \times 60\ kg\ person}$$

$$ADI = \frac{2.9 \times 500}{1 \times 60} = 24\ (\mu g/kg\ bw)$$

In conclusion, the microbiological ADI is 24 µg/kg bw, equivalent to 1.45 mg for a 60 kg person.

Because the ADI is far below the level at which toxicological effects occur it is considered that this ADI is protective for both microbiological and toxicological effects. Therefore, the microbiological ADI will be used for the risk assessment.

2.1.7. Observations in humans

No data was available. Bambermycin is not authorised as a human medicinal product.

2.1.8. Findings of EU or international scientific bodies

No relevant evaluations by EU or international scientific bodies were identified.

2.1.9. Overall conclusions on the ADI

The pharmacological activity of bambermycin is limited to its antimicrobial action and consequently a pharmacological ADI is not required. A microbiological ADI of 24 µg/kg bw (1.45 mg per person) was calculated.

A toxicological ADI was not derived, due to various shortcomings in the studies provided. However, because the microbiological ADI is far below the level at which toxicological effects occur, it is considered that this ADI is protective for both microbiological and toxicological effects. Therefore, the microbiological ADI is considered the overall ADI that will be used for the risk assessment.

2.2. Residues assessment

2.2.1. Pharmacokinetics in target species

Limited data on the time dependent plasma concentration of bambermycin in rabbit were submitted. During an unlabelled residue study, plasma samples were taken, but determination of bambermycin was not possible due to analytical problems.

During a radiolabelled study in rabbits after a single oral dose of approximately 1 mg/kg bw (following a 10 day feeding period with food including 9 mg of non-labelled bambermycin/kg feed), blood concentrations of total radioactivity were measured. These blood concentrations were very low, a maximum of 0.003 µg bambermycin equivalents/ml (limit of quantification = 0.0027 µg equivalents/ml) was measured at 24 and 48 hours after administration. Too few samples had concentrations above the limit of quantification to allow for the calculation of pharmacokinetic parameters.

In the pivotal radiolabelled study (see Section 2.2.2) on the mass balance and residues of bambermycin in rabbits, plasma radioactivity concentrations were measured at time points 4, 48, 120 and 240 hours after the last dose of a 14 day dosing period, in which the animals were orally dosed at 2 mg/kg bw per day. Mean plasma levels (expressed as µg bambermycin equivalents/l) showed hardly any decline, varying from 150 µg/l at 4 hours to 110 µg/l at 240 hours. A reliable estimate for t1/2 could not be calculated, but clearly exceeds 10 days.

2.2.2. Residue depletion studies

From an unlabelled residue study, no time-dependent course of the residues in tissues could be obtained. In this study four groups of 6 rabbits were used. The animals weighed between 1.9-2.3 kg (at the start of the dosing period), and were housed individually in cages. Each group consisted of 3 male and 3 female animals. One group (group 2) was given bambermycin twice daily at an oral dose of

approximately 4 mg/kg bw for 14 days (28 doses), and two groups (groups 1 and 3) were given bambermycin twice daily at an oral dose of approximately 0.8 mg/kg bw for 14 days (28 doses). The remaining group (group 4) received no bambermycin and was used as a control. The purity of the bambermycin used in this study was reported to be 88.4%.

The animals of groups 1 and 3 were killed 6 hours and 5 days after the last dose, respectively. Group 2 was killed approximately 6 hours after the last dose, while group 4 was killed 2 days after the last dose was given to group 3. Blood samples from groups 1, 2 and 4 were taken at 4, 11, 12 and 13 days after the start of the dosing period. Urine and faeces were collected quantitatively from group 3 from the start of the dosing period, until 5 days after the end of the dosing period (only the faeces samples were analysed). At slaughter, liver, kidney, fat and muscle from groups 1, 3 and 4 were sampled, as well as urine from group 2 *post mor*tem. Bambermycin concentrations were determined in faeces, organs and tissues using fully validated analytical methods (no suitable analytical method could be developed for the determination of bambermycin in plasma). Liquid chromatography mass spectrometry (LC-MS/MS) was used to detect the various components of bambermycin (flavophospholipol A, as well as the minor components A12, C1, C3 and C4). As levels of bambermycin in plasma were not measured, no pharmacokinetic parameters such as Cmax, Tmax or T1/2 could be determined, leaving the fate of the compound after absorption unknown. For the 6 rabbits slaughtered 5 days after administration, in which daily faecal excretion of bambermycin was determined, the recovery of bambermycin varied from 82.5 – 86.6% of the administered dose. The excretion via the urine was not measured. The concentrations of bambermycin in liver, kidney and muscle at 6 hours and 5 days after cessation of treatment were fully comparable, showing a possible long half-life of bambermycin in the rabbit, and a possible prolonged excretion via the urine. The highest concentrations of bambermycin were found in liver and kidney, and were approximately 80 µg/kg in both organs at both time points. The concentrations of bambermycin were much lower in muscle (approximately 4 µg/kg at both time points) and below the limit of quantification in most fat samples.

In the pivotal radiolabelled study on the metabolism and residues of bambermycin, [^{14}C]-bambermycin was orally administered twice per day to 4 groups of rabbits for 14 days. The daily dose was 2 mg/kg bw. Each group consisted of 2 male and 2 female rabbits. At time-points 4 hours (group 1), 48 hour (group 2), 120 hour (group 3) and 240 hour (group 4) after the final dose a group of animals was slaughtered. The radioactivity in excreta and urine was determined daily (for group 4 only) using standard liquid scintillation methods. In all groups the total radioactive residue (TRR) in edible tissues (liver, kidney, muscle and fat) and blood plasma was quantified. Further, using validated LC-MS/MS method, the concentration of flavophospholipol A was determined in the edible tissues (liver, kidney, muscle and fat) of all four groups. Also pooled samples of liver and kidney from all 4 groups were extracted and the extracts were analysed using high performance liquid chromatography with radiochemical detection. The aim was to separate each extract into its components. Muscle samples were not extracted as residues were too low to obtain metabolite profiling.

Residues: Mean radioactivity levels in liver, kidney and fat were comparable for rabbits slaughtered at approximately 4 hours after the last dose. Compared to levels in these tissues, radioactivity levels were lower in plasma and in muscle. For all sample types, there was a very slow decline over time in mean radioactivity. Over a period of 10 days values of radioactivity (expressed as µg bambermycin equivalents/kg) in liver, kidney, muscle, fat and plasma, declined from 260 to 190; from 309 to 220; from 57 to 50; from 290 to 210 and from 150 to 110 µg/kg, respectively. No decline but a relatively constant concentration could be observed for the tissue concentrations of flavophospholipol A. Over a period of 10 days mean concentrations of flavophospholipol A in liver, kidney, muscle and fat varied from 35 to 97; from 35 to 57; from 4 to 5 and from 4 to 8 µg/kg, respectively.

Excretion/balance: Approximately 72% to 84% of the total dose radioactivity was excreted via the faeces, while excretion via the urine was determined to be about 1.4-1.8%. Approximately 20% percent of the dose was not recovered. It could be estimated that 10 days after dosing the radioactivity left in the carcass of an animal accounted for approximately 0.2% of the total dose. Since the missing part of radioactivity could not be found in the carcass, it is most likely that this part is excreted as $^{14}CO_2$ after partial degradation of bambermycin in the digestive tract.

Selection of marker residue and ratio of marker to total residues

A marker residue has not been defined because a 'No MRL required' classification in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 was proposed.

2.2.3. Monitoring or exposure data

No monitoring or exposure data relevant to the use of bambermycin in rabbits were available in addition to the data described elsewhere in the residue section.

2.2.4. Analytical method for monitoring of residues

No analytical method for monitoring is available. This is acceptable because maximum residue limits for bambermycin in rabbits are not necessary for the protection of human health (see section 3.1). The analytical method used in the residue depletion study was sufficiently validated.

2.2.5. Potential effects on the microorganisms used for industrial food processing

The substance is not intended for use in dairy animals and therefore potential effects in dairy products were not investigated.

2.2.6. Findings of EU or international scientific bodies

Evaluations of bambermycin from other EU or international scientific bodies are not available.

3. Risk management recommendations

Rabbits are considered a minor species, and therefore the guideline on safety and residue data requirements for veterinary medicinal products intended for minor uses or minor species (EMEA/CVMP/SWP/66781/2005), and the note for guidance on the risk analysis approach for residues of veterinary medicinal products in food of animal origin (EMEA/CVMP/187/00-FINAL), were taken into account for the evaluation of the reduced data package which complied with the requirements of the guidelines mentioned above.

3.1. Availability of alternative medicines and other legitimate factors

Availability of alternative medicines

Altenative veterinary medicines for the treatment of Enzootic Rabbit Enteropathy (ERE) are available in the EU. For example, the centrally authorised VMP Econor has this indication on the SPC.

Technological aspects of food and feed production (potential effects on the microorganisms used for industrial food processing)

Rabbit meat is not further processed to food or feed products using microorganisms.

Conditions of use

The low amounts absorbed show a very slow depletion from the tissues. Therefore, the CVMP considers it important to restrict the use of bambermycin to oral use only. Other routes of administration may result in a high systemic exposure and the residues are likely to persist for a long period of time. In view of the large margin between the maximum consumer intake and the ADI, there is no need for further restrictions to ensure consumer safety.

Other factors that should, if applicable, be taken into consideration in support of the MRL recommendation

Bambermycin is used worldwide as a growth-promoting antibacterial in animal feeds. From 2006, the use of bambermycin in feedstuffs for rabbits, laying hens, chickens for fattening, turkeys, piglets, pigs, calves and cattle for fattening was banned in the EU (Regulation 1831/2003/EC). The *in vivo* effect of bambermycin is mainly by contributing to the gastro-intestinal microbiota equilibrium by reducing colonization of pathogens, such as *Salmonella enterica*, *Clostridium perfringens* and *Fusobacterium* spp. While availability of an authorised VMP containing bambermycin increases the possibility of its misuse, this is expected to be adequately mitigated by clear product information as well as existing restrictions and controls on the use of antimicrobial veterinary medicinal products in the EU, such as the prescription-only status. In addition, due to its molecular weight (>1500 g/mol), bambermycin is not expected to be absorbed from the gastro-intestinal tract to any significant extent. Therefore, numerical MRLs are not expected to be effective in detecting misuse of bambermycin in food producing species.

3.2. Elaboration of MRLs

Calculation of theoretical daily intake of residues

Because the microbiological ADI for bambermycin was considered the overall ADI to be used for the risk assessment, the total residues (parent and metabolites) with antimicrobial activity is considered to be the relevant part of the total residue for the consumer exposure assessment. However, no information is available on the microbiological activity of the individual metabolites. The CVMP therefore concluded that the total radioactive residues of bambermycin could serve as a worst case estimate of the microbiologically active residue.

The maximum concentrations of total radioactive residues, expressed as mg bambermycin equivalents/kg, taken from the most recent residue depletion study in rabbits, were as follows: 0.057 mg/kg in muscle (at 4 hours), 0.31 mg/kg in fat (at 5 days), 0.26 mg/kg in liver (at 4 hours), and 0.32 mg/kg in kidney (at 4 hours). The theoretical maximum consumer intake of total residues is calculated using the food factors of the standard food basket:

Organ/tissue	**Maximum concentration (mg/kg)**	**Food factor (kg/day)**	**Intake (mg/day)**
Muscle	0.057	0.3	0.0171
Fat	0.31	0.05	0.0155
Liver	0.26	0.1	0.026
Kindney	0.32	0.05	0.016
Total			**0.075**

Only a maximum of 0.075 mg per day (total residues) can theoretically be ingested by consumers. In conclusion, due to the low oral absorption of bambermycin, the total residues in a standard foodbasket made up of rabbit tissues, in a worst-case scenario, equates to approximately 5% of the conservatively established microbiological ADI.

Therefore, no MRLs are necessary for the protection of human (consumer) health.

4. Considerations on possible extrapolation of MRLs

In line with Article 5 of Regulation (EC) No 470/2009, the CVMP considered the possibility of extrapolating the maximum residue limits recommended for rabbit tissues to other food producing species and commodities.

In accordance with Article 6 (e) of Commission Regulation (EU) 2017/880, a 'No MRL required' status can only be extrapolated from a minor species to other species if the metabolism in the extrapolated species and the reference species is similar. In vitro metabolism data indicated that in rat, chicken, pig, cow and the rabbit the metabolic pathways (phase I and phase II) were generally comparable. However, these data were limited and the metabolism could not be confirmed in vivo. Therefore, the CVMP considers that, in line with Commission Regulation 2017/880, extrapolation is not justified.

5. Conclusions and recommendation for the establishment of maximum residue limits

Having considered that:

- the microbiological effects of bambermycin occur at exposure levels far below those producing toxicological effects and consequently the microbiological ADI of 24 µg/kg bw (1.45 mg per person) is established as the overall ADI,
- the theoretical maximum daily intake of residues represents 5% of the ADI,
- the establishment of maximum residue limits for bambermycin in rabbits is therefore not necessary for the protection of human (consumer) health,
- extrapolation to other food producing animal species is not possible because there is no information to confirm a similar metabolism and a similarly low absorption in other species,

the Committee concludes that the establishment of maximum residue limits for bambermycin in rabbits is not necessary for the protection of human (consumer) health and therefore recommends the inclusion of bambermycin in table 1 of the Annex to Commission Regulation (EU) No 37/2010 as follows:

Pharmaco-logically active substance	**Marker residue**	**Animal species**	**MRLs**	**Target tissues**	**Other provisions**	**Therapeutic classification**
Bambermycin	NOT APPLICABLE	Rabbit	NO MRL REQUIRED	NOT APPLICABLE	For oral use only	Anti-infectious agent/ Antibiotics

The theoretical maximum daily intake of residues from rabbit tissues represents approximately 5% of the ADI.

1. Background information on the procedure

Submission of the dossier:	19 April 2017
Steps taken for assessment of the substance	
Application validated:	10 May 2017
Clock started:	11 May 2017
List of questions adopted:	7 September 2017
Consolidated response to list of questions submitted:	20 December 2018
Clock re-started:	21 December 2018
List of outstanding issues adopted:	21 February 2019
Consolidated response to outstanding issues submitted:	18 March 2019
Clock re-started:	19 March 2019
CVMP Opinion adopted:	16 April 2019