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Reflection paper on dose review and adjustment of established veterinary antibiotics in the context of SPC harmonisation

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Executive summary

The Committee for Medicinal Products for Veterinary Use (CVMP) has created a reflection paper on dose review and adjustment of established veterinary antibiotics. Established veterinary antibiotics are not always used at the authorised dose. Doses may need to be reviewed and adjusted in order to maintain effectiveness and to limit the selection of resistant mutant target pathogens. However, a change in dose may have implications for target animal safety (TAS), withdrawal periods (WP), the environmental risk assessment (ERA) and, if applicable, for the user safety assessment (URA). This implies the need for many studies, but Marketing Authorisation Holders may not have the resources to perform them. Thus, requiring such data may lead to decreased product availability, which could have a negative impact also on animal health and may lead to overreliance on other anitbiotics. The present paper aims to reflect on non-experimental approaches for dose review and adjustment, and to evaluate the consequences on TAS, WP, ERA and URA, with the final objective to improve the Summary of Product Characteristics of veterinary antibiotics authorised in the EU.

Dose review and adjustment of products could be helpful in the process of the harmonisation of veterinary medicinal products (VMPs) throughout the EU. While the intention of Article 70 of Regulation 2019/6 is harmonisation at the level of individual reference products, an adjusted dose developed by the described methodology may also be applicable to silimar products (same pharmaceutical form, route of administration and authorised indication) with certain differences in formulation (e.g. concerning their excipients).

Non-experimental approaches are proposed, namely pharmacokinetics/pharmacodynamics (PK/PD) integration for dose review and adjustment, PK modelling for WP adjustment, and scientific review approaches to address the safety of both target animals and the environment. Where needed, CVMP consulted with additional experts from academia, regulators and industry. The approaches were tested in two case studies: (1) the treatment of respiratory infections in pigs by administration of amoxicillin (AMO) in drinking water; (2) the treatment of respiratory infections in (lactating) cattle by injection of oxytetracycline (OTC). The latter case study was expected to be more difficult due to formulation-specific pharmacokinetics and varying WPs for tissues and milk and considering residues at the injection site. Anonymised relevant data for these case studies were kindly provided by AnimalhealthEurope and the European Group for Generic Veterinary Products (EGGVP). The scenarios selected for the two case studies were based on discrepancies in the dose regimens between the SPCs for the relevant products; under real-life conditions, if review suggests an adjustment to a new dose that is above the approved dosing regimens, there should be empirical evidence supporting a potential lack of effectiveness of the previous doses (e.g. lack of efficacy reports, reduction in target pathogen susceptibility).

The case studies were conducted simultaneously with, and helped the development of, the non-experimental approaches. Consultation processes have led to further improvements of the proposed methodologies. The consequence of this is that the case studies may not be completely compatible with the proposed revised methodologies in all aspects. Therefore, the case studies should be seen as an illustration only. Also, the case studies were based on a limited amount of data, gathered from public literature and provided by industry, and consequently sometimes assumptions (e.g. dose-linearity, half-lives, completeness of distribution at MRL level) were accepted that would normally require a more robust scientific foundation. In conclusion, the case studies only illustrate how non-experimental approaches could work and that these may be helpful in addressing the problem statement explained in the first paragraph.

The PK/PD analysis, as performed in the case studies, indicated that the dose for AMO, under the conditions of the exercise, should be 40 mg/kg bw, which is twice the dose for most of the currently authorised products. For OTC, different adjusted doses had to be calculated for the 10% vs 20%

formulations, due to different pharmacokinetics of the formulations considered. For the 10% formulations, the adjusted daily dose was 10 mg/kg bw for 3-5 days, which was equal to the currently authorised doses for most products. For the 20% formulations, the adjusted dose was two doses of 20 mg/kg bw, given 36-48 hours apart. This dose level was the same as for most authorised products; however, the recommendation of a second dose is currently not part of most of the authorisations. The calculation of adjusted WPs considering the calculated adjusted dose regimens was based on tissue residue depletion with overall tissue half-lives of 2 days for AMO and 6 days for OTC. Dose increases did not give rise to any TAS or ERA concerns, except in relation to local reactions for OTC, which would limit the injection site volume.

As noted above, the case studies only considered part of the information that would be considered necessary to initiate a review of diverging approved dosing regimens, which renders the case studies not immediately actionable.

This Reflection Paper should not be read as a guideline. It is intended to provide general principles rather than detailed instructions. It is acknowledged that science will evolve and that further changes to the proposed methodologies may be possible.

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1. Introduction

The Committee for Medicinal Products for Veterinary Use (CVMP) has investigated non-experimental approaches with the purpose to review and adjust doses of established veterinary antibiotics. The considerations and results are published in this reflection paper as a basis for possible future work on the subject.

1.1. Background

Safeguarding the continued availability of established veterinary antibiotics is important for the veterinary sector. The main reason for this is that likely very few new antibacterial active substances will be developed for use in veterinary medicine. In addition, due to concerns about antimicrobial resistance (AMR) in humans and animals, there is a pressure to limit the veterinary use of some antibiotics (e.g. fluoroquinolones, 3rd- and 4th-generation cephalosporins, and colistin). However, the availability of the older veterinary antibiotics is essential to keep a range of safe and effective treatment options for bacterial diseases in animals in the EU. The strategy of the EU regulatory network is to preserve the established antibiotics for veterinary medicine by ensuring that the conditions of use are harmonised and aligned with the principles of responsible use.

It is acknowledged that established veterinary antibiotics are not always used in accordance with the authorised Summary of Product Characteristics (SPC). One of the reasons could be that the SPC recommendations are no longer up to date. In some cases, emerging antimicrobial resistance (AMR) has resulted in changed susceptibility distributions of the pathogens for which these antibacterial products are indicated. As a consequence, the posology described in the authorised product information of these products may require a critical evaluation in order to be updated for the desired level of effectiveness and to limit the selection of resistant pathogenic bacteria, under modern animal production conditions.

Evidence that a review of the posology could be needed may result from use of a product in the field, susceptibility patterns of the target pathogens, and from pharmacokinetic and clinical data. Should there be a need to review and adjust the posology, this should ideally be supported by data on dose finding, dose confirmation, and field efficacy data. A change in the posology of a product, in particular an increase in the dose or in the dosing frequency, can have implications for target animal safety (TAS), and also, in the case of food producing species, for the withdrawal periods (WP), as well as for the environmental risk assessment (ERA) and possibly the user safety assessment (URA). If the review and adjustment of posology is handled via variations using current dossier requirements for new marketing authorisations, then this would require a substantial update to the authorisation dossier. It is considered unlikely that this would be a viable approach: most Marketing Authorisation Holders (MAHs) will not have the resources for this, and consequently this approach may lead to a decreased availability of established veterinary antibiotics, which could have a negative impact on animal health and it may lead to overreliance on other antibiotics.

The CVMP recognised that the current regulatory environment does not stimulate the realisation of the desired dose review and adjustments. CVMP wished therefore to explore if non-experimental approaches to improve the SPCs of established veterinary antibiotics could be identified in lieu of new clinical, safety and residue data. In this context, "non-experimental approaches" refer to approaches other than animal studies. The CVMP recognised that such options might be less optimal (as compared to a new full dossier), but yet may still be helpful in improving the posology in the SPCs, which would in turn facilitate harmonisation of national authorisations of individual products across EU Member States (MSs).

It was recognised that non-experimental approaches may be useful to improve the posology and to address the safety issues that may be associated with a dose increase. However, such approaches might not be possible in all situations or for all veterinary antibiotics, for example, in cases where the available data are inappropriate or insufficient or in cases of non-linear PK. In order to test the non-experimental (e.g. modelling) approaches, it was agreed that the CVMP would initiate a pilot project with data input from industry.

1.2. Scope

This reflection paper comprises the development and testing of non-experimental scientific approaches for dose review and adjustment using PK/PD modelling techniques, and for assessments of safety for consumers, target animals and the environment; these approaches can be used as tools for adjusting and improving the label instructions of established veterinary antibiotics authorised in the EU, for example in the context of SPC harmonisation. Proposals for selection and prioritisation of candidate antibiotics for dose review and adjustment will be made. Whilst recommendations for future implementation of dose review and adjustment can be made, the selection of regulatory procedures for SPC harmonisation and the legal implications are outside the scope.

This Reflection Paper should not be read as a guideline. It is not intended to provide detailed instructions. Likewise, the case studies, including the calculations, should not be regarded as reflecting the only possible or definitive methodology, and neither do these case studies constitute calls to action for either of the substances considered.

1.3. Aim of the reflection paper

The general aim of the reflection paper is to consider the use of modelling or other approaches as a substitute for clinical data, residue depletion data, ERA data, and TAS data, as a tool for the review and adjustment of the posology for established veterinary antibiotics in the context of harmonisation of product literature of individual products.

Specific objectives included:

- to agree on the rationale/objectives for the review and adjustment of the posology for established veterinary antibiotics;
- to establish criteria for selection of products for which doses should be reviewed;
- to obtain a common understanding of the applicability of PK/PD modelling and other sources of information for posology review and adjustment;
- to obtain an agreement on the PK/PD techniques and applicability to be used for dose review and adjustment in the context of harmonisation of established veterinary antibiotics;
- to obtain an agreement on the acceptability of PK techniques for withdrawal period extrapolation in case of dose review and adjustment as a practicable approach in the context of harmonisation of established veterinary antibiotics;
- to obtain an agreement on the approach to be used for the evaluation of the impact of posology review and adjustment on target animal safety in the context of harmonisation of established veterinary antibiotics;
- to obtain an agreement on the approach to be used for the evaluation of the impact of posology review and adjustment on environmental safety in the context of harmonisation of established veterinary antibiotics;

- to discuss the possible approaches for the regulatory processes to effectuate the harmonisation of the product literature and consider the impact and implications on the future product development and improvements.
- to explore possibilities for funding under Horizon 2020 or other funding sources, for studies to fill gaps in data for off-patent veterinary antibiotics related to reviewing and adjusting dosing with respect to minimising risks from AMR where progress is not possible without generation of additional data.

1.4. Development and testing of the approaches

A PK/PD modelling approach for the dose review and adjustment, a PK modelling approach for the adjustment of the withdrawal periods, and data review approaches to address the safety of both the environment and target animals were developed. Where needed, the group consulted additional experts from academia, regulators, and industry. These approaches are described in chapters 3, 4, 5, and 6, respectively.

Whereas an adjustment of the dose can theoretically have an impact on the user safety risk assessment (URA), it was not considered necessary to develop specific methodologies for the URA, because there will be no issue regarding replacing "new studies" by e.g. modelling approaches. Although higher doses or a different strength of the VMP may have an inpact on URA as well, it is expected that the relevant toxicity data will already be available and that any increase in exposure can be compared to established PODs (Points of departure) using the principles of existing guidance . Moreover, the CVMP notes that the formulations and strength of the products will not change and that therefore critical user exposure scenarios (e.g. spilling of droplets on skin) may not change qualitatively and quantitatively. It is also expected that the most important risks for users have already been identified on the label and that the existing label warnings would only need to be adjusted to cover the situation where a higher dose is used. Of course, there is always the possibility to further address the URA where needed, but no specific methodology was developed.

Whilst the approaches need to be scientifically robust, they also should be practically applicable and fit for purpose. Therefore, the approaches were tested in two case studies. The case studies were selected based on the expectation that one would be relatively easy and the other one would be relatively difficult, so they could be used to demonstrate both the capabilities and the limitations of the approaches. The treatment of respiratory infections in pigs by oral administration of amoxicillin in the drinking water was selected as the relatively easy case study. The treatment of respiratory infections in cattle, including lactating cattle, by parenteral administration of oxytetracycline was selected as the relatively difficult case study. The difficulties for the latter case study were expected to be related to formulation-specific pharmacokinetics and to withdrawal periods for meat (including injection sites) and milk. Relevant data for these case studies were kindly provided by AnimalhealthEurope and EGGVP. The case studies for amoxicillin and oxytetracycline are presented in Annex 1 and Annex 2, respectively. It should be noted that the case studies were presented as an illustration of how the methodology could work, and were based on a limited dataset and should in no way be regarded as providing final conclusions for these molecules. In addition, the case studies, including the calculations, should not be regarded as reflecting the only possible or definitive methodology, and should not be read as a guidance document. Within the scope of this reflection paper, discussions and conclusions have been kept at high level.

This Pilot Project was performed to test the feasibility of the various non-experimental methods. It should be noted that the outcome of the dose review was based on a limited amount of data, gathered from public sources or provided by industry. Therefore, the numerical results (e.g. adjusted dose, WT

etc.) are merely indicative, and may not reflect a final outcome (e.g. after a referral in which all related VMP authorised in the EU are included).

The case studies were conducted simultaneously with and helped the development of the non-experimental approaches. Consultation processes have led to further improvements of the proposed methodologies. The consequence of this is that the case studies may not be completely compatible with the proposed revised methodologies in all aspects. Therefore, the case studies should be seen as an illustration only. Also, the case studies were based on a limited amount of data, gathered from public literature and provided by industry, and consequently sometimes assumptions (e.g. dose-linearity half-lives, completeness of distribution at MRL level) were accepted that would normally require a more robust scientific foundation. In conclusion, the case studies only illustrate how non-experimental approaches could work and that these may be helpful in addressing the problem statement explained in Chapter 1.1.

1.5. Acknowledgements

Ludovic Pelligand and Alain Bousquet-Melou are gratefully acknowledged for providing their expertise.

2. General considerations

2.1. Criteria for selection of products for which doses should be revised and adjusted

It is acknowledged that the established veterinary antibiotics authorised in the EU might not always have the optimal dose on the label today. However, this may not be the case for all products. Therefore, not all veterinary antibiotics need to be reviewed. To select the candidates for which a dose review and adjustment may be needed, the following criteria are proposed:

- the existence of different dosage recommendations for the products in the SPCs,
    - within a product from the same MAH between MSs.
    - or between similar products without obvious reasons (such as differences in formulation)
- evidence of lack of efficacy from pharmacovigilance data, national treatment guidelines, literature
- evidence of decreased susceptibility or increased resistance of target pathogens.

A further prioritisation of the selected candidates is proposed, by scoring on Antimicrobial Advice Ad Hoc Expert Group (AMEG) categorisation, administration route, use, and specific evidence of AMR risks, in accordance with the table below.

Table 1. Scoring table for prioritisation of selected candidates for dose review and adjustment

Priority	AMEG categorisation	OIE categorisation	Administration route	Antibiotic consumption (in accordance with ESVAC data)*	Specific evidence of AMR risk
1	Category B ++	VCIA ++	Group oral ++		Expert judgement
2	Category C +	VHIA +	Parenteral or individual oral +		
3	Category D /	VIA /	Topical/local** /		

* Stratification to be further developed

**The PK/PD approach has not been considered for topical/locally applied products within this reflection paper

The scores are graded as "/" (nil), "+"and "++".

2.2. Collection, integration, and application of data: the hour glass approach

In this reflection paper, the dose review, adjustment and harmonisation are considered at the level of the veterinary medicinal product, not at the level of the pharmacologically active substance. The decision was based on the following scientific and practical considerations.

1. Although products with the same active ingredient may be indicated for the same condition in the same target animal, the difference in formulation and route or method of administration may result in different absorption characteristics and therefore a different pharmacokinetic profile. Consequently, in some cases a different posology may be needed to attain a similar plasma concentration of the active ingredient.
2. A product-by-product approach will result in safe and effective posologies, with a minimal market disturbance.

Whereas a product-by-product approach is used, the modelling and review approaches will benefit from the input of all relevant information across products, and in addition the information from other sources such as published papers. Therefore, the data will be collected at the level of an *animal species-disease indication-route of administration-pharmaceutical form* level (as in the case studies, see 1.4.). The information will be integrated in the review approaches (ERA and TAS) and in the selection of model parameters (dose and WP). It should be noted that the integration of data from different dossiers would not be legally possible in the context of procedures for a single veterinary medicinal product. However, in procedures where more products are included, such as an article 35 referral procedure, this would be possible. Information integration will facilitate the optimal estimation for the relevant parameters. Following the integration of the information, the outcome of the (modelling) approaches will be applied to the individual products. For example, in case of dose-linearity, a 2-fold increase in dose that requires an extra 3 days withdrawal period, would result in the addition of 3 days to the authorised withdrawal periods, which can be different for the different products. In this way, the current difference in authorised withdrawal periods will not be disturbed. This approach was designated as the *hour glass approach* which is depicted in Figure 1.

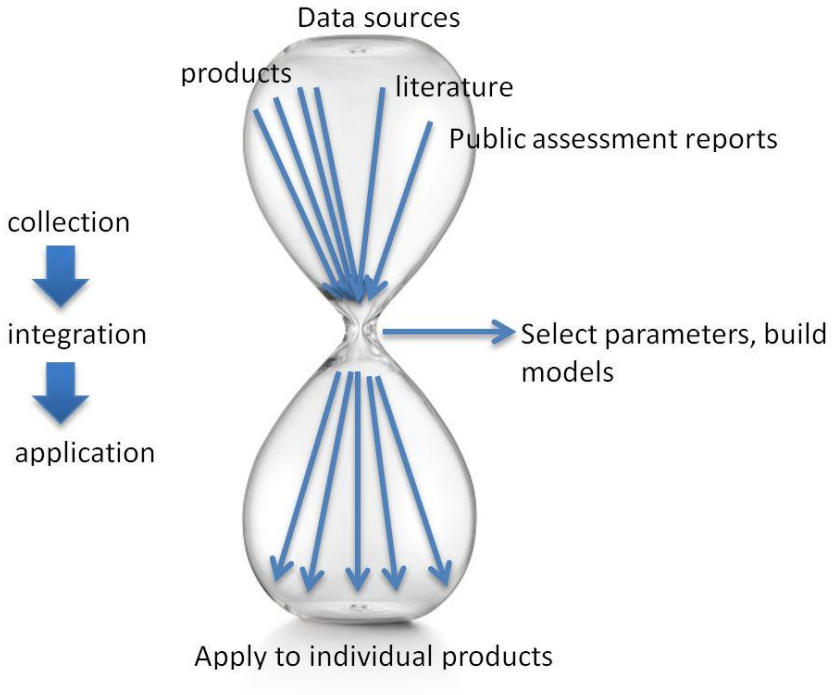


Figure 1. The hour glass approach

3. PK/PD approach for dose review and adjustment

3.1. Background to the evaluation of the applicability of PK/PD modelling approaches to address doses

In the EU, the evaluation of doses for new veterinary medicinal products is in accordance with the requirements of Directive 2001/82/EC. The revised guideline for the demonstration of efficacy for veterinary medicinal products containing antimicrobial substances (EMA/CVMP, 2016) specifies the data required to demonstrate the therapeutic efficacy of a veterinary medicinal product (VMP) containing an antibacterial agent for (a) given indication(s) using an appropriate therapeutic regimen.

To be effective, the dose of an antibacterial agent must be selected considering the pharmacodynamic (PD) effects of the active substance on the target bacteria as well as its pharmacokinetic (PK) particulars exerted in the target animal. For all substances with systemic activity, next to other pharmacodynamics parameters, the *in vitro* susceptibility data (Minimal Inhibitory Concentration, MIC) should be compared with the concentration of the substance in the relevant biophase (target tissue), if available from pharmacokinetic studies. Based on MIC data, and target animal PK data, an analysis for the PK/PD relationship may be used to support the selection of a dose regimen as well as to interprete criteria relevant for resistance development. The overall assessment of the PK/PD relationship should be sufficiently comprehensive to assess with reasonable confidence whether or not the investigational antibacterial substance, when used at the selected dose regimen, would show clinical efficacy against claimed target pathogens that appear to be susceptible *in vitro*. It is acknowledged that the PK/PD analyses will be based on PK data obtained from healthy animals or from diseased animals that have been experimentally infected with the target pathogens. The purpose of the PK/PD approach is to consider possible non-experimental models that could be useful for dose review and adjustment in order to achieve efficacious antibiotic treatment of animals infected with the target pathogens.

3.2. Scientific appropriateness and the applicability of (modelling) approaches to address doses

In the last 20 years during product development of new antibiotics, the prospective PK/PD approach has been recognised as an important tool for the establishment of dose regimens based on preclinical models coupled with pharmacokinetic/pharmacodynamic analyses (Drusano, 2016). According to guideline EMA/CVMP/627/2001-Rev.1 (EMA/CVMP, 2016), use of the PK/PD relationship can be made to justify the dosages to be used in dose-determination studies or in some cases where the PK/PD relationship is well established using validated approaches, it may be possible to omit dose-determination studies and to confirm the efficacy of one or few dose regimens in clinical trials (dose confirmation and clinical field studies). The PK/PD approach is also used retrospectively in the process of establishing clinical breakpoints by EUCAST (Mouton et al., 2012). With the increase of knowledge about the relationship between antibiotic exposure, AMR selection and bacteriological and clinical cure, it is recommended to review available data to investigate the dosage regimen of established veterinary antibiotics and to assess their potency against target pathogens.

Mathematical models have been developed to describe the evolution of concentration-time curves and to assess the effect on bacteria using parameters observed *in vivo* or extrapolated from *in vitro* or *ex vivo* studies. These models are used to analyse data obtained from different experimental studies and to simulate different exposure conditions (Nielsen and Friberg, 2013). Based on the analysis of clinical trials, experimental *in vitro* and *in vivo* studies, and mathematical models, a relationship between clinical and bacteriological targets and PK/PD was established (Ambrose et al., 2007).

The relationship between a pharmacokinetic and a pharmacodynamic parameter to predict clinical efficacy is labelled as a PK/PD index (PDI). Minimal inhibitory concentration (MIC) is the most used pharmacodynamic parameter. The MIC corresponds to the first concentration where no visible growth of bacteria is observed under standardised conditions. Three pharmacokinetic parameters are commonly used in PK/PD integrations (Annex 4):

- the total concentration integrated over a given time interval (area under the curve, AUC),
- the highest (peak) concentration (C_{max}) observed,
- the time during which the concentration exceeds a specific threshold (time above MIC, T>MIC).

PK/PD assessments are based upon the MIC of the target pathogen and the free antibiotic concentration available in the host biophase or serum/plasma, because only the free (unbound) fraction has antibacterial activity. An italic *f* (for free) is added when indices are based on unbound product concentration. The notation of the three PK/PD indices have been standardised (Mouton et al., 2005) into *f*AUC/MIC, *f*C_{max}/MIC and *f*T>MIC. If there are no subscripts indicating a time interval, it is assumed that the calculations of AUC and T>MIC were based on a 24-hour interval at pharmacokinetic steady-state conditions.

PK/PD indices can be viewed as predictors of clinical efficacy. Correlation between PK/PD indices and clinical and bacteriological cure were determined from experimental models with laboratory animals. Retrospective and prospective clinical trials in human medicine have studied this correlation for different pathologies and show a good agreement between experimental and clinical observations (Ambrose et al., 2007). Based on the review of this observation for different antibiotic classes, a consensus was reached to propose target values of the PDI (PDT) predicting a high level of cure (>80-90 %), some examples for are given below.

Betalactams (e.g. penicillins, cephalosporins) exhibit time-dependent antimicrobiological effects, meaning that maximizing *f*T>MIC will enhance bacterial killing. In general, betalactams require 40-80% *f*T>MIC of the dosage interval to achieve bactericidal activity depending on the individual subclass

and the target bacterial species (Ambrose et al., 2007). For fluoroquinolones which are concentration-dependent, two different PDI *f*AUC_{24h}/MIC and *f*C_{max}/MIC were described in the literature predicting a high level of cure (>80-90 %). *f*AUC_{24h}/MIC predicts efficacy against gram-negative bacteria if a PDT of 70 to 125 is reached. C_{max}/MIC is also considered as a relevant clinical predictor for fluroquinolones if target value >10 is reached (Ambrose et al., 2007; Schentag, 2000). For aminoglycosides, the *f*C_{max}/MIC is used as best predicator of therapeutic efficacy. It is generally agreed that to obtain a clinical response of >90% in patients and reduce the risk of emergence of resistance, *f*C_{max}/MIC needs to be 8-12 (Craig, 1998; Moore et al., 1984). It should be noted that the *f*AUC/MIC and *f*C_{max}/MIC are correlated. Thus, recent updates to the knowledge of PK/PD relationships have shown that the *f*AUC/MIC could also be a good PK/PD index for aminoglycosides (Nielsen et al., 2011; Toutain et al., 2017).

It is important to note that all three PK/PD indices are correlated in the sense that C_{max}/MIC describes an intensity, T>MIC describes a duration, and AUC/MIC is a combination of intensity/duration. The calculation of the three PK/PD indices is always derived from the same PK data. The PK/PD index should ideally be used in combination with clinical information to determine an improved dose and dosing regimens. It must be considered as a simplification when it is used in isolation. To note that, different dosing regimens could result in the same PK/PD index value. The PDT for a certain antibiotic-bacteria combination is determined by plotting the value of a specific endpoint (typically $\log_{10}$ CFU/ml after 24 hours of treatment) versus plasma/serum exposures using different doses and/or dose intervals (EMA/CHMP, 2016).

It should be noted that recently, some scientific evidence has established that the AUC_{24h}/MIC index could also be used for time-dependent antibiotics, as for example for phenicols (Manning et al., 2011) or beta-lactams (Kristoffersson et al., 2016; Nielsen et al., 2011). These recent updates to the knowledge of PK/PD relationships have shown, using mathematical physiological models, that when the half-life of the antibiotic is long (e.g. 1.5-3.5 hours), the AUC_{24h}/MIC index is at least as effective as the T>MIC index for predicting antibacterial activity. These new insights in PK/PD relationships could be of importance for those veterinary medicines which are long-acting formulations. Thus, the use of AUC/MIC as a universal PK/PD index could facilitate the finding of an improved dosage regimen for certain long-acting formulations (Toutain et al., 2017) because it can be adressed with simpler computational tools than for other index (i.e., C_{max}/MIC, T>MIC).

3.3. Proposed approach to address doses

It is assumed that in regard to dose review and adjustment, products will be harmonised in groups dependent on:

- Active substance
- Target animal species
- Disease
- Route of administration
- Pharmaceutical form

Refer to Annex 3 for an overview of the PK and PD data that could be used for the proposed modelling approach to address doses.

Refer to Annex 4 for an overview of the general definition of PK, PD and PK/PD indices.

3.3.1. Step 1: Determine the PK for the active substance according to the route of administration, the target animal species and indication

Formost pathogens of clinical interest that are located extracellularly the biophase for antibiotics is the extracellular fluid (Greko et al., 2003; Schentag, 1991). Extracellular fluids are difficult to sample but if there is no barrier to impede drug diffusion, the concentration of free antibiotic in plasma approximates its free concentration in the extracellular space (Toutain and Bousquet-Melou, 2002). Irrespectively, using plasma concentrations for PK/PD integration is a simplified approach that may not always be the appropriate surrogate for the target tissue biophase.

The simplest relationship between the dose and the PK parameters is given by the following equation:

Equation 1. $$Dose = \frac{Clearance}{Bioavailability} \times C_{Target}$$

Where "Dose" is the dose of antibiotic by time unit. "Clearance" is the PK parameter describing the volume of blood cleared from the antibiotic by time and "Bioavailability" is the fraction of dose reaching blood. "C_{target}" is the mean plasma concentration required to obtain the effect. This equation can be used for any type of products. In the case of antibiotics, the target concentration must reach the target value of the PK/PD index (PDT) correlated with their effectiveness.

Protein binding of antimicrobials may affect the clinical efficacy of therapy. Only the non protein bound fraction of a drug in plasma can penetrate and equilibrate with the extravascular space. Penetration into the extravascular space is important as the majority of bacterial infections occur in the interstitial fluid of tissues or in other body fluids than blood. Moreover, it was shown that only the non protein bound fraction of an antimicrobial is microbiologically active. Standardized MIC determination were performed with a protein binding close to 0 (Zeitlinger et al., 2011).

The values of the PK parameters (clearance, fraction unbound (*f*), bioavailability), determine the link between plasma exposure and the dose. Concerning the PK component, to address dose using PK/PD integration, a review of all products with the same active substance, the same route of administration, the same type of formulations will have to be done for each target animal species and indication. The following points should be considered to determine if PK data are applicable for PK/PD integrations:

- Is there a dose linearity?

  Dose linearity is required to analyse a range of doses for simulation of AUC.

- Is there a difference in bioavailability between products?

  The bioavailability can vary dependent of the formulation. Differences in bioavailability should be taken into accout by a population pharmacokinetic analysis.

- Is the free plasma concentration representative for the target tissue biophase?

  It is assumed that for most target tissues, the free plasma concentration is representative of the extravascular phase and is in rapid equilibrium. For certain tissues with a strong blood barrier (e.g. brain), free plasma concentration is not applicable and different pharmacokinetic approaches may be necessary to model infections in such tissues.

3.3.2. Step 2: Define the target bacteria and determine the MIC

All PK/PD indices are based on MICs which are a measure of the net effect on growth and antibiotic induced bacterial killing over the incubation period. The MIC is determined at a fixed time and at a fixed concentration using consistent medium and growth conditions. MIC testing has been highly standardized (e.g. CLSI, EUCAST) to avoid potential errors due to different testing methodologies.

Recent MIC testing methods, if well validated and representing/mimicking more closely *in vivo* conditions can be also used, where agreed to be suitable for the gaining MIC data used for PD analysis.

To determine the pharmacodynamic effects of the active substance against the target pathogen bacteria, two types of information are required:

The mode of action (bacteriostatic, bacteriocidal) of the active substance as well as the mechanism of action i.e. the relationship between concentration and bacterial killing rate must be defined. According the pharmacological class of the active substance, the bacterial killing can be time-dependent and/or concentration-dependent.

The MICs used for PK/PD integrations should preferably be based on recent MIC distribution profiles of the target pathogens. To determine the MIC distribution of the wild type (WT) target bacterial population against the active substance, an epidemiological cut-off value (ECOFF) needs to be established. The ECOFF corresponds to an antibiotic MIC distribution that separates the bacterial population into a wild type population where acquired and mutational resistance mechanisms are absent and a non-wild type population for that acquired or mutational resistance to the antimicrobial substance is present. According to EUCAST, the ECOFF is defined as the MIC value identifying the upper limit of the WT population. Both, WT and non-WT micro-organisms may or may not respond clinically to antimicrobial treatment.

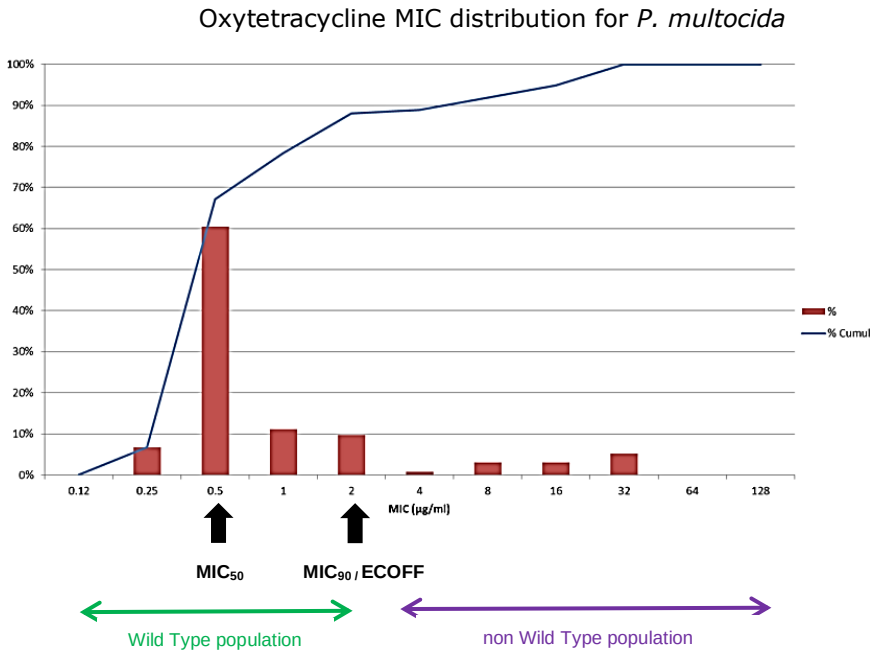


Figure 2. Comparison of MIC_{50}, MIC_{90} and ECOFF values. MIC_{90}: Minimum Inhibitory Concentration required to inhibit the growth of 90% of susceptible the population. MIC_{50}: Minimum Inhibitory Concentration required to inhibit the growth of 50% of the susceptible population.

In regard to the PD component, to address the dose using PK/PD integration, a review of the PD data and scientific papers to support the mode and mechanism of action and to provide the MIC distribution will have to be done. The following points should be considered:

- What kind of information on the pharmacodynamics of the active substance or of other substances of the same pharmacological class is available? What is the mode and mechanism of action against the target bacterial species?
- Are data available to describe the recent MIC distribution of target pathogens?
- Is the MIC determined by standard methods?

- Are time-kill curves available obtained on strains representative of the target bacterial species?
- Which is the least susceptible target pathogen, i.e. the dose-limiting bacterial target species for the indication for which the dose is determined?

3.3.3. Step 3: Define the PK/PD index (PDI)

The PK/PD index is the key parameter in the modelling of dose (Annex 4). Three PDIs are commonly used (Mouton et al., 2012):

- AUC/MIC: the ratio between the total concentration integrated over a given time interval (area under the curve, AUC) and MIC,
- C_{max}/MIC: the ratio between the highest concentration (C_{max}) observed and MIC
- T>MIC: time above MIC, the period of time during the concentration exceeds the MIC.

To support the choice of an appropriate PDI applicable for PK/PD integrations of an antibacterial substance or class, a review of the scientific literature will have to be done by taking also into account the target animal species and relevant target bacterial pathogens. The following points should be considered:

- What is the mechanism of action of the active substances against the target bacteria (time or concentration dependent)?
- What is the pharmacokinetic profile of the active substance?
- What is the amount of protein binding of the active substance?
- Which PK/PD index is considered best predictive to achieve clinical efficacy in treatment of the respective indication in the target animal species?

The PK/PD index correlated best to predict clinical efficacy should be used in the first place, provided PK data are available allowing to establish the ratio of this PDI. For the situation that this approach is not feasible and under the condition that the half-life of the antibiotic is long, the AUC/MIC could be used as a point of departure for the PK/PD analysis to define a daily dose. Results of this analysis could subsequently be refined with the T>MIC or the C_{max}/MIC as a function of the antibiotic class.

The 'mutant selection window' (MSW) is a concept well described in the scientific literature (Zhao and Drlica, 2001) for certain classes of antibiotics (e.g. fluoroquinolones). It postulates that an antibiotic concentration zone exists where resistant mutants are selectively amplified. The lower limit of the MSW is the lowest concentration that inhibits the growth of the susceptible cells and is often approximated by the MIC. The upper limit is the minimum concentration that inhibits growth of the least-susceptible single-step mutant subpopulation, the mutant prevention concentration (MPC).

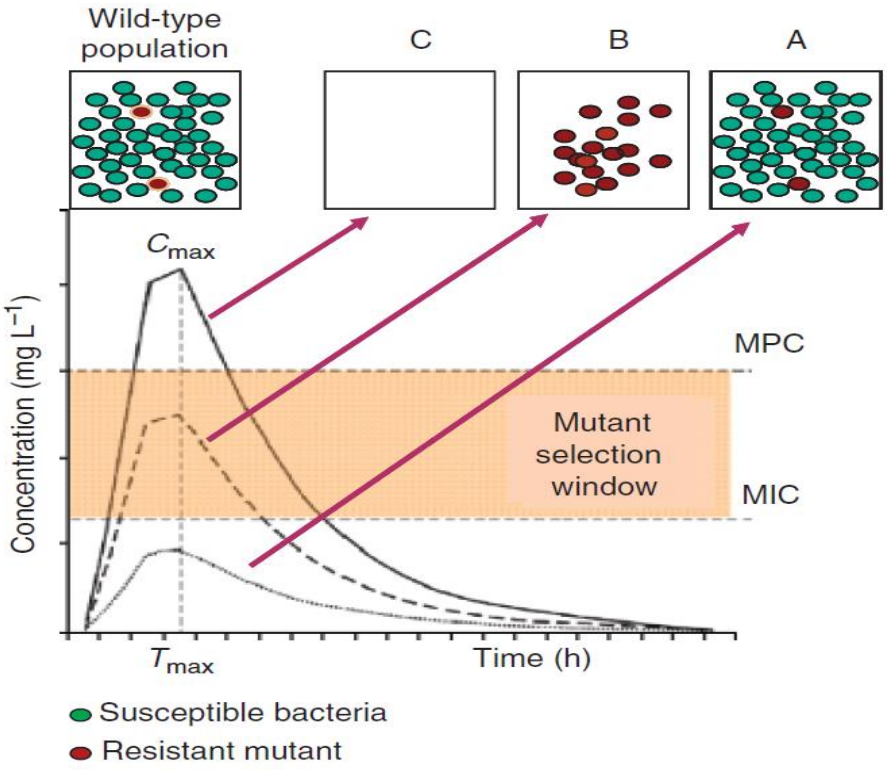


Figure 3. Concept of mutant selection window (based on Cantón and Morosini (2011))

This MSW also updates the classical concept of "sub-inhibitory" concentrations favouring the emergence of resistance, although the threshold to be considered is not the MIC of the majority wild pathogen population but the MIC of the least susceptible pathogenic sub-population, which in fact corresponds to the MPC.

To take into account the notion of concentration preventing mutation in a PK/PD modelling, it is necessary first to define MPC distribution values for each molecule/bacterial species combination. It will allow obtaining three new PK/PD indices by replacing the MIC by the MPC:

- AUC/MIC → AUC/MPC
- T>MIC → T>MPC
- C_{max}/MIC → C_{max}/MPC

Currently, MIC distributions are well standardised notably for surveillance monitoring programs and the information is easily accessible. However, applying MPC principles, when available, may serve to optimise antibiotic therapy and reduce resistance selection.

3.3.4. Step 4: Set a target value for the PDI (PDT)

After selecting the appropriate PDI of the antibiotic class, the numerical target value (PDT) to be achieved under steady-state conditions to predict clinical efficacy must be established. Different target values of the PDI are described (Lees et al., 2015). They vary according to the antibacterial effect (bacteriostatic, bactericidal), the clinical context (clinical burden, immune response), the prevention of mutant selection for the targeted pathogen for certain antibiotic classes (fluoroquinolones, aminoglycosides), and the protection against toxicological outcomes (aminoglycosides).

Studies from peer-reviewed journals may be used to support the choice of target value (PDT) for the selected PDI. The sources and search strategy should be documented. The following points should be considered:

- What is the clinical context of treatment (severe or mild infections)?
- What is the expected clinical outcome (risk of relapse)?
- Is there a risk of mutant selection for the target pathogen?
- What is the therapeutic objective of the treatment (bacteriostatic, bactericidal, magnitude of the bacterial reduction e.g. 2-4log)?

In case no data are available for the target animal species, experimental or pre-clinical trials of non-target animal species or pharmacological and clinical data obtained in human medicine can be used to deduce the PDT. For example, the PDT can be derived from time kill curve studies performed *in vitro* allowing for characterization of the whole concentration-effect relationship between the active substance and target pathogens.

3.3.5. Step 5: Set a probability of target attainment (PTA) for the PDIvalue

The probability of target attainment (PTA), also historically termed Target Attainment Rate (TAR), is defined as, the probability that at least a specific value of a pharmacodynamics index (e.g. 30% *f*T>MIC; *f*AUC/MIC of 100) is achieved at a certain (minimum inhibitory) concentration in Monte Carlo simulations (Mouton et al., 2005). When a PDT has been identified, it is necessary to assess whether this applies across a typical animal species population. Therefore, a statistical approach is taken to simulate individual animal PK profiles for which the inputs include measures of central tendency statistics for PK parameters and their associated variance. Using simulations it is possible to estimate the PTA when MICs of the substance are within a range observed for the bacterial pathogens relevant to the intended clinical uses (EMA/CHMP, 2016).

The acceptable level of PTA is still under debate. Values of 99%, 95% or 90% have been used. Based on expert considerations (Toutain et al., 2017), it was considered that for the purpose of dose review and adjustment of VMPs a PTA of 90% would be acceptable provided the population PK/PD model takes into account simultaneously the population PK and recent MIC distribution profiles representative for the target bacterial population intended to be treated.

3.3.6. Step 6: Model of the relationship between dose, PDI and probability of target attainment (PTA)

Dependant on the PK and PD data available, the relationship between the dose and the PDI can be defined by use of two approaches.

- The first approach is based on a summary of PK parameters (C_{max}, AUC, clearance, fraction unbound, etc.) describing peak concentration (C_{max}) or overall exposure (AUC). If these PK data are available and fulfill quality criteria (peer-review papers, data from dossier), a statistical analysis can be performed to derive an overall mean and standard deviations of each parameter from the pool. The collection of data for a systematic review can be performed taking into account the rules commonly applied for a meta-analysis (transparency, literature search protocol, elibility criteria, etc.). A formula (e.g. equation 1) for the relation between dose and AUC, Cl/F or C_{max} can be used to estimate distribution of the PDI. This allows to calculate the PTA for the selected PDT (C_{target}). This approach can be used to estimate the

range of a daily dose based on the PDIs AUC/MIC and C_{max}/MIC under the assumption of dose linearity. This approach cannot be applied for the T>MIC since for this PDI pharmacokinetic data would be required that describe concentrations along time.

- The second approach takes pharmacokinetic raw data (time, concentration) into account. The collection of data for a systematic review can be performed taking into account the rules commonly applied for a meta-analysis. Pharmacokinetic raw data can be derived from pharmacokinetic studies with different dosage regimens, formulations and individual characteristics (age, weight, sex). Based on these data a population pharmacokinetic analysis using on non-linear mixed effect algorithms can be performed to estimate the distribution of the PK parameters. The influence of different animal characteristics and formulation characteristics can be considered in the analysis, when used as covariates. By use of this model, the statistical distribution of the PDI can be computed and the PTA for a PDT can be calculated. This approach can be applied to analyse the 3 PDIs (AUC/MIC, T>MIC, C_{max}/MIC).

In both cases, a Monte Carlo Simulation (MCS) of at least 5000 cycles (Mouton et al., 2012) should be performed to build distribution with a good convergence to the initial estimates. The range of doses tested should consider good veterinary practices and should be based on pragmatic approaches by taking into account the feasibility of treatments under field conditions. The number of daily doses and interval between doses need to be determined and justified.

3.3.7. Step 7: Set a clinical breakpoint (CBP) based on the dose

Before setting a new CBP three critical MIC values need to be determined:

- Epidemiological cut-off: MIC value of the upper limit of the wild type population for each bacterial target species
- PK/PD cut-off: is the maximal MIC value reaching the PTA of the selected PDI
- Clinical cut-off: MIC value reflecting clinical outcomes to discriminate between clinical failure and success. Individual clinical, bacteriological and pharmacokinetic data are required to discriminate clinical outcomes dependent on the MIC of isolates and the level of exposure.

The CBP reflects the concentration value determined by considering all three critical MIC values. To ensure that a dose leads to an optimal exposure, a CBP should not cut the wild type distribution of the target pathogens. If a dose is defined, a CBP can be set in relation with the PTA for different values of MIC (Mouton et al., 2012). However, in the absence of clinical data reflecting the clinical outcomes, only a PK/PD breakpoint can be established.

3.3.8. Step 8: Define an improved daily dose

After complying with all the previous steps, applying available PK and PD data in the computation, the results of the PK/PD integration approaches should allow to define an adjusted daily dose that aims to reach a PTA of 90 % for the least susceptible target pathogen.

4. PK approach for withdrawal period adjustment

4.1. General considerations on the calculation of withdrawal periods

In general, the methods of calculating withdrawal periods (WPs) could be defined as: a mutually agreed way, to use and treat the experimental data of residue depletion studies in order to calculate a WP. These methods have been harmonised in CVMP guidelines, with the aim to:

- ensure consumer safety;
- guarantee a level playing field for MAHs regarding the estimation of WPs.

It is acknowledged that the data requirements for residue depletion studies are a balance between scientific robustness and feasibility. From a scientific point of view, a large amount of residues data would be needed to cover all aspects and variables involved. Therefore, ideally, multiple residue depletion studies would be needed in order to cover the large variation under field conditions, such as different breeds, different animal life stages with different ages and body weights, different housing and feeding conditions, and different health status. However, in view of the costs involved and the number of experimental animals needed, such data requirements are considered not practicable, and therefore, as a pragmatic approach, only one standardised residue depletion study for each veterinary medicinal product is normally required. Although this approach may have scientific limitations in terms of predictability under various field conditions, it is considered that the resulting WPs are adequately protective for consumers in view of the safety margins that already exist in the consumer safety assessment (ADI/MRLs).

4.2. Current situation regarding withdrawal periods for established antibiotics

With respect to the available residue data used for the adjustment of the WPs for established veterinary antibiotics, the following observations can be made:

- Dossiers of established veterinary antibiotics often contain old residue studies. These studies may be non-GLP, using older analytical techniques and numbers of animals and sampling times may be lower than requested according to current guidance.
- Older residue depletion studies often failed to meet the statistical demands of the required first order kinetical decay (e.g. due to low numbers of time points in the elimination phase), which led to the use of the so-called alternative method, applying chosen safety factors.
- Even when the same residue depletion data were available, the same products may have different WPs in the different Member States, e.g. by using different safety factors.
- Full residue studies are often available for reference (originator) products only: For generic products bioequivalence data are sufficient, with the exception of products with the potential to leave local residues for which injection site residue data are required.
- Most of the more recent residue depletion studies do comply with required statistical criteria. They are often designed to minimise inter-animal variance. This may have the consequence, that they are not fully representative of field conditions.

4.3. Proposed algorithm to address the extrapolation of withdrawal periods

The proposed method for the estimation of WPs in this reflection paper is similar to the algorithm used by FARAD (Food Animal Residue Avoidance Databank) since 2002. Both make use of long established and validated pharmacokinetic principles. The Extrapolated Withdrawal-Interval Estimator (EWE) algorithm from FARAD provides a tool for adjusting so called withdrawal intervals (WDI) in case of off-label use of VMPs (Martín-Jiménez et al., 2002). After calculation of the new dose, the terminal tissue half-life is used to calculate the new WP.

Because in this reflection paper, an adjusted dose would be established via the outcome of the PK/PD-modelling, only the extrapolation part of the model is needed, with the inclusion of an F_{rel} factor to account for possible differences in bioavailability between the old and new dose.

The following approximative formula for estimating the WP corresponding to the adjusted dose is proposed:

Equation 2. $WP_{new} = WP_{old} + [\log_2(F_{rel} \times D_{new}/D_{old}) \times T_{1/2}(\text{final phase})]^{\text{rounded up}}$

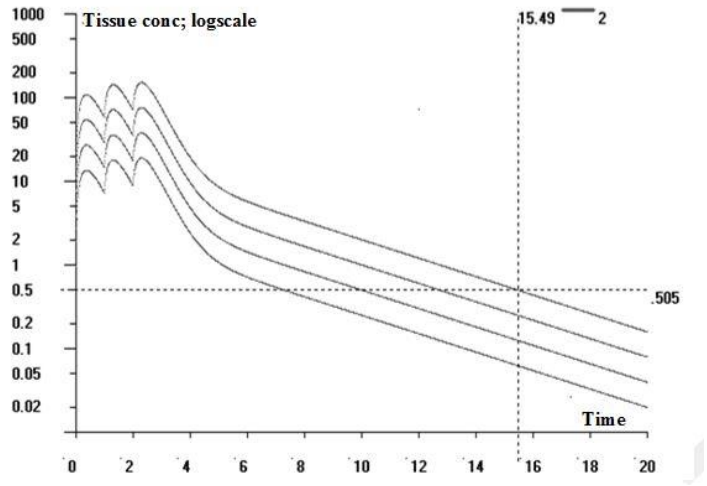
Where:

F_{rel} =	Relative bioavailability new dose/old dose (a default value of 1 is used, but may be adjusted if needed);
$T_{1/2}$(final phase) =	half-life (days, hours) in WP determining tissue(s) after distribution is complete
WP =	Withdrawal period (days, hours)
D =	Dose (mg/kg); it is assumed that the dosing frequency and duration will not change. However, if the dosing interval and/or duration would change, use could be made of FARAD subroutines, to calculate the new dose (D_{new}).

Since the outcome of Equation 2 strongly depends on $T_{1/2}$, it should be chosen with care. Using a mean $T_{1/2}$ from different studies for the particular WP-determining tissue might lead to an underestimation of WP_{new}; a worst-case estimation might be reasonable. If the WP-determining tissue at the adjusted dose level is not known, the largest of all tissue specific half-lives can serve as an estimate.

Within this reflection paper only dose variations are considered and no extra label use (e.g. other routes of administration, other target animal species), therefore the conditions to be fulfilled are:

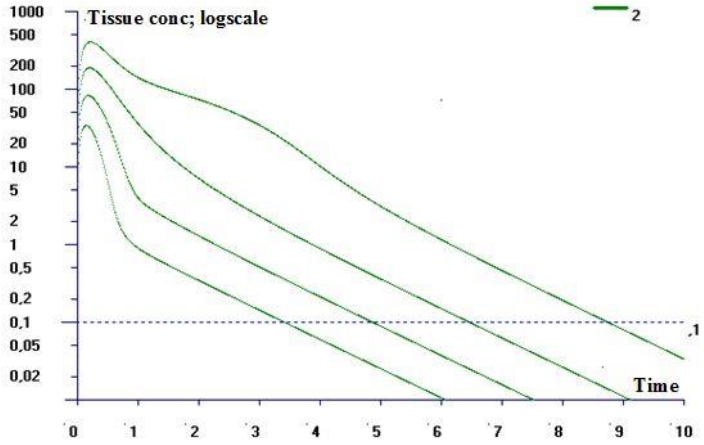
- Linear kinetics/dose proportionality (for all ADME-processes) apply within the dose extrapolation range in each tissue
    - (see Figure 5 for simulations in case of non-linearity)
- At MRL-level, tissue distribution is complete in each tissue, i.e. there is a log-linear decrease of concentrations in terminal elimination phase
    - (see Figure 6 for simulations in case of non-complete distribution)



Dose	WP	Difference in WP
D	7.4	-
2D	10.1	2.7
4D	12.8	2.7
8D	15.5	2.7

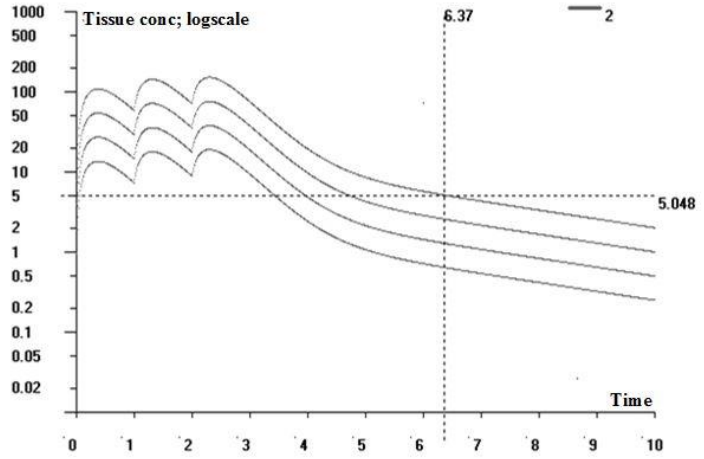
Figure 4. Theoretical simulations, under the conditions: linear kinetics and complete distribution at MRL level. Proportional increase of WP at various doses

Figure 4 shows the linear increase of the WP under the conditions mentioned above. If bioavailability remains unchanged, doubling the dose leads to the addition of one half-life (in this example 2.7 days). Figure 4 and Figure 5 show the effect of violations of the assumption on the change in WPs.



Dose	WP	Difference in WP
D	3.4	-
2D	4.9	1.5
4D	6.5	1.6
8D	8.7	2.2

Figure 5. Theoretical simulations under the conditions: Non-linear kinetics, resulting in a non-linear increase of WP at higher doses.



Dose	WP	Difference In WP
D	3.5	-
2D	4	0.5
4D	4.9	0.9
8D	6.4	1.5

Figure 6. Theoretical simulations under the conditions Linear kinetics, tissue distribution not complete at MRL-level, resulting in non-linear increases of the WP at higher doses.

Differences in tissue specific half-lives might lead to a change in the WP-determing tissue (see Figure 7). This needs to be carefully considered, i.e. WPs corresponding to the adjusted dose need to be calculated for all relevant tissues based on their respective half-lives.

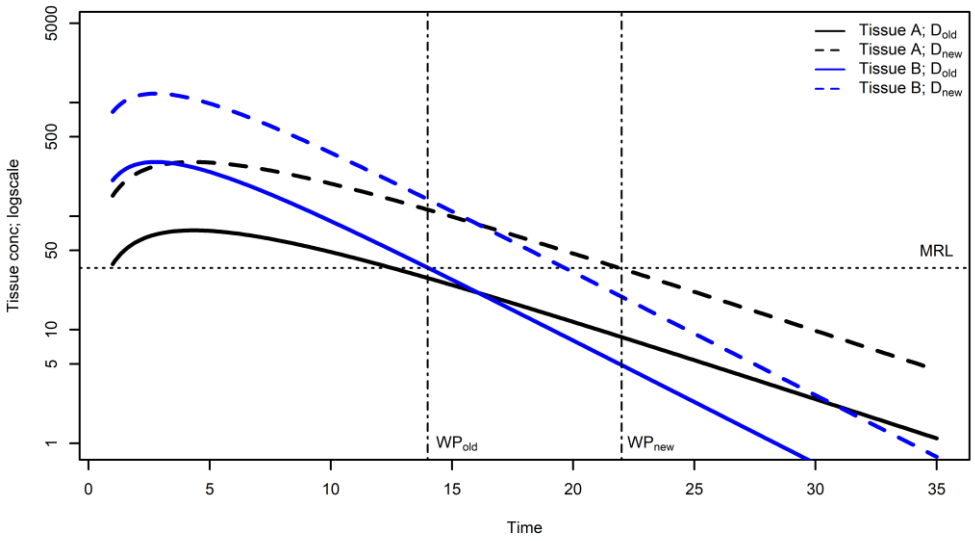


Figure 7. Theoretical simulations under the conditions: linear kinetics, equal bioavailability and tissue distribution complete at MRL-level, but a change in the WP-determining tissue due to differences in tissue specific half-lives, while for the old dose tissue B is WP-determining (its curve crosses the MRL-line later than that for tissue A – for the new dose tissue A becomes WP-determining due to the lower steepness of the curve, i.e. the longer half-life). Equation 2 is applicable, if the half-life of the new WP-determining tissue is taken as input.

It has to be noted that the reliability of the new WP depends on the quality and quantity of the information available for its estimation. The current guideline on the calculation of WPs recommends a statistical approach that is based on the estimation of a 95% upper confidence limit of the 95th sample percentile (95/95 tolerance limit), if the corresponding statistical assumptions are met. However, the necessary data for the statistical approach is usually not available for the adjusted dose. Therefore, the described algorithm (Equation 2) provides a simplified method to obtain an adjusted withdrawal period based on extrapolation of the available data. However, it does not take into account the additional uncertainty due to extrapolation, which is inherently accounted for by the statistical approach due to the convex nature of the tolerance limit curve. However, if the extrapolation is not too far away from the original dose, it can be expected that the described approach performs reasonably well.

As stated above, older residue depletion studies often failed to meet the statistical demands of the required first order kinetical decay (e.g. due to low numbers of time points in the elimination phase), which led to the use of the so-called alternative method, applying chosen safety factors. However, if full residue depletion studies are available, in case of dose proportionality the WP for the *k*-fold dose could also be determined by applying the statistical approach outlined in the guideline (Guideline on determination of withdrawal periods for edible tissues) to the *k*-fold of the individual residue concentrations (if necessary, corrected for the relative bioavailability F_{rel} by multiplying the *k*-fold residue concentrations with F_{rel} – cf. Equation 2.

The addition of an appropriate safety span to the obtained WPs can be considered to account for all uncertainties due to the WP estimation not being based on appropriate residue depletion data.

4.4. Proposed steps to address the extrapolation of withdrawal periods

It is proposed to conduct the extrapolation of WPs in accordance with the following stepwise procedure:

1. Establish the general pharmacokinetic particulars of VMP/active substance/residues involved, such as:
    a. Do linear kinetics (dose proportionality) apply for the intended dose range in each tissue that might be WP determining (yes/no)
    b. Relative bioavailability of the new dose (default $F_{rel}=1$)
    c. General ADME particulars (e.g. active transport)?
    d. Is tissue distribution completed at MRL-level (yes/no), in particular: is the final elimination phase log-linear (yes/no)?
2. Establish terminal half-lives in each tissue, that might be WP-determining; (for milk or eggs please refer to 4.6.)
    a. Data from the following sources can be considered:
        i. Dossier data
        ii. EMA Public Assessment Reports (if available)
        iii. FARAD database
        iv. International Journals (peer reviewed)
        v. Publications by public committees (e.g. JECFA/EFSA)
3. If conditions (linear kinetics, complete distribution and log-linearity of final eliminaton phase) are fulfilled, calculate the WP (extrapolated):
    a. Apply algorithm (Equation 2) to each VMP separately, calculating a new WP. There has to be a check whether other tissues (than the original WP-determining tissue) may become critical for the WP, as a result of possible differences in $T_{1/2}$ between the tissues; if the WP-determining tissue at the adjusted dose level cannot be determined (e.g. due to insufficient data), the longest of all tissue specific half-lives should be used as a worst case. If the relevant tissue for WP_{old} or WP_{new} or both is injection site, applicability of Equation 2 should be checked with particular care (see 4.5).
4. If conditions are not fulfilled or are not known to be fulfilled, one of the following options can be used to allow for setting WPs for the adjusted dose, perform further kinetic modelling.

Please note that the proposed steps are not exhaustive, as this was not the aim of this reflection paper. If e.g residue depletion studies are available, the guideline's approach could be applied to the *k*-fold of the individual residue concentrations, adjusted for relative bioavailability. Depending on the residue kinetic data available and the pharmacokinetic particulars, the use of other models or methods may be required, including the application of safety spans to cover uncertainty.

4.5. Injection sites

In general, the points mentioned in section 4.4. should also be adressed for injectable products, i.e. Equation 2 can be used if there are data available indicating that the conditions mentioned above are fulfilled even when higher volumes per injection site are necessary to achieve the adjusted dose. If those data are not available or if the data show that altering the volume per injection site leads to different residue kinetics, Equation 2 should not be used for injection site data. The following considerations should be taken into account:

If the injection site is the WP determining tissue, for instance doubling the dose by injecting a same amount and volume of the product at another location leads theoretically to the same withdrawal period if the injection site would remain the WP determining tissue (see Figure 8), i.e. if diffusion from injection sites is not impacted by a change in the concentration gradient between injection site and plasma, which means that the diffusion does not significantly decrease if plasma concentrations increase. Hence, in this case, no new withdrawal period has to be determined. However, due to the increase of the dose, residues in all other tissues will increase – thus, it has to be checked whether another tissue could become WP determining. If another tissue than the injection site is WP determining at the old dose, the volume at each additional injection site should be at most the volume that was originally specified per injection site. This ensures that the injection site will not become WP determining. In this case a new withdrawal period can be determined by means of the described algorithm (Equation 2) as outlined in Section 4.4.

Note that adjusting the dose by increasing the number of injection sites without increasing the volume per site restricts the factor by which a dose might be increased because higher numbers of injections may give rise to animal welfare issues.

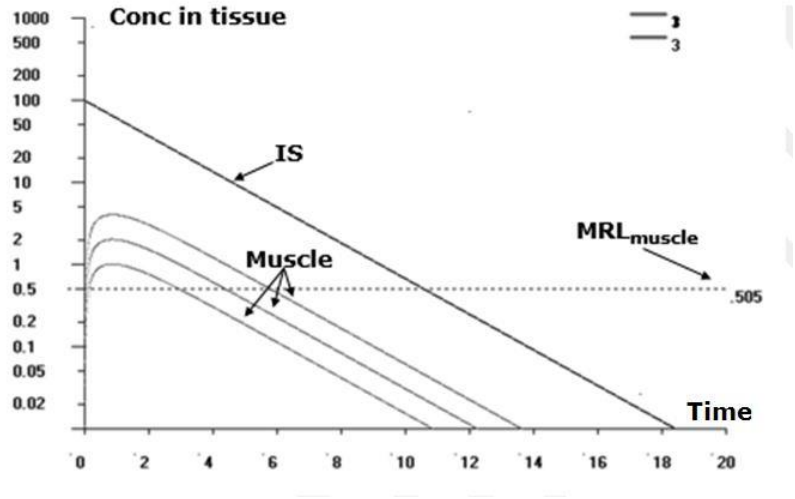


Figure 8. Theoretical simulations where the Injection sites remain WP determining at various doses, resulting in the same WP for all doses.

4.6. Withdrawal period estimation for eggs and milk

With regard to milk and eggs one has to keep in mind, that in contrast to tissue, there are usually several measurements from the same animal over time. This provides additional information for the assessment, but also introduces dependency between observations, that has to be taken into account in the analysis. Therefore, the usual approach used for withdrawal period determination in tissue yields biased results, as it does not take into account this dependency. Instead, it is recommended to use methods that are established for withdrawal period determination for milk, such as the so called time to safe concentration (TTSC) method or linear regression for each individual animal. For WP determination for eggs, so far no guidance exists, but the milk methods mentioned above might also be applicable in case of eggs (if the individual eggs can be assigned to individual hens). Note that the regression method results in one individual half-life per animal, while in the TTSC approach there are generally no half-lives determined.

Depending on the data available, there are different procedures for adjusting the withdrawal period non-experimentally based on the established methods. In the following, four procedures are described, that take into account different levels of information on $Dose_{old}$ (Scenario 1-4).

For all procedures, assume dose proportionality, and assume $Dose_{new} = Dose_{old} \times k$ (i.e. $\log_2(k)$ half-lives are added to WP_{old} if Equation 2 is applied to estimate WP_{new}).

1. One has individual residue concentrations $conc_{old}$ at all time points – then one simply can apply an established method such as TTSC to $conc_{old}$ multiplied by k (note: this is only possible, if $conc_{old} \times k <$ MRL at the last time point).

2. One has individual $TTSC_{old}$ and individual $t_{1/2}$ – then one can apply Equation 2 to each individual animal using their individual $TTSC_{old}$ and individual $t_{1/2}$; i.e. add to each individual $TTSC_{old}$ $\log_2(k)$ times the individual $t_{1/2}$ to obtain individual $TTSC_{new}$ values. From these WP_{new} can be determined analogously to the TTSC method.

3. One has individual $TTSC_{old}$, but just one (ideally worst-case) $t_{1/2}$ is known – then one can proceed as in 2. but replacing the individual half-lives by the single known one.

4. Just WP_{old} and one (ideally worst-case) $t_{1/2}$ are known – then one can apply Equation 2 to WP_{old} and the one $t_{1/2}$ as already described in the text.

In any case, one has to be aware that the obtained WP_{new} might be inaccurate or biased. However, one can expect that the more information is used, the more accurate the results are.

Since this project potentially should cover WPs in milk and eggs as well, the proposed algorithm was also tested on residue depletion data in regarding these food commodities, obtained from literature. The substances used in the examples below have no EU MRLs in eggs or milk, however, they are only used to illustrate the underlying pharmacokinetic principles. The data correspond to Scenario 4 (see above).

Example on residues in eggs

The example for eggs was taken from Liu et al. (2017), in which residues of amoxicillin in eggs were determined following doses of 25 and 50 mg/kg bodyweight. For the purpose of this example, dose proportionality and complete distribution were assumed.

Table 2. Comparison of the predicted WP and the experimentally derived WP using data from Liu et al. (2017)

Dose mg/Kg	WP egg (days)	WP 50 mg/kg calc according to Equation 2 based on 25 mg/kg dose and $T_{1/2}$= 1.5 days
25	6	
50	8	8

The authors used the Japanese MRL of 10 µg/kg in chicken eggs, and calculated the WP usingused the statistical method for tissues (WT1.4) from the CVMP guideline (EMA/CVMP, 1996) for the calculation of the WP on the residue data for the 25 and 50 mg/kg bw dose. However, the experimental design does not justify the use of this method, because the data are not independent. In this case a more appropriate method would have been the Time To Safe Concentration (TTSC) method which was developed for withdrawal periods for milk (EMA/CVMP, 2000b).

For this Reflection Paper, these residue data in eggs were also analysed using a Physiologically Based Pharmacokinetic (PBPK) model for eggs that was recently developed (Hekman and Schefferlie, 2011).

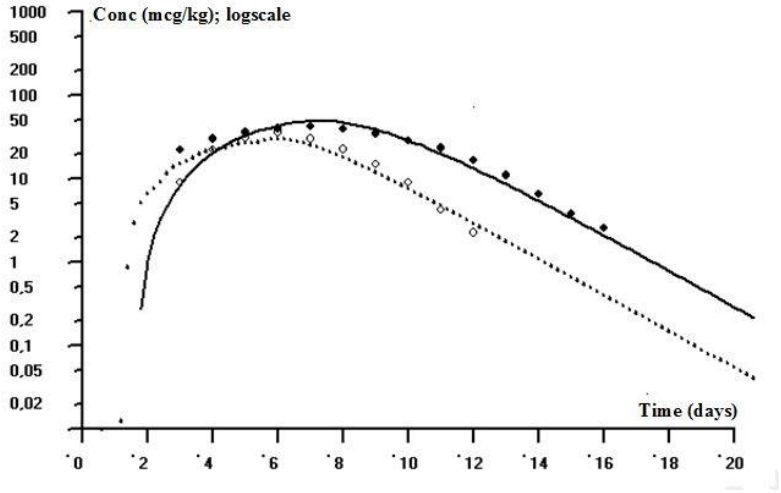


Figure 9. Fits of the time dependent course of amoxicillin residues in albumen (open circles) and yolk (closed circles) after 50 mg/kg bw during the first 5 days via the drinking water. Parameters for egg formation, kinetics (1 compartment) and transport rates of amoxicillin in to albumen (Kw) and yolk (Ky) were kept constant: e.g. $T_{1/2\ elimination}$= 1,6 days; Kw/Ky= 0,54. It is clear that in yolk as well as in albumen the curves eventually run parallel

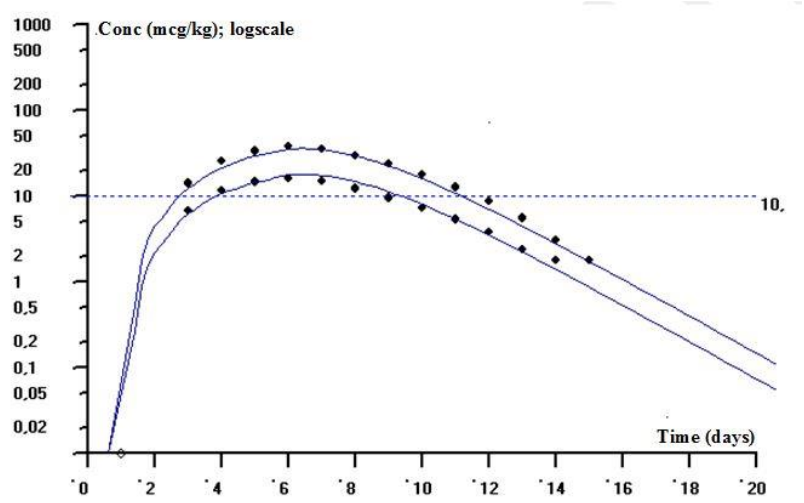


Figure 10. Fits of the time dependent course of amoxicillin residues in whole egg, Dose: 25 and 50 mg/kg bw during the first 5 days via the drinking water. Parameters for egg formation, kinetics (1 compartment) and transport rates of amoxicillin into albumen (Kw) and yolk (Ky) were kept constant: e.g. $T_{1/2\ elimination}$= 1,6 days; Kw/Ky= 0,54

The analysis by Liu et al. (2017) using WT1.4 and the fits according to the PBPK-model (see Figure 9 and Figure 10) indicate that the final phase of the residue depletion curve is log-linear, although this could not be confirmd by statistical tests due to the lack of individual data. As stated above, dose proportionality and complete distribution were assumed, such that the use of Equation 2 for calculating the WP when using the higher dose is justified.

Example on residues in milk:

The example for milk was taken from Malreddy et al. (2013). This example relates to residues of gabapentin in milk following oral administration to lactating cattle at a dose of 10 and 20 mg/kg bodyweight, using an 8 hour milking scheme and a fictive MRL of 0.1 µg/ml.

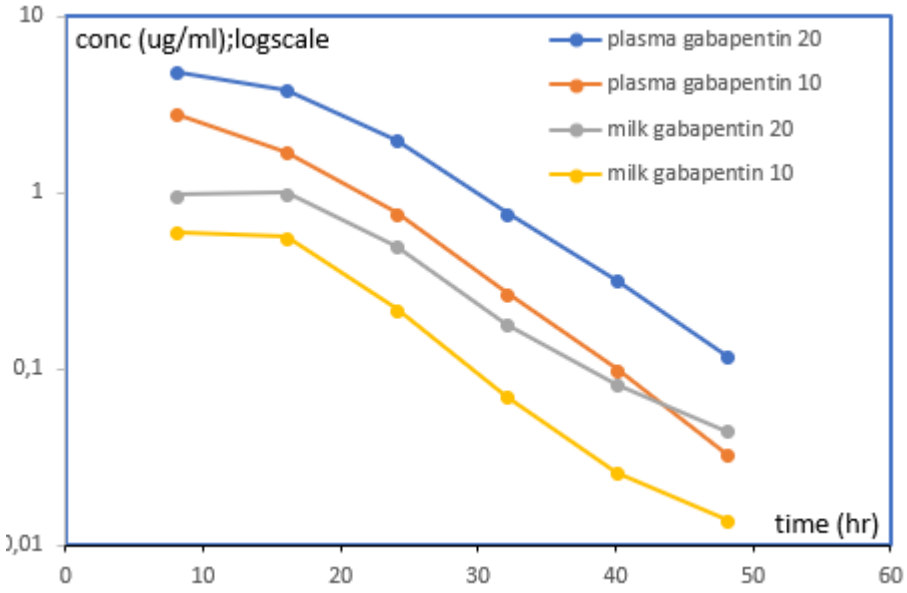


Figure 11. Mean plasma and milk concentrations of gabapentin following 10 and 20 mg/kg bodyweight PO administration; based on Malreddy et al. (2013)

Table 3. Comparison of the predicted WP and the experimentally derived WP using data from Malreddy et al. (2013)

Dose mg/kg	**WP milk (h)**	**calculated WP (h) based on the 10 mg/kg dose and mean $T_{1/2}$= 6.2 h (lin regression)**
10	32	-
20	40	40

From Figure 11 it can be observed that the final phase of the residue depletion curve is log-linear. This example also shows the applicability of the algorithm used in this example, where the new WP for the 20 mg/kg bw dose is calculated using the $T_{1/2}$ of the 10 mg/kg bw dose ($T_{1/2}$: 6.2 hours) resulting in the same withdrawal period as when the WP is calculated based on the actual measured residue concentrations in tissues for the 20 mg/kg bw dose. Note that in this example we pragmatically determined the WP by determining the time point when the mean residues fell below MRL. This is not in line with the guidelines as instead of means tolerance limits have to be considered, but these were not available for this example. However, as the tolerance limit curves are almost parallel to the mean residue concentration curves, the difference between the two tolerance limit curves should be approximately the same as that one between the two mean curves.

These examples show how the proposed algorithm could be applied to adjust withdrawal periods for milk and eggs. They were based on a limited amount of data, gathered from public literature and provided by industry, and consequently sometimes assumptions (e.g. dose-linearity, half-life determination, complete distribution at MRL) and pragmatic calculations were accepted that would normally require a more robust scientific foundation.

5. Approach for addressing risks for the environment

5.1. Introduction

In the EU, the Environmental Risk Assessment (ERA) is conducted for all veterinary medicinal products in accordance with VICH and CVMP Guidelines. Typically, the ERA is conducted in two phases. In Phase I, products with a low environmental exposure are filtered out; these products do not need further assessment and substance related environmental fate and effect data are not strictly required. Examples of products with a low environmental exposure are products for companion animals only and products that result in a Predicted Environmental Concentration in soil (PEC_{soil}) of less than 100 µg/kg, based on a worst-case estimation. In Phase I can also terminate compounds with PEC_{soil} above 100 µg/kg if data show extensive metabolism in the target animal (ADME study) or complete degradation in manure (EMA/CVMP/ERA, 2011). In Phase II, starting with Tier A, a basic set of environmental effect data in representative species is produced, to estimate Predicted No Effect Concentrations (PNECs) for up to three environmental compartments: soil, surface water and, if needed, groundwater.

PECs for these compartments are also calculated. When the PECs are for all environmental compartments below the relevant PNECs, no further assessment is needed. If any of these PECs is above the PNEC for that compartment and therefore possible risk is identified as RQ is above 1, then PEC refinement based on metabolism, excretion and the environmental fate of the substance can be taken into account. When the PECs after refinement are below the relevant PNECs, no further assessment is needed. If no data for refinement are available or if PECs after refinement is still above the PNEC for that compartment, then further data on fate and effects are required for the relevant environmental compartment(s) in Tier B. It should be noted that a PEC in groundwater (PEC_{gw}) ≥ 0.1 µg/l triggers further risk assessment also taking into account guideline on assessing the environmental and human health risks of veterinary medicinal products in groundwater (EMA/CVMP/ERA, 2018). As a general rule, when the PECs for all environmental compartments are below the relevant PNECs, no further assessment is needed. However, if any of these PECs is above the PNEC for that compartment, then further data on fate and effects are required for the relevant environmental compartment(s) in Tier B. In Tier B, also the risk for sediment-dwelling organisms will be calculated if needed. This tiered approach progresses from a crude worst-case risk estimation to a refined, more realistic risk estimation. In the situation where following a full ERA a risk for the environment cannot be ruled out, i.e. the PEC is higher than the PNEC, this should be considered in the overall benefit/risk balance for the product, and risk mitigation measures (RMMs) may need to be recommended in the product literature.

The presence of antibiotics in the environment may influence the distribution and perseverence of AMR in the environment. Thus, dose review and adjustment may increase the risks due to AMR in the environment. However, currently there is no assessment procedure for AMR in the environment and the relative risks of this route for humans, compared to other routes, are still mainly unknown. Thus, the assessment of increased AMR risk via the environment is currently not further taken into account.

5.2. The impact of dose review and adjustment on the ERA

5.2.1. The relation between the dose and the PEC

The total dose (in mg/animal for the entire treatment) is one of the inputs into the models used to calculate the PECsoil. The PECs for the other environmental compartments are linked to the PECsoil. The relation between the dose and the calculated PECsoil is linear, meaning that a certain increase in the total dose will result in the same relative increase of the PECsoil. This will be the case for the initial

PECsoil (as calculated in Phase I) as well as for the refined PECsoil (as calculated in Phase II). Likewise, most PECs for the other environmental compartments that are calculated in Phase II Tier A have a linear relationship with the dose. Irrespective of wether the relationship is linear or not, an increased dose leads to an increased exposure of the environment. However, using the standard spreadsheets, recalculating the PECs is easy and fast. Once more sophisticated models (FOCUS models) are used in Phase II at the end of Tier A, the relation between the dose and the PECs for groundwater, surface water and sediment is non-linear due to the use of parameters as the KOC or the DT50 in the Tier B models. Therefore, in case the initial assessment in Tier A indicates a risk, these PECs will need to be recalculated.

5.2.2. The importance of triggers

As explained above, the ERA follows a tiered approach using triggers; when one of the triggers is exceeded, a further targeted assessment in the next Tier is required. The main trigger in phase I is based on environmental exposure (the PEC_{soil}) and the main trigger in Phase II Tier A is based on environmental risk (the Risk Quotient (RQ), i.e. the PEC/PNEC; when the RQ ≥ 1, further assessment is required in Tier B). Another trigger in Tier A is exposure of groundwater at concentrations of ≥ 0.1 µg/L. When this trigger is exceeded, an RQ for groundwater will be calculated using the available Tier A data for aquatic species, and the risk for humans via consumption of drinking water will be assessed. It should be noted that the CVMP guideline on groundwaterspecifies that the risk for groundwater communities should also be assessed if the PECgw is under the trigger value but the PNEC for surfacewater organisms is ≥ 1 µg/L). When the RQ for groundwater is ≥ 1, even after refinement of the PEC_{gw}, the applicant should propose adequate risk mitigation measures and if no suitable risk mitigation measures can be applied, the risk for groundwater has to be addressed in the benefit/risk evaluation.

The tiered approach implies that the final conclusion on the risk for the environment for a product with an optimised (higher) dose will remain unchanged when no triggers are exceeded that were not exceeded for the previous (authorised) dose.

5.2.3. Possible data gaps as a result of trigger crossing

In general, there can be three situations where an optimised (higher) dose will result in the need for additional ERA data: (1) when the PEC_{soil} exceeds the Phase I trigger for the new dose but not for the old dose; (2) when the RQ in Phase II Tier A is equal or higher than 1 for the new dose but not for the old dose; and (3) when the concentration in groundwater is equal or higher than 0.1 µg/L for the new dose but not for the old dose. In situation (1), according to the guidelines, a basic set of (Tier A) fate and effect data for the active ingredient(s) is required, whereas in situations (2) and possibly (3) the guideline may require further Tier B studies (e.g. long-term studies), further PEC-refinement and/or risk mitigation. A pragmatic strategy for dealing with ERA-related data gaps in the context of dose review and adjustment will be necessary.

5.3. Proposed approach to address the ERA

It is anticipated that the worst case PEC_{soil} calculated in Phase I exceeds the trigger value for the majority of the established veterinary antibiotics at the currently authorised doses, in particular for the oral products. Whereas the Phase I guidance allows for the provision of data (not obligatory) to show extensive metabolism of the substance in animals or extensive degradation in their excreta, experience has shown that such a complete metabolism or mineralisation does generally not take place for the established antibiotics. Therefore, in most cases, the starting position will be that Phase II data are available.

It is also envisaged that the established veterinary antibiotics are not likely to fulfil PBT or vPvB criteria. Therefore, the PBT assessment shall be outside the scope of the ERA in the context of dose review and adjustment.

The environmental risks for products with an optimised dose can be addressed in a stepwise approach. As explained above, the need for additional assessment of environmental risk(s) depends on the individual situation, for example on whether or not triggers are exceeded. The stepwise approach is explained below and is schematically illustrated in the decision tree (Figure 12).

5.3.1. Step 1: Determine the assessment situation

The first step of the revised dose assessment includes a comparison between the ERA situation for the authorised dose and for the optimised dose. There may be different authorised doses for the same or similar products, and as a general rule, the available ERA(s) covering the highest (total) dose for the relevant target species will be used for the comparison.

If the product with the optimised dose still has a lower dose than the product with the highest authorised dose, no further ERA action is required. If the optimised dose is higher, but the outcome of the initial assessment with the optimised dose is that the ERA can stop in Phase I (e.g. PEC_{soil} <100 µg/kg, or complete mineralisation of the active ingredient(s) in either the animals or in their excreta occurs), then it can be concluded that no further assessment is necessary. The risks for the environment have been sufficiently addressed for the optimised dose, and no further action is required. If this is not the case, then proceed to step 2 (see the decision tree below).

5.3.2. Step 2: Retrieve Tier A ERA data and identify data gaps

All substance related Tier A data will be collected from the dossiers of the relevant authorised products. If sufficient Tier A data are available, then proceed to step 4, otherwise proceed to step 3 before continuing to step 4.

5.3.3. Step 3: Fill data gaps

A. Substance specific Tier A data that are not available from the marketing authorisation (MA) dossiers may be retrieved from the published literature, from public assessment reports for VMPs authorised in the EU or elsewhere, or from any other published assessments by any regulatory body. In the context of the dose optimisation for established veterinary antibiotics, published endpoints may be sufficient In addition, the concerned Marketing Authorisation Holders (MAHs) may be asked if they have any additional studies that have not been submitted previously. The suitability of the additional information may be judged on a case-by-case basis; also, information other than GLP/OECD studies can be considered according to VICH GL 38. See chapter 2.2. for an explanation on the use of data integration from different veterinary medicinal products.

If the data retrieved under A are still insufficient to conduct the Tier A risk assessment, then the required information may in the future be estimated, for example by the use of validated (Quantitative) Structural Activity Relationships ((Q)SARs) or by using a "read across" procedure, i.e. taking on board relevant information from similar substances. A scientific justification in terms of reliability and relevance must be given for any tools used for the estimation. It is noted that such approaches are not covered in existing guidelines and therefore not allowed for the regular ERA. However, with proper justification, these apporaches may be accepted for this specific purpose.

B. If certain data are still insufficient (e. g. phys-chem data), then the data gap may be taken into account in the overall B/R assessment and in the consideration of RMMs (step 8).

5.3.4. Step 4: Calculate the Tier A Risk Quotients

On the basis of the Tier A data, the RQs for the different environmental compartments are calculated. For groundwater, the RQ is only calculated in cases where the PEC_{gw} is at or above 0.1 µg/L (it should be noted that the CVMP guideline on groundwater specifies additional situations for which a risk assessment for groundwater is required). When necessary, further PEC refinements are carried out in accordance with the guidelines.

If the outcome of step 4 is that the Tier A RQs are lower than 1 for all environmental compartments, then it can be concluded that no further assessment is necessary. The risks for the environment have been sufficiently addressed for the optimised dose, and no further action is required. The assessment stops at this point. If this is not the case, then proceed to step 5.

5.3.5. Step 5: Retrieve Tier B ERA data and identify data gaps

All substance related Tier B data will be collected from the dossiers of the relevant authorised products. This information should be limited to the relevant data for the compartment(s) for which the RQ was 1 or higher in Tier A. If sufficient Tier B data are available, then proceed directly to step 7, otherwise proceed to step 6 before continuing to step 7.

5.3.6. Step 6: Fill data gaps

The same procedure as indicated under step 3 should be followed for the relevant Tier B data.

5.3.7. Step 7: Calculate the Tier B RQ

On the basis of the Tier B data, the RQs for the relevant environmental compartment(s) including sediment and, if needed, groundwater are calculated. It should be noted that the PECs for groundwater, surfacewater, and sediment will need to be recalculated in Tier B because the models used in Tier B can result in PECs that are not lineary related to the dose. Again, it is recommended to perform any possible refinements, where needed.

If the outcome is that the Tier B RQ is lower than 1 for the relevant compartment(s), then it can be concluded that no further assessment is necessary. The risks for the environment have been sufficiently addressed for the optimised dose, and no further action is required. The assessment stops. If this is not the case, then proceed to step 8.

5.3.8. Step 8: Benefit/Risk and Risk Mitigation Measures

Because the RQ is 1 or higher for one or more environmental compartments following a Phase II Tier B assessment, or the PECgw exceeds 0.1 µg/L for substances that are within the scope of points 1 to 6 of Annex VIII to the WFD, and no further refinements of the risk assessment are possible, a risk for the environment cannot be excluded. If no feasible RMM is found to lower this risk to an acceptable level the presence of a risk for the environment has to be taken into account in an overall B/R assessment for the product and the RMMs should be considered.

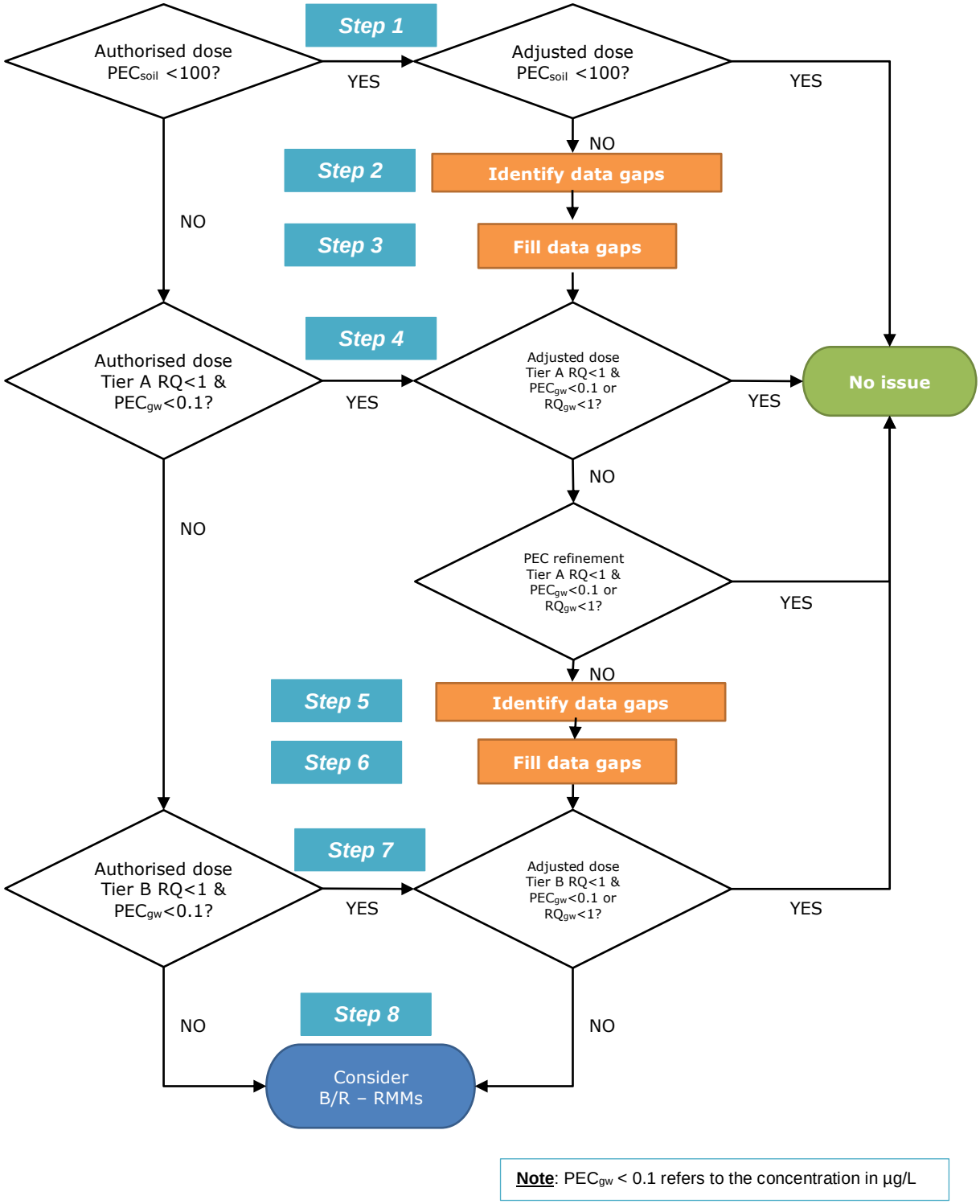


Figure 12. Decision tree for addressing the environmental risk assessment for increased doses

6. Approach for addressing risks for the target animal

6.1. Background to the evaluation of target animal safety

In the EU, the evaluation of target animal safety for new veterinary medicinal products is in accordance with the requirements of Directive 2001/82/EC, as amended.

The general principles for the conduct of Target Animal Safety (TAS) studies for regulatory submissions are laid out in VICH GL 43. TAS studies have the objective to investigate the safety of an investigatory product in the target species, to identify the target organs for toxicity and to establish a margin of safety (MOS) for the proposed dose regimen. These studies are conducted in healthy experimental animals representative of the species/category (e.g. piglets, sows) in which the product will be used, administered the final formulation of the VMP by the proposed administration route and at the recommended dose and suitable multiples thereof for an extended duration of time. For products that are intended to be used in animals for breeding, then effects on reproduction and viability of the off-spring are also investigated. It is noted that VICH-compliant studies are unlikely to be available for products authorised before 2009.

As the safety of a product may also be dependent on the characteristics of the animal that is treated, such as age, breed and the presence of underlying diseases, then observations on harms under conditions of clinical field use are also required as evidence for safety in sensitive sub-populations of the target population.

In addition to the TAS data provided to support new MA applications, once a product is authorised, data on adverse events (AE) are regularly collected through the pharmacovigilance reporting system. These AE data are provided in periodic safety update reports (PSURs) and are also monitored through signal detection. PSURs include data on AEs following off-label use, including use at doses above the approved dose.

6.2. The impact of dose improvement on the evaluation of target animal safety

On the basis that, in the context of this reflection paper, any change to the dose of an antibiotic will be based on PK/PD modelling, then it is assumed that any adverse impact on safety will be in most cases as a consequence of an increase in the dose (mg/kg) administered and/or an increase in the frequency of dosing. An increase in total dose over a given period of time will result in a reduction in the MOS for a product. In some cases the frequency of administration may impact safety, for example a high dose of gentamicin administered once daily has been recommended compared to more frequent dosing in order to limit nephrotoxicity (EMA/CVMP, 2015). PK/PD modelling cannot be used to determine the duration of treatment to achieve a clinical cure; hence treatment duration generally will not be changed unless the PTA is reached for only a very short time (see oxytetracycline case example). In each case it would be necessary to assess if an acceptable MOS for each product can be retained with the new dose/regimen. What is an 'acceptable' MOS is determined by the benefit-risk for the product, taking into account any additional risk management measures that could be applied.

It has been suggested that in order to improve the evidence base for decision-making concerning target animal safety, the outcomes of studies from similar products may be pooled (see chapter 2.). In this respect, pooled studies will be useful for establishing the toxicity and MOS. When pooling outcomes from different products, consideration should be given to the fact that the formulation, pharmaceutical form and route of administration may all affect the bioavailability and pharmacokinetics of the active substance.

In addition to the impact of dose change on safety of the active substance, consideration also needs to be given to the safety of a concurrent increase in exposure to the specific excipients included in the formulation of each product. It is anticipated that problems with toxicity of excipients would be less likely as most commonly used excipients have a wide margin of safety; nevertheless, this should still be considered.

For intra-muscular and sub-cutaneous injections, an increase in dose volume could furthermore affect local tolerance. For orally administered products, then palatability of feed/water could be affected.

6.3. Proposed approach to address target animal safety

It is assumed that in regards to the approach and correction factors required for dose review and adjustment, groups of products will be reviewed dependent on:

- Active substance
- Target animal species/category
- Route of administration
- Pharmaceutical form
- Disease indication

The SPCs will then be harmonised at the level of individual reference products and their generics so that differences in the bioavailability of the active substance from products that have not been demonstrated as bioequivalent can be taken into account (see 2.2. above).

Annex 6 provides an overview of the data considered useful for reviewing target animal safety. The review can be done in a step-wise manner as explained below.

6.3.1. Step 1: Determine the target animal safety profile for the active substance and establish the MOS for the active substance according to the revised dose, pharmaceutical form and route of administration

Review the TAS studies for all products with the same active substance and pharmaceutical form that are administered by the same route of administration to the concerned target animal species/category.

6.3.1.1 Margin of Safety studies

The aim is to:

- Confirm the target organs and toxicity profile of the active substance.
- The new MOS should be estimated based on the improved dose relative to the dose for which no/an acceptable level of AEs was observed in the TAS.

When pooling studies within different product groups as outlined above, some attention may need to be given to the relative bioavailability and differences in the PK profile for the active substance from different product formulations (for example, long-acting compared to immediate release injections). When calculating the MOS, studies from different products should only be pooled if the PK profiles are similar. Strict bioequivalence is not necessary, also considering that TAS studies are not anyway able to determine a precise MOS due to the dose multiples used. However, the degree of pharmacokinetic variability that can be accepted will be dependent on the (new) therapeutic index of the active substance. Relevant information may be found in the pharmacokinetics studies for the individual products. Assumptions may also be made regarding the relative bioavailability of different formulations

based on the principles for biowaivers outlined in the CVMP's Guideline on the conduct of bioequivalence studies (EMA/CVMP, 2000a).

In accordance with convention, the TAS studies are likely to have been conducted at 0x (negative control), 1x, 3x and 5x the highest originally recommended treatment dose (ORTD) for three times the proposed duration. If signs of toxicity were already seen in either the 1x or 3x groups, it may be difficult to conclude that an acceptable MOS remains for the increased dose. Pooling studies from different products may increase the data available as different doses/dose multiples may have been used. An acceptable MOS is dependent on the benefit-risk for the product.

Additional risk management measures, if needed, could include strengthening of SPC warnings and advice on overdose. If the risk due to the new MOS cannot be mitigated, then a dose change using this methodology will not be possible.

6.3.1.2 Reproductive Safety studies (where applicable): VICH GL 43 requires studies only to be conducted at 0x and 3x ORTD. The new MOS should be determined based on the increased dose. If this dose is lower than 3x ORTD and no adverse reactions were observed at 3x ORTD, then it is probable that reproductive safety could be accepted for the improved dose, albeit with a lower margin of safety. Further information to support a decision may also be available from laboratory animal reproductive toxicity studies and pharmacovigilance post-marketing. However, if there is a lack of reproductive safety studies or the margin of safety of the improved dose is below 3, additional risk management measures should be implemented including strengthening of warnings in SPC 4.7 (NtA, Volume 6C) e.g. restrictions on use in breeding animals or only according to the benefit/risk assessment by the responsible veterinarian.

6.3.1.3 Local (injection site) safety: Consideration should be given to injection-site safety, which may have been investigated at 1x ORTD, only. See also Step 6. Additional risk management measures, if needed, could include restrictions on the maximum volume of injection at individual sites, and/or bodyweight of animal to be treated.

6.3.1.4 Evidence for reduced palatability at higher doses should also be noted. See also Step 6. Additional risk management measures, if needed, could include SPC warnings regarding the maximum inclusion rate in feed/water.

Step 1a: If needed supplementary data may be availabale from:

Dose determination (and occasionally dose confirmation) studies that may have investigated doses higher than the ORTD in the target species.

Useful safety information (from target and non-target species) may also be available from studies presented in other sections of the dossier (see Annex 6). Data from non-target species will provide additional information on target organs and toxicity profile.

TAS studies conducted with products of a different pharmaceutical form or administered via a different route of administration or to different target species may provide additional information regarding the toxicity of the active substance. Consideration would need to be given to the similarity of pharmacokinetic profiles before these studies could be used to derive a MOS for a different pharmaceutical form, target species or administration route.

6.3.2. Step 2: Safety in the target population

Review the safety data from the clinical field trials for all products with the same active substance and pharmaceutical form that are administered preferably by the same route of administration to the concerned target animal species/category. The following points should be considered:

- Is there a relationship to dose, dosing frequency or treatment duration for the observed adverse events?
- Is there evidence of a decreased MOS in sensitive sub-populations (e.g. age groups)?

Although formulation differences affecting bioavailability should not be overlooked, the main purpose of field data is to investigate the potential impact of a dose/regimen change on safety across the diversity of the target population characteristics and in the presence of disease. Additional risk management measures, if needed, could include strengthening of SPC contraindications or warnings relating to sensitive sub-populations.

6.3.3. Step 3: Safety based on post-marketing pharmacovigilance

Review the Eudravigilance veterinary database (EVVet) for all products with the same active substance and pharmaceutical form that are administered by the same route of administration and in the same species with focus on reports where the product has been administered at overdose (subject to availability). The main purpose is to gain a general impression of the safety of the products when used under field conditions; some specific information regarding the safety of increased doses may be available.

6.3.4. Step 4: Safety based on published literature and authorisations in third countries (if needed)

If needed, studies from peer-reviewed journals, reports from scientific institutions and textbooks may also be used to provide supporting evidence for the safety of the increased dose and experience from field use. In this case, the sources and search strategy should be documented.

Information on target animal safety is available in the published SPCs of EU-authorised products. If supporting data are available (e.g. proprietary studies, pharmacovigilance reports, literature) they should be assessed in Steps 1 to 4. If the origin of the information is not verifiable, it should be reviewed critically.

In addition, similar products may be authorised in other e.g. VICH-participating countries where they are used with different dosing regimens. SPCs and assessment reports relating to these products may be publicly available.

6.3.5. Step 5: Conclude on the safety of the increased dose of the active substance according to the pharmaceutical form and route of administration

Based on the totality of the data considered under steps 1 to 3, and 4 if necessary, a conclusion should be made on the safety of the increased dose of the active substance according to the pharmaceutical form and route of administration in the target species.

Consideration should also be given to additional risk management measures as indicated above.

6.3.6. Step 6: Further considerations for the conclusion on the safety and benefit-risk for individual products

- ***Excipients*** - Consideration should be given to the systemic and local safety of the excipients in the individual formulation in relation to any impact of the concurrent dose increase. Information on the product excipient formulation is available from Part 2 of the dossier. Product-specific information,

e.g. injection site safety studies, should be reviewed. Further information on the MOS of excipients is available from public sources (e.g. MRL summary reports, Codex reports, GRAS list).

- ***Indications*** – If the change in the MOS could impact on the benefit-risk, then the indications for individual products need to be part of this consideration, for example, consideration may have to be given to the severity of the concerned disease and availability of alternative treatments.

6.3.7. Step 7: The conclusions above are incorporated into the final benefit-risk for the dose increase for each individual product

6.4. Data sources

- Target Animal Safety studies, including reproductive and injection site safety as appropriate
- Pharmacological studies for individual products
- Pre-clinical studies (e.g. dose determination)
- Clinical field trials in the target population
- Eudravigilance veterinary and PSURs
- Detailed information on the product composition and formulation
- Laboratory animal and human safety studies – reproductive toxicity and special studies
- Literature searches
- Information on authorisations of similar products in other e.g. VICH participating countries

An overview of the TAS-related data considered useful is presented in Annex 6.

7. Discussion and conclusions

7.1. Dose review and adjustment by PK/PD analysis

Case studies analysis

For the purpose of the pilot study, the PK/PD index AUC_{24h}/MIC is considered for tetracyclines (Andes and Craig, 2007) and amoxicillin (Lees et al., 2015) as a first step to define a dose. For amoxicillin, we refined the analysis consider also T>MIC (Rey et al., 2014). This use of PK/PD indices in the application of the methodology will allow review of advantages (such as applicability, feasibility) and drawbacks (such as data requirements, complexity) of the PK/PD index used.

The calculation of AUC/MIC is simple to perform and allows back calculation to set a dose or a breakpoint. It can be obtained from summary tables (T1/2, AUC, Cmax, Tmax) derived from non compartmental analyses of PK data (time, concentrations). It requires a good pharmacokinetic dataset to estimate AUCs and does not require extensive pharmacometrics as it can be obtained from noncompartmental analysis. The calculation of time above MIC requires PK data (time concentrations) to estimate the intersection between MIC and curves or robust estimates of the distribution of pharmacokinetic parameters (means and variances) of pharmacokinetic models established after analysis ofdifferent experimental studies. An expertise in pharmacometrics using nonlinear mixed effects is needed for this step. The time to maintain MIC is not a simple parameter but a variable function of different conditions and depends not only on the dose but also from the shape of the time concentration curve. Thus, it cannot be derived from a simple formula and needs to be computed for Monte Carlo studies. The use of population pharmacokinetics, allows simulation of probable product

exposures which can be obtained with any dosage regimen. This is important for a time-dependent antibiotic such as amoxicillin for which the input rate (absorption) is at least as important as the total administered dose. Indeed, the time to maintain MIC will be highly dependent not only on the dose administered but also on the formulation, the route of administration and the inter-individual PK variability (for example in body weight, sex, age, social rank).

As an example, for pigs, for oral *ad libitum* administration, plasma concentrations are related to the feeding and water intake behaviour. This behaviour can be modified by disease state. The pharmacokinetic data set used by Rey et al. (2014) was obtained with healthy animals as it was submitted for marketing authorization for a veterinary medicine. Infection could modify the feeding and water intake behaviour and also product disposition. As discussed in the paper by Rey et al. (2014), the effect on disposition must vary according to the type of disease. Exposure of diseased animals could increase or decrease in comparison with healthy animals. Both PK/PD indexes AUC/MIC and T>MIC are dependent on animal status, product bioavailability, disposition and clearance.

The use of a PK/PD approach requires a definition of the PTA to be achieved such as:

T>MIC: 40% of 24 hours greater than the MIC of 90% of the pig population

AUC/MIC: Ratio expected for bacteriostatic or bactericidal effect of 90% of the pig population.

The relationship between T>MIC and antibacterial efficacy has been determined *in vitro* in several experimental animal studies (Craig, 1998) and retrospective analysis of clinical trials in human medicine seems to confirm those findings (Ambrose et al., 2007). For AUC/MIC, the targets were derived from *in vitro* activity of amoxicillin in serum on a limited set of P. multocida strains (Lees et al., 2015). The choice of this index was justified in the paper because a concentration-dependent killing profile was observed *in vitro* in serum and confirmed in ex vivo studies. In addition, it was shown that for antibiotics like the β-lactams, where efficacy has been found to be correlated to T>MIC, the best PK/PD index shifts towards AUC/MIC as half-life increases (Nielsen and Friberg, 2013) while for an AUC/MIC dependent antibiotic a decrease in half-life will lead to a shift into a T>MIC relationship. When the half-life was increased to be higher than 2 h in laboratory animal experimentations, the AUC/MIC became the most important PK/PD index (Nielsen et al., 2011).

Mechanisms based on PK/PD modelling based on *in vitro* studies are also proposed as a flexible and powerful tool to describe the effect of antibacterial agents. The simulations are based on a model characterizing *in vitro* time-kill curve experiments combined with a pharmacokinetic model. The approach selected the previously PK/PD indices for different classes of antibacterial product. The target level and adjusted dosing regimen should be based on quantitative description of the full time course of PK as well as PD and tailored to the population to be treated (Nielsen et al., 2011).

For oxytetracycline, the PK/PD index was AUC/MIC with a target value from *in vitro* studies for a 24 h exposure. The PDT were derived form studies performed in CAMHB which are closed to the conditions of determination of MIC in standardized method instead of value determined in other media (serum, transudate). The choice of a PDT for a period of 24 h instead of a PDT to reach by interval of time between administrations can be discussed but it allows a comparison of different dosage regimen on a daily basis.

The definition of most of the PDT are derived from studies conducted in laboratory animal disease models, verified in human clinical trials and used in human medicine (Toutain et al., 2017). Target values of these indices indicate the systemic exposure, normalized by MIC, required against each pathogenic bacterial species. The target value itself does not depend on animal or human characterisitics but on the antimicrobial effect on the bacterial target.

7.1.1. PK/PD and prevention of resistance

The 'mutant selection window' (MSW) is a concept well described in the scientific literature (Zhao and Drlica, 2001) for certain classes of antibiotics (e.g. fluoroquinolones). It postulates that an antibiotic concentration zone exists where resistant mutants, are selectively amplified. The lower limit of the MSW is the lowest concentration that inhibits the growth of the susceptible cells and is often approximated by the MIC. The upper limit is the minimum concentration that inhibits growth of the least-susceptible single-step mutant subpopulation, the mutant prevention concentration (MPC).

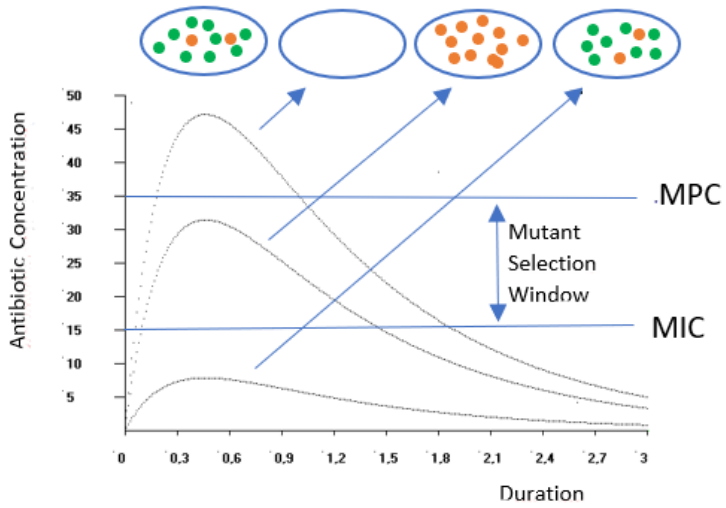


Figure 13. Concept of mutant selection window (based on Cantón and Morosini (2011))

This MSW also updates the classical concept of "sub-inhibitory" concentrations favouring the emergence of resistance, although the threshold to be considered is not the MIC of the majority wild pathogen population but the MIC of the least susceptible pathogenic sub-population, which in fact corresponds to the MPC.

Then, to clearly take into account the notion of concentration preventing mutation in a PK/PD modelling, it is necessary first to define MPC distribution values for each molecule/bacterial species combination. It will allow obtaining three new PK/PD indices by replacing the MIC by the MPC:

- AUC/MIC → AUC/MPC
- T>MIC → T>MPC
- C_{max}/MIC → C_{max}/MPC

Currently, MIC distribution is well standardised notably for surveillance monitoring programs and the information is easily accessible. However, applying MPC principles, when available, may serve to optimise antibiotic therapy and reduce resistance selection.

7.1.2. Limitations of the modelling approach

7.1.2.1. Impact on gut microbiota

One of the main challenges in relation to AMR is to reduce the exposure of intestinal microbiota to antimicrobials in order to control the dissemination of resistant bacteria and resistance determinants in the environment. Consequently, dose review and adjustment should aim to lower antibiotic exposure of the treated animals over time. Although the proposed PK/PD approach could be a useful tool to

determine doses that are effective in the treatment of bacterial target pathogens, the methodology does not consider the potential impact of antibiotics on gut microbiota.

7.1.2.2. Use of the MIC as a PD indicator

The PK/PD relationship is based on the determination of a MIC as an indicator of effectiveness. MIC testing has been highly standardized (e.g. CLSI, EUCAST) to avoid potential errors due to different testing methodologies. However, MIC values may differ if they are tested in other conditions. Also, MIC testing requires a 2-fold dilution approach which provides only an approximate inhibitory value. Even though the MICs are determined *in vitro* in a standardized environment they are not always representative for the site of infection. It should be noted that those aspects are currently under investigation notably studies comparing *in vitro* MIC obtained either in a standardized broth medium or in serum or biological fluid such as transudate/exudate. Evidence suggest that potency of certain antibiotics measured in serum (as MIC) differs markedly from MICs determined in artificial broths and may need also to be considered for the dose adjustment (Dorey et al., 2017; Dorey and Lees, 2017; Lees et al., 2018). In addition, in numerous situations, the MICs are not predictive of *in vivo* antibacterial activity as for example for intracellular pathogens or in a biofilm environment (Ferran et al., 2016). Furthermore, additional mechanisms of action presented by some antibiotics (e.g. anti-inflammatory, immunomodulatory activities) are not covered by MIC (Fischer et al., 2011).

Next to that, to establish a reliable susceptibility distribution profile of the target bacterial population an appropriate, at best a statistical sufficient, number of strains needs to be investigated.

7.1.2.3. Host immune response

The PK/PD relationship does not take into account the immune response of the host which can have an effect on the growth and multiplication of bacteria as well as on their clearance from the target animal. The efficacy and memory effect of the immune response are dependent of several conditions (e.g. inoculum size, immune status of the host). A relationship between the bacterial population and the immune cell population can be described and added in a more complex model in order to relate the dosage regimen (dose, frequency, duration of treatment) to the recovery rate and the risk of relapse. At this stage of research on PK/PD modelling, such models are still under investigation (Gjini and Brito, 2016).

7.1.2.4. Duration of treatment

Until now, one of the main limitations of the PK/PD methodology to revise the dosages of older antibiotics is that it aims to determine a dose but does not give any information on the duration of treatment. Limiting the treatment duration of an antibiotic to the minimum time necessary can help to reduce costs and adverse effects. The main benefit is however, to reduce the duration of antibiotic exposure on the commensal microbiota, which is an essential element in preventing the selection, amplification and circulation of resistant bacteria/resistance determinants as well as their release into the environment. A number of studies have assessed the impact of the antibiotic treatment duration on the amplification of resistance within the commensal flora. An increase in the daily dose could in principle warrant a reduction of the treatment duration in a certain number of cases. Nevertheless, confirmatory clinical trials would be needed to verify that a reduction of the treatment duration indeed leads to bacteriological or clinical cure of the animals in the absence of relapse.

7.1.2.5. Need for a clinical confirmation

According to the revised efficacy guideline (EMA/CVMP, 2016), the application of a PK/PD relationship can be accepted for the purpose of dose determination in new antibiotic products but a clinical

confirmation is always required to assess the efficacy of the proposed dose. If the outcome of the PK/PD modelling in old antibiotics would lead to the recommendation of a substantial increase of the daily dose, a regulatory process needs to be established that can be applied in order to change the dose regimen of established products. For products that have been on the market for a long time and are widely used in the veterinary field, it is unlikely that new efficacy studies can be requested according to the current legislation. Thus, an important requirement of the PK/PD modelling approach is the availability established PDIs, PDTs and corresponding PTAs for certain type of infections in animals that can be reliably correlated with clinical efficacy.

7.1.2.6. Mode of administration

The method proposed thus far considers the intake of the medicinal product to be "perfect". For injection routes, this is hardly a problem, provided that good hygiene measures are followed and needles and syringes suited to the dosage are used. In contrast, bioavailability studies by the oral route are all based on the forced drenching of animals. While pets receive their antibiotic by drenching, oral treatments of livestock food-producing animals are most often collective and based on "voluntary" intake by the animals, either by a solid medium via medicated feed, or by a liquid medium via drinking water. For oral *ad libitum* administration, plasma concentrations are related to the feeding and water intake behaviour (depending on e.g. the health status, the animal social rank), meaning that it induces individual pharmacokinetic variabilities, which the presented approach cannot take into account.

- ***Administration via feed***

When feeding *ad libitum*, the amount of feed consumed is more variable than the amount of water drunk. The main limitation is therefore, the feed intake of each animal within the batch since this can lead to a greater variability of serum concentrations following administration of the same antibiotic (Soraci et al., 2014).

- ***Administration via drinking water***

Compared to feed, administration via drinking water presents several advantages. For example, treatment durations for these products are usually shorter than for VMPs with same active substances which are administered via feed. This lowers the exposure on the commensal flora. It is also easier to target smaller batches of animals and treatment can be initiated earlier compared to medicated feed. Although drinking water medication has higher compliance in animals, limitations and uncertainties can be linked to inaccuracy of the dosage and quality of the medicated water.

7.1.3. Data requirements

In order to use the PK/PD integration approach for the dose review and adjustment of established veterinary antibiotics, the following data are considered essential:

- PK data
    - PK raw data from studies of individual products
    - Mean and SD values for AUC related to different dose regimens
    - Mean and SD values for each PK parameter (CL, F, f …)

- PD data
    - MIC distribution for each target bacteria

- PDIs and PDTs for different type of infections that can be reliably correlated with clinical efficacy in animals

Furthermore, the following data would be desirable:

- Time-kill curves
- PK/PD modelsling
- Literature data
- *In vivo* experiments - correlation between prediction and clinical outcome

7.1.4. Conclusions on the PK/PD integration

7.1.4.1. The importance of the dose review and adjustment of established veterinary antibiotics

The importance of revising the dosages is based on a need to improve the doses of older antibiotics because repeated exposure to inappropriate concentrations represents a major risk in terms of antimicrobial resistance development. An improved dosage must be determined to ensure the efficacy of the treatment, but also to prevent the emergence, selection and/or dissemination of resistant micro-organisms and resistance determinants in a bacterial population in the animal. Inter-individual variability, in terms of exposure to the antibiotic, is certainly one of the risk factors with the greatest influence on the emergence of antibiotic-resistant organisms. Accordingly, a PK/PD approach should take the inter-individual variability into account. Consequently, and in order to determine an improved dose, the methodology for revising the dosages of older antibiotics is based on a PK/PD approach that integrates the variability of both parameters, the pharmacokinetics (clearance, bioavailability) and pharmacodynamics (in terms of MIC). The PK/PD integration based on Monte Carlo Simultion allows to integrate the inter-individual variation to estimate an improved dosage regimen for most (90%) of the individual cases.

The current doses of established antibiotics generally provide a clinical benefit as demonstrated by their long period of usage. Nevertheless, the dose regimens were not adjusted and reviewed to avoid the risk for selection, emerge and spread of antimicrobial resistant bacterial target pathogens or commensal microbiota.

7.1.4.2. The feasibility of the PK/PD approach

The PK/PD approach requires consolidated data available for both, the pharmacokinetics of the antibiotics in the respective animal species, and the pathogens' susceptibility to antibiotics, in the form of MIC distributions. Established PK/PD indices and PDTs are central to the PK/PD methodology applied to antibiotics, because they are essential to predict the probability of therapeutic success in potentially varying clinical situations. Currently, such data are limited, in particular for older antibiotic classes. Ideally PK/PD indices (and their threshold values) should be confirmed in clinical trials performed in the target species. For old antibiotics, the PK/PD integration approach on dose review and adjustment is eligible, only when the substance belongs to an antimicrobial class for that scientific evidences from experimental and clinical trials is available supporting the setting of PDI and PDT.

7.2. Withdrawal Period adjustment by PK analysis

7.2.1. Case studies analysis

For the purpose of testing the approach of adjustment of the WP using an algorithm based on PK modelling (see chapter 4.), two case studies were performed. The idea was to test a simple case (amoxicillin products for oral use in pigs) together with a more difficult one (injectable oxytetracycline products for use in cattle; including dairy cattle). However, as it turned out, both cases each had their own specific difficulties.

Whilst the problem how to deal with the injection site and the i.m versus s.c. administration had to be addressed in the oxytetracycline case, the amoxicillin case turned out to be unexpectedly difficult, due to lack of usable residue data.

Since the depletion of residues of amoxicillin after oral administration to pigs is very rapid, most of the old residue studies that could be found in registration dossiers, only confirm that the residues are already below LOD after a few days.

For oxytetracycline, the erratic sampling of the injection site caused some fitting problems as well as the fact, that increasing the dose could pose a challenge regarding the maximum amount of injections that would be practical versus the maximum weight of an animal to be treated.
In both cases it was not possible to check and validate all preconditions pointed out in section 4.4 (i.e. linear kinetics (dose proportionality) for the intended dose range in each tissue that might be WP determining, relative bioavailability new dose vs. old dose, tissue distribution completed at MRL-level). However, possibly the particular challenges could be overcome by the use of the hour glass approach. Including and combining data and insights from multiple sources (FARAD, literature, published thesis's, registration files, *etc.*) might be helpful to find the relevant PK parameters to make worst-case estimations of (for example) half-lives. Furthermore, the uncertainty in the calculated adjusted WPs may be compensated by the use of safety spans, where necessary.

The two cases show that it may be possible to use non-experimental approaches for extrapolating the WP, if the relevant data are available. It should be noted that the methodology for WP extrapolation needs to remain flexible, in order to be able to handle various datasets that differ in the amount and quality of the data available. This flexibility also allows for possible improvements of the calculations.

7.2.2. Concluding remarks on Withdrawal Period extrapolation

WPs in the EU are usually based on residue depletion studies (as the "golden standard"). However, in the context of dose adjustments, usually new residue depletion studies are not feasible. Therefore, data and insights from multiple sources (FARAD, literature, published thesis's, registration files, etc.) could be used and combined in order to find the relevant PK parameters and to eventually make a worst-case estimation of the terminal half-life for the relevant tissues. The use of multiple information sources and established pharmacokinetic principles ensures the scientific basis for the proposed extrapolation approach. Furthermore, uncertainties in the calculated adjusted WPs may be compensated by the use of safety spans, where necessary.

As already pointed out, it should be noted that the third step in the proposed extrapolation-process is to apply the algorithm to each VMP separately. This would mean that the absolute differences in the existing withdrawal periods will remain.

7.3. Addressing environmental risks by a data review approach

7.3.1. Case studies analysis

The environmental risk assessment for the case studies on amoxicillin in pigs and oxytetracycline in cattle turned out to be fairly easy. For amoxicillin, the doubling of the dose from 20 to 40 mg/kg bw per day for 5 days did obviously increase the PECs with a factor of 2. The Risk Quotients remained below 1 when the duration is maximally 5 days, and above 1 when the duration is 7 days. It was considered that the duration of 3-5 days may be sufficient for products with the same indication, which would justify the limitation of the duration to maximally 5 days, in order to limit the exposure to the environment. For oxytetracycline, there was already an ERA for a single dose of 20 mg/kg bw. Dose review and adjustment resulted in two regimens: 2 x 20 mg/kg bw for the LA formulations, or 5 x 10 mg/kg bw for the SA formulations, both of which would increase the environmental exposure as compared to the existing ERA. However, even with these posologies the Risk Quotients remained below 1, and therefore there was no trigger crossing and consequently no need to enter another Phase or Tier of the ERA.

7.3.2. Conclusions on the ERA data review

A data review approach was set up and tested in two case studies. The case studies showed that the data review approach was feasible, and that there were no additional concerns for the environment with the new adjusted doses. This conclusion was reached without the need for additional experimental studies.

It has to be recognised that the case studies were easy in the sense that there was no trigger crossing when going from the current dose to the adjusted dose. Therefore, the data review approach as outlined in chapter 5. , was not tested to the full extent. There may be other cases where the approach can be more challenging. Nevertheless, within the limitations of this pilot, the approach was successful.

7.4. Addressing target animal safety by a data review approach

7.4.1. Case study analysis

The data review methodology proposed to address target animal safety was not followed comprehensively in the two case studies due to the lack of availability of pivotal study data for these old products from either pharmaceutical companies or regulatory agencies, and the time needed to perform searches to fill data gaps from publicly available material. Although the methodology could be time consuming, the expectation is that it would be followed until sufficient evidence is available to give confidence in the conclusions.

In regards to the amoxicillin case study, only two proprietary TAS studies were available that, although not to current VICH requirements and performed in only a small number of animals, gave a reasonable level of evidence to support a margin of safety for the proposed revised dose in the target species. No specific studies could be found on a basic literature search to support field safety in pigs; however standard texts and reports representing use of the substance over decades in laboratory species and humans give reassurance of a wide margin of tolerance. It was possible to fully identify the target organs and toxic profile of the substance based on the totality of the data available.

For the oxytetracycline case study, the CVM Freedom of Information summary reports provided the most informative data on systemic tolerance; although it has to be considered that this is only available in high level summary format. For oxytetracycline, the adjusted dose regimens suggested by PK/PD modelling fell within the range of doses approved in the EU; however, the margin of safety for

renal effects would have to be taken into account for any further dose increase. The proprietary studies provided to the project by industry related to injection site safety with the focus being on local tolerance and injection volume, rather than dose. These studies clearly highlighted that local tolerance is likely in practice to be the key dose-limiting factor for oxytetracycline injectable formulations, with some variability between different formulations according to excipient composition.

For both the amoxicillin and oxytetracycline studies, no proprietary data were available from either field safety studies or post-marketing pharmacovigilance. Outside the pilot project scenario, these data should be sought to give greater confidence in the final conclusions.

7.4.2. Conclusions on the TAS data review

For the amoxicillin formulations, the data review approach can give reasonable confidence that the proposed dose increase to 40 mg amoxicillin/kg x 5 days in drinking water would be adequately tolerated in pigs for the treatment of respiratory disease. Amoxicillin is a well-established molecule with a wide margin of safety in many species and with further probing of dossiers sufficient data are likely to be available to draw conclusions on the safety of the dose increase in pigs. The oral formulations are administered as solutions and have relatively simple excipient formulations, and therefore safety can be extrapolated between them with a degree of confidence.

Although no increase in dose outside of the EU-approved ranges was suggested for oxytetracycline injections, the possibility of a hypothetical dose increase was explored. Especially, if the margin of safety for an active substance is not large, the necessity of supporting data would be increased and a wider review of product dossiers may be necessary. Overall, a lack of available data may limit the applicability of the approach.

For this case, the oxytetracycline injectable formulations are more complex than the oral amoxicillin solutions. The data review methodology identified that local injection site reactions may be dose-limiting in practice. Local tolerance can vary according to individual product composition and would have to be considered on a product-by-product basis; therefore, proprietary studies would be required to establish the maximum injection volume where not already stated in a product's SPC. Where data are not available, a default value would need to be established according to the worst-case scenario. If restriction of injection volume would lead to an impractical number of injection sites, a simple risk management solution would be to limit the maximum bodyweight of animals to be treated. In conclusion, use of the data review approach may be possible for the evaluation of TAS, but the need for individual product reviews could be burdensome.

7.5. Regulatory processes to effectuate the harmonisation of the product literature

The main purpose of the reflection paper was to develop and test a novel approach for dose review and adjustment, and its possible impact on WPs, ERA, TAS and URA, without the need for conducting further experimental studies. This approach may be useful to review and improve the situation of established veterinary antibiotics where the authorised dose may not be effective anymore. At the same time, application of this approach will lead to a certain level of harmonisation between authorised products across the EU. In this respect, this approach may also be used as part of other regulatory harmonisation exercises for antibiotics (e.g. possibly initiated by the new EU legislation on veterinary medicines).

A number of general principles for the regulatory implementation of this approach and the related harmonisation of VMPs (discussed below) were defined, but the appropriate regulatory procedures, the

appropriate legal basis, and other related legal issues were not defined or discussed. The latter points need further discussion.

7.5.1. Selection of candidates

Chapter 2.1. offers a method to select and prioritise (groups of) established veterinary antibiotics for which dose review and adjustment may be required. Application of this method allows putting resources where they are most needed and provides clarity on the order at which the products will be reviewed, which would facilitate short and long term planning of related work at the sides of regulators and industry.

7.5.2. Extent of harmonisation

As explained above, the dose review and adjustment of products or groups of products will lead to a certain degree of harmonisation. The minimum desired level of harmonisation would be a harmonisation of individual products with authorisations in different Member States (i.e. at product level). This has been explained in chapter 2.2. (the hour glass method). However, because of the group-wise analysis, some aspects such as the adjusted dose, may be applied to products within the same group, as was done for the case studies with amoxicillin and oxytetracycline. This may in particular apply to similar products which have been licensed nationally some time ago, resulting in different summaries of product characteristics, but which are essentially similar.

7.5.2.1. Same-product harmonisation

The same product with authorisations in different Member States can have differences in the indications (i.e. inclusion of certain diseases), the causal organisms (i.e. inclusion of certain pathogens), the dose, the withdrawal periods, and the special warnings and precautions for use. There are several possibilities for within-product harmonisation, and the selected level of harmonisation has consequences for the approaches to address dose, WP, ERA, and TAS and for the final outcome. For example, one could calculate a dose for each disease, or even per causal pathogen for these diseases, resulting in differentiated adjusted doses that can be applied to the authorisations depending on which diseases/pathogens has been already licensed in the various Member States. However, such an approach would require many calculations for the doses and withdrawal periods and may also have different outcomes for ERA and TAS, depending on the highest label dose. Moreover, in practical terms this may not offer advantage since for first line antibiotic treatment is often started before the causative pathogen has been identified and many infections (including respiratory disease) are syndromes with mixed bacterial etiology. In addition, differences of the SPC of the product between Member States would remain. Another possibility would be to aim for the largest possible denominator and thus a full harmonisation per product. That would include the sum of all authorised indications/pathogens for which a dose review and adjustment was possible applied to all authorisations of this product across the EU, irrespective of the current indications authorised in the individual MSs. This approach is not only easier to apply but would maximise the availability of efficacious veterinary antibiotics for various diseases at the same time. A full harmonisation per product is preferred, resulting in identical SPCs in all MSs where the product is authorised. A full harmonisation also implies a single WP for meat and offal, and single WPs for milk or eggs, where applicable. It should be noted that current WPs for the same product can be very different between MSs. Therefore, the establishment of a single WP will require the selection of a "Reference WP" that can be used as a starting point for the extrapolation. Is proposed that this Reference WP will be scientifically established on the basis of available residue data, and not on the shortest or the longest WP by default.

7.5.2.2. Between-product harmonisation

As explained in chapter 2.2. , there are scientific and practical reasons to harmonise at the level of individual products. Nevertheless, the analysis conducted according to the hour glass method may reveal that certain products in a group are so similar that for the same indication and the same species, the same adjusted dose could apply. However, as indications can differ between products it is proposed not to harmonise indications across these products. For example, if product A has only respiratory tract infections on the label, and similar product B has both respiratory tract infections and urinary tract infections on the label, then the respiratory tract infections could be harmonised between products when possible (i.e. they will have the same adjusted dose), but product A will not get the urinary tract infections indication. In addition, it is proposed that WPs are not harmonised across (similar) products. Where differences in excipient formulation could have an impact on tolerance, this aspect needs to be considered on a product-specific basis.

7.5.3. Level of assessment

Established veterinary antibiotics have been authorised through national, decentralised, or mutual recognition procedures, and therefore have national marketing authorisations. Therefore, in principle, any changes to the marketing authorisations fall within the remit of the National Competent Authorities (NCAs). However, it should be noted that:

- the process of dose review and adjustment, WP, ERA and TAS requires input from National Competent Authorities through the authorisation dossiers from all MSs;
- the process of dose review and adjustment, WP, ERA and TAS will result in a certain degree of harmonisation across the EU MSs and would be consistent with the well-established principle of mutual recognition within the Community;
- the techniques for dose review and adjustment, WP, ERA and TAS must be applied in a consistent manner for all relevant (groups of) established veterinary antibiotics throughout the Community;
- the regulatory process of dose review and adjustment must be conducted in a consistent manner for all relevant (groups of) established veterinary antibiotics throughout the Community;
- the implementation of the outcome of the dose review and adjustment must be consistent across all MSs concerned.

Therefore, it is advised that the organisation, assessment and decision will be executed at the central European level. Given the scientific nature of the work, the assessment could be well done in the CVMP.

7.6. Need for further research

One of the objectives of this Reflection Paper was to explore possibilities for funding under Horizon 2020 or other funding sources, for studies to fill gaps in data for off-patent veterinary antibiotics related to improving dosing with respect to minimising risks from AMR where progress is not possible without generation of additional data. Non-experimental approaches for dose review and adjustment, WP, ERA and TAS were developed. It is envisaged that the data that are needed as input for these approaches will be available for the vast majority of the established veterinary antibiotics and therefore possibilities for funding were not further investigated.

As explained in 7.1.2.5. , a dose derived by PK/PD analysis should ideally be confirmed by clinical data, however this cannot be expected in the context of improving the situation of the established veterinary antibiotics, for the reasons mentioned in chapter 1.1. . The same reasoning applies to the WP, ERA and

TAS. In this context, it should be noted that the strength of the hour glass method is in the integration of data from all authorisation dossiers and other available data, providing presumably a very data-rich basis for the modelling and review approaches.

Whereas the PK/PD methodology allows for adjusting the dose, it will not provide the answer to the question for how long the PTA should be reached for a clinical cure. Therefore, in principle, the length of treatment is not optimised using PK/PD modelling. As a result, the treatment duration will not be changed in principle. However, there may be cases where the PTA is reached only relatively shortly, in which case the treatment duration may need to be extended, although it is recognised that this extension can be somewhat arbitrary. In the case study for the LA oxytetracycline formulations, a second dose was introduced to achieve the PTA to be reached for at least 3 days. In order to strengthen decisions related to treatment duration, collection and/or generation of scientific data on this aspect will be helpful.

8. CVMP Recommendations

1. It is recommended that there is a continued dialogue between regulators and industry to discuss the possible procedures and legal implications in relation to the implementation of the recommendations of this report.
2. It is recommended that the implementation of the recommendations of this report will take place at the central level, i.e. that CVMP will conduct the scientific assessment. It was noted that the outcome could result in an e.g. Commission Decision.
3. It is recommended to develop a clear procedure to establish a list of the candidate products for dose review and adjustment, with a prioritisation of these candidates, in line with the principles discussed in chapter 2 of this report. In establishing the actual list, it is recommended that relevant stakeholders are consulted. For example, the FVE can be consulted to obtain information of dosages used in the field, and VetCAST can be consulted to obtain information products for which, according to their knowledge, the current dosing regimens is not in line with PK/PD principles.
4. It is recommended that selected candidate products for dose review and adjustment are grouped at the *animal-species-disease-route of administration-pharmaceutical form* level.
5. It is recommended to follow the hour glass approach (see chapter 2) for collection and integration of data and for the application of model outputs.
6. It is recommended that procedures for dose review and adjustment, withdrawal periods, ERA, and TAS, result in harmonisation at product level and where applicable also between similar products as outlined in paragraph 7.5.2.2.
7. It is recommended that the dose review and adjustement and the consideration of withdrawal period, ERA, and TAS, can be conducted using non-experimental approaches, such as presented in chapters 3, 4, 5, and 6 of this report.

9. Glossary

ADME	Absorption, Distribution, Metabolism, Excretion
AE	Adverse Event: any observation in animals, whether or not considered to be product-related, that is unfavourable and unintended and that occurs after any use of VMP (off-label and on-label uses). Included are events related to a suspected lack of expected efficacy according to approved labelling or noxious reactions in humans after being exposed to VMP(s).

AMEG	Antimicrobial Advice Ad Hoc Expert Group
AUC	Area Under the Curve: the total concentration integrated over a given time interval
AMR	Antimicrobial resistance
B/R assessment	Benefit-risk assessment: A process of assessing benefits and risks in accordance to the benefit-risk assessment policy. This assessment includes the mitigation of risks from a proposal of benefit-risk management options. The benefit-risk balance is the outcome of the benefit-risk assessment.
CBP	Clinical breakpoint: A selected MIC value to distinguish between treatable and non-treatable organisms
CLSI	Clinical and Laboratory Standards Institute
C_{max}	The maximum (or peak) serum concentration that a product achieves in a specified compartment or test area of the body after the product has been administrated
CVMP	Committee for Medicinal Products for Veterinary Use
DDDvet	Defined Daily Doses for Animals; The DDDvet is the assumed average dose per kg animal per species per day
Dose review and adjustment	A process using established PK/PD modelling techniques that defines a dosing regimen where an adequate ionised concentration of the antimicrobial active substance would accumulate at the target site and at a predictable concentration above modern MIC values for the target pathogen(s).
ECOFF	Epidemiological cut-off value: measures of a antibiotic MIC distribution that separate bacterial populations into those representative of a wild type population, and those with acquired or mutational resistance to the molecule.
EGGVP	European Group for Generic Veterinary Products
EMA	European Medicines Agency
ERA	Environmental Risk Assessment
EUCAST	The European Committee on Antimicrobial Susceptibility Testing
FARAD	Food Animal Residue Avoidance Databank. FARAD is part of the Food Animal Residue Avoidance & Depletion Program in the US, which has served the veterinary profession for more than 35 years. FARAD is supported by the USDA National Institute of Food and Agriculture (NIFA).
f	free or unbound fraction
GLP	Good Laboratory Practice
GRAS list	A list of substances that are generally recognised as safe. This list is available on the website of the US Food and Drug Administration (FDA): https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/
Horizon 2020	Horizon 2020 is a EU Research and Innovation programme with nearly €80 billion of funding available over 7 years (2014 to 2020)
LA	long acting

MAH	Marketing Authorisation Holder: A person or entity who/which holds the authorisation of a VMP.
MBC	Minimum Bactericidal Concentration
MIC	Minimum Inhibitory Concentration: the lowest concentration of a chemical which prevents visible growth of a bacterium.
MOS	Margin Of Safety, also called the *therapeutic window* (or pharmaceutical window) of a product, is the range of dosages which can treat disease effectively without having toxic effects.
MRL	Maximum Residue Limit. The maximum concentration of residue resulting from the use of a veterinary medicinal product (expressed in mg/kg or µg/kg on a fresh weight basis) which may be accepted by the Union to be legally permitted or recognised as acceptable in or on a food.
MS	Member State of the European Union
NCA	National Competent Authority
OECD	Organisation for Economic Co-operation and Development
OIE	World Organization for Animal Health
ORTD	Original Recommended Treatment Dose
PBT	Persistent, Bioaccumulative and Toxic
PEC	Predicted Environmental Concentration
PD	Pharmacodynamics
PDI	PK/PD-index: The quantitative relationship between a pharmacokinetic parameter (such as AUC, peak level) and a microbiological parameter (such as MIC)
PDT	target value of the PK/PD index
PK	Pharmacokinetics
PK/PD modelling	*in silico* modelling of PK and PD. It integrates a pharmacokinetic and a pharmacodynamic model component into one set of mathematical expressions that allows the description of the time course of effect intensity in response to administration of a drug dose
PK/PD integration	integration of a PD parameter (usually MIC) to calculate an antimicrobial activity (PK/PD Index) using PK descriptors and or PK model.
PNEC	Predicted No Effect Concentration
PSUR	Periodic Safety Update Report: A periodical scientific report on adverse events and other issues within the scope of pharmacovigilance that have been reported to a MAH during a specific period.
PTA	Probability of Target Attainment
QSAR	Quantitative Structural Activity Relationship
Read across	Read-across is a technique for predicting endpoint information for one substance, by using data from the same endpoint from (an)other substance(s).

RMM	Risk Mitigation Measure
RQ	Risk Quotient, i.e. PEC/PNEC ratio
SA	short acting
Signal Detection	A pharmacovigilance procedure to detect safety signals. A safety signal is information on a new or known adverse event that may be caused by a medicine and requires further investigation.
SPC	Summary of Product Characteristics
TAS	Target Animal Safety
vPvB	very Persistent and very Bioaccumulative
VCIA	Veterinary Critically Important Antimicrobial Agents
VHIA	Veterinary Highly Important Antimicrobial Agents
VIA	Veterinary Important Antimicrobial Agents
VICH	VICH is a trilateral (EU-Japan-USA) programme aimed at harmonising technical requirements for veterinary product registration. Its full title is the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products.
VMP	Veterinary Medicinal Product
WFD	Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for the Community action in the field of water policy. In short: EU Water Framework Directive.
WP	Withdrawal Period. The withdrawal period is the time after the last administration of the veterinary medicinal product during which the animal must not be slaughtered or during which milk or eggs must not be taken for human consumption, ensuring that residues will not exceed the MRLs.
WT	wild type

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11. Annex 1: Case study amoxicillin

The case studies were conducted simultaneously with, and helped the development of the non-experimental approaches. Consultation processes have led to further improvements of the proposed methodologies. The consequence of this is that the case studies may not be completely compatible with the proposed revised methodologies in all aspects. Therefore, the case studies should be seen as an illustration only. Also, the case studies were based on a limited amount of data, gathered from public literature and provided by industry, and consequently sometimes assumptions (e.g. on dose-linearity, half-lives, completeness of distribution at MRL level) were accepted that would normally require a more robust scientific foundation. In conclusion, the case studies only illustrate how non-experimental approaches could work and that these may be helpful in addressing the problem statement explained in Chapter 1.

11.1. Introduction

Amoxicillin is a very commonly used beta-lactam antibiotic in veterinary medicine. In the EU amoxicillin is licensed as various formulations (powder, granules, tablets and suspensions for injection) for a variety of animals (food-producing and non-food producing).

This case study shall be limited to the oral administration of amoxicillin to pigs, by medicated drinking water.

The reason for selecting amoxicillin for dose review is based on the fact that there are different approved dosage recommendations for the same or similar products in the EU. However, the CVMP did not look for and therefore does not have and is not aware of any evidence to suggest that the currently approved doses are not effective under conditions of field use.

Amoxicillin is a broad-spectrum, semisynthetic aminopenicillin antibiotic with bactericidal activity. Amoxicillin binds to and inactivates penicillin-binding proteins (PBPs) located on the inner membrane of the bacterial cell wall. Inactivation of PBPs interferes with the cross-linkage of peptidoglycan chains necessary for bacterial cell wall strength and rigidity. This interrupts bacterial cell wall synthesis and results in the weakening of the bacterial cell wall and cell lysis.

Amoxicillin is usually available as amoxicillin trihydrate.

The approved doses vary widely between 10–20 mg/kg bw, to be given once or twice daily for 3-7 consecutive days. Most commonly a daily dose of 10–20 mg/kg bw is recommended for 3-5 days. It should be noted that the dose can be expressed in amoxicillin base or amoxicillin trihydrate. The conversion factor to the trihydrate is 1.15 and to amoxicillin 0.87.

Licensed products are indicated for a wide variety of infections of the respiratory, gastro-intestinal and uro-genital tract as well as skin and joint diseases. This case study will focus on the indication for respiratory disease which is most commonly caused by *Actinobacillus pleuropneumoniae*, *Glaesserella (previously Haemophilus) parasuis*, *Pasteurella multocida*, *Streptococcus suis* and *Bordetella bronchiseptica*.[1]

[1] From the clinical signs of the disease no firm conclusion can be drawn to the causative agent apart from typical influenza virus infections (peracute-acute disease, rapid sprading) or an acute *Actinobacillus pleuropneumoniae* infection by a highly virulent strain (acute outbreak, circulation problems, bloody froth, quick spreading - pers. communication K.-H. Waldmann, 2017). Thus, from a clincial perspective, swine respiratory disease is often a mixed infection whereby the causative pathogen cannot be readily identified form the clinical signs. *Bordetella bronchiseptica* can cause monocausal infections although this is rather uncommon.

11.2. Dose review and adjustment

11.2.1. Determination of the PK parameters

- Summary of PK data from published literature

PK parameters can be derived from published papers and available information in marketing authorisation dossier (Annex 4). For the purpose of the pilot study, a review of published papers was performed (Table 4).

Table 4. Overview of published scientific papers for amoxicillin

Reference	Intravenous administration dose (mg/Kg)	Oral administration dose (mg/Kg)
Agersø and Friis (1998a)	9	10
Agersø and Friis (1998b)	9	
Martinez-Larranaga et al. (2004)	20	20
Hernandez et al. (2005)	15	15
Reyns et al. (2009)	20	20
Godoy et al. (2011)	15	5/9/10/15/18
Krasucka and Kowalski (2010)		28

The pharmacokinetic parameters extracted from the papers are the mean value and standard deviation of the clearance, the bioavailability and the apparent clearance. An overall mean and standard deviation for each parameter were calculated from the pool.

Equation 3. $$mean_{all} = \frac{\sum mean_i \times N_i}{\sum N_i}$$

Equation 4. $$SD_{all} = \sqrt{Var_{all}} = \sqrt{\frac{\sum (Var_i \times (N_i - 1))}{\sum (N_i - 1)}}$$

Where $mean_{all}$ is the mean of the pool, $mean_i$ the mean reported for the i^{th} study, Var_{all} the variance of the pool, var_i the variance for the i^{th} study.

- For amoxicillin in pigs, clearance is 0.5 ± 0.18 $L.h^{-1}.kg^{-1}$ and oral bioavailability is 0.33 ± 0.12.
- The free fraction of amoxicillin in plasma was set at a mean value of 0.7 ranged 0.6 to 0.8.

The availability of PK raw data or in this case study, the summary of PK parameters allows performing a meta-analysis for a given product using a non-linear mixed effect model (Figure 14 and Table 5). This approach allows integrating variability of biological origin (e.g. breed, sex, age, health status) and non-biological origin (e.g. study design, tested dose).

In a peer reviewed paper (Rey et al., 2014), amoxicillin concentrations in function of time were obtained from four different sources (three pharmaceutical companies, one academic laboratory). Five formulations administered by oral routes were analysed and a common pharmacokinetic model was established. It is a two-compartment model with a zero-order input rate (K0) between lag time (Tlag) and end time (Tend).

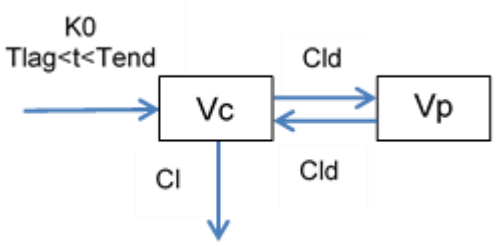

Figure 14. Diagram of pharmacokinetic model for amoxicillin administered orally to pigs. Cl= clearance of elimination, Vc= Volume of central compartment, Vp=Volume of peripheral compartment, Cld=Clearance of distribution.

The data were analysed using software for non-linear mixed effect model. A covariate analysis was performed taking into account the formulation as the main covariate able to account for the individual intervariability. A diagonal Ω matrix was assumed.

Table 5. Pharmacokinetic parameters obtained for a population pharmacokinetic model for 5 formulations of amoxicillin administered orally in pigs at 20 mg/kg bw. Population geometric mean.

Model/Formulation	M1	M2	M3	M4	M5	CV %
Lag time (h)	0.094	0.194	0.194	0.194	0.194	40.3
Duration of the zero order of absorption (h)	1.73	1.73	1.73	6.23	1.73	29.9
CL/F (L/kg/h)	3.1	3.1	1.55	3.1	1.55	23.4
Cld/F (L/kg/h)	0.297	0.297	0.297	0.297	0.297	98.1
Vc/F (L/kg)	3.54	3.54	3.54	3.54	3.54	34.6
Vp/F (L/kg)	3.56	3.56	3.56	3.56	3.56	66.4
AUC24 (mg.h/L)	6.32	6.32	12.34	6.33	12.34	
T≥0.1 µg/ml	5.57	5.57	12.1	9.00	12.1	

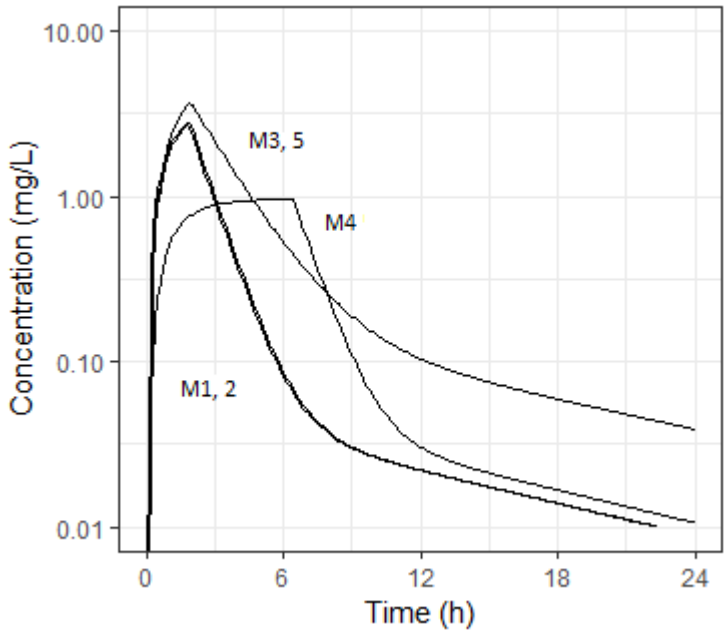

Figure 15. Simulation of a dose of 20 mg/kg based on mean parameters for the 5 formulations presented in table 5 (based on Rey et al. (2014)).

In the original publication, the target for the T>MIC was set at 40% of a period of 24h. Figure 15 shows the simulation obtained with the PK model for the mean value parameter of each formulation. The parameters of formulation 2 were chosen for the pilot study because they represent the worst-case scenario in terms of exposure (AUC and T>MIC).

11.2.2. Define the target bacteria

The therapeutic indication targeted is swine respiratory disease with the following list of targeted pathogens.

- *Actinobacillus pleuropneumoniae,*
- *Bordetella bronchiseptica,*
- *Glaesserella (Haemophilus) parasuis,*
- *Pasteurella multocida,*
- *Streptococcus suis*

The amoxicillin MIC distributions for these pathogens were derived from the CEESA VetPath survey (de Jong et al., 2014; El Garch et al., 2016) which corresponds with isolates obtained from acute respiratory disease cases from 9 EU countries between 2002 and 2012. The MICs distribution of the two studies where merged in order to increase the numbers of strains for each target pathogens, this will increase the accuracy of the distribution used for the PD component of the modelling.

Table 6. Merged amoxicillin MIC distribution frequencies of swine respiratory target pathogens isolates from the EU (de Jong et al., 2014; El Garch et al., 2016)

MIC (µg/mL)	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128
P. multocida (n=382)		1	56	290	26	2		1				2	4
A. pleuropneumoniae (n=378)		54	36	145	113	2	1	1	2	2	7	3	12
H. parasuis (n=68)	23	21	10	10	3						1		
B. bronchiseptica (n=118)								1	14	64	21	9	9
S. suis (n=333)	226	92	4	7	3						1		

The mode of action of amoxicillin is considered as time dependent as for other compounds of the class of betalactams.

11.2.3. Define the PK/PD index

For amoxicillin, two PDI were investigated in the peer-reviewed scientific papers, the AUC/MIC (Lees et al., 2015) and T>MIC (Rey et al., 2014)**.** According to the process previously described, the point of departure will be the definition of a daily dose using AUC/MIC and T>MIC will be used to refine the dosage regimen.

11.2.3.1. AUC/MIC

When the efficacy of the antibiotic is correlated with the AUC_{24h}/MIC, the following equation gives the relationship between the target concentration and the threshold value of the PDI:

Equation 5. $$C_{Target} = \frac{\left(\frac{AUC}{MIC}\right)_{Critical\ value}}{24} \times \frac{MIC}{f}$$

Where $\left(\frac{AUC}{MIC}\right)_{critical\ value}$ is the critical value of the PDI expressed in hours, *f* is the free unbound fraction of the antibiotic in plasma, MIC the minimal inhibitory concentration for the bacteria targeted by the treatment.

When combining Equation 5 with Equation 1, it allows calculating the daily dose necessary to maintain an antibiotic level of exposure reaching the PK/PD value targeted.

Equation 6. $$Daily\ Dose = \frac{Clearance}{Bioavailability} \times \frac{MIC}{f} \times \left(\frac{AUC}{MIC}\right)_{Critical\ value}$$

Different values of the AUC/MIC indices are described (Lees et al., 2015). They vary according the antibacterial effect (bacteriostatic, bactericidal) and the clinical context (clinical burden, immune response). Values for the PDT were derived from a study performed in calf with amoxicillin against Pasteurellaceae (Lees et al., 2015). They correspond to 3 different levels of activity against bacterial strains observed determined from *in vitro* time kill curves.

Table 7. Target value of PK/PD AUC/MIC for amoxicillin and mean plasma concentration at steady state (Css) (based on Lees et al. (2015)).

AMOXICILLIN			
Target	**Bacteriostatic**	**Bactericidal 2-log reduction of bacterial population**	**Bactericidal 4-log reduction of bacterial population**
AUC24h/MIC	28	45	60
Mean Css	1.2 x MIC	2 x MIC	2.5 x MIC

11.2.3.2. Time above the MIC - T>MIC

Amoxicillin belongs to the class of beta-lactams and the time to maintain the MIC is considered as a good predictor of efficacy. For amoxicillin in pigs, a study was performed to investigate the Monte-Carlo simulation to analyse the distribution of time to maintain different values of MIC and different dosage regimen (Rey et al., 2014). For the pilot study, we applied this approach for comparison with the simplest one (being AUC/MIC). To estimate the T>MIC, it is necessary to simulate the concentration in function of time to sum the period dt of time where C(t) is higher than MIC using a PK model.

Equation 7. $$T > MIC = \int_0^{24} I \times dt$$

Where I=1 if C(t)≥MIC and I=0 if C(t)<MIC.

11.2.4. Set a target value for the PDI

According to Mouton *et al.*, for antibacterial agents where efficacy is primarily correlated with the %fT>MIC, such as beta-lactams, the PK/PD breakpoint can be derived directly from a PDT such as 40% (static PDT) to 60% (1-2 log reduction) over a period of 24h (Mouton et al., 2012).

Table 8. Summary of the PDI and PDT for amoxicillin (based on Lees et al. (2015)).

	Bacteriostatic	**Bactericidal (2 log reduction)**
AUC/MIC*	28	45
T>MIC**	40%	60%

* These targets are defined from one peer-reviewed paper and derived from *in vitro* studies.

** These targets are defined from a general consensus in human medicine about beta-lactam PDI.

11.2.5. Model of the relationship between dose and PDI target attainment

11.2.5.1. AUC/MIC

For amoxicillin in pigs, clearance is 0.5 ± 0.18 $L.h^{-1}.kg^{-1}$ and oral bioavailability is 0.33 ± 0.12.The free fraction of amoxicillin in plasma was set at a mean value of 0.7 ranged from 0.6 to 0.8. The Monte Carlo Simulation was performed with @Risk software. The model was used to determine the Probability of Target Attainment for the PDIs for a daily dose of 10, 20 and 40 mg/kg bw for different values of MICs ranging from 0.025 to 128 µg/mL. The following figure reports the probability of attainment of the PDT in function of the distribution of MIC for the targeted bacteria.

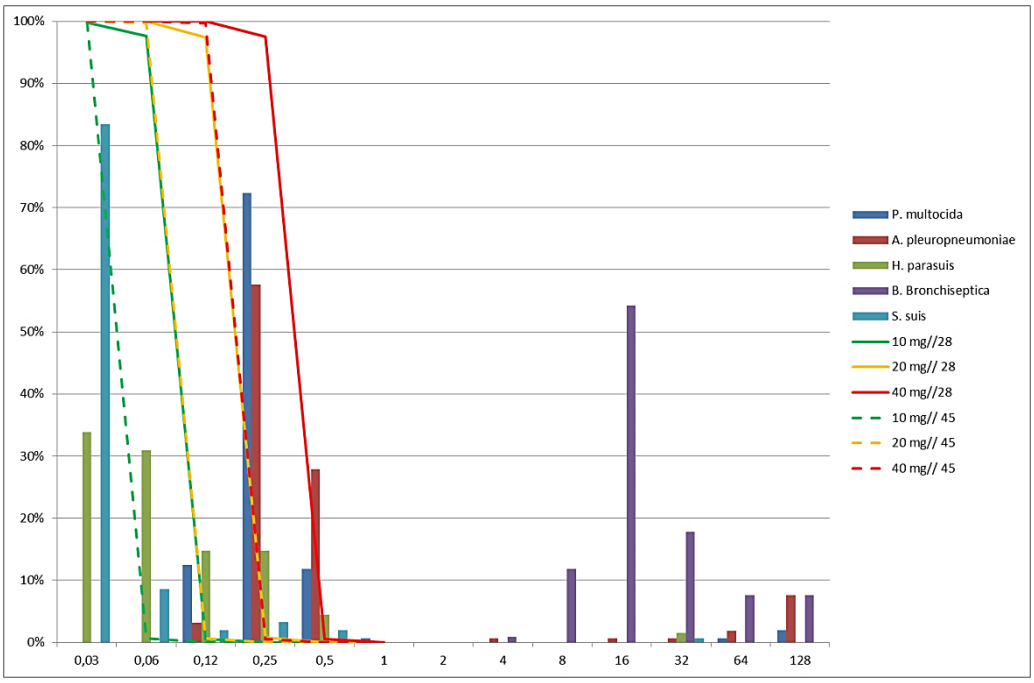

Figure 16. Graphic representation of probability of target attainment for different daily dose (10, 20, 40 mg/kg bw) according to target value of the PDI (AUC/MIC) according MIC levels and the MIC distribution for the targeted bacteria

The three doses tested (10, 20 and 40 mg/kg bw) have a dramatic low probability to reach the PTA of 90% for strains with MIC above 1 µg/mL. Then, we investigated the PTA for bacterial species corresponding to most of the strains with a MIC equal or lower than 1 µg/ml.

The three doses have a probability of target attainment higher than 90% for *S. suis* for both bacteriostatic and bactericidal activity. The doses of 20 and 40 mg/kg bw are able to achieve a PTA above 90% for *H. parasuis* only for a bacteriostatic activity. To achieve a bactericidal activity a dose of 40 mg/kg bw is required. For *P. multocida* and *A. pleuropneumoniae,* a dose of 40 mg/kg bw leads to a bacteriostatic activity with a simulated PTA around 90%. With the proposed dose and due to the high MIC values for *B. bronchiseptica,* amoxicillin never reaches the PK/PD objectives for this target pathogen. *B. bronchiseptica* should be deleted from the therapeutic indication of amoxicillin administered by the oral route to pigs when one is adjusting the dose.

Table 9. Overview of probability of target attainment according to target value of the PDI (AUC/MIC) and the MIC distribution for the targeted bacteria and for different daily dose. *Red font: daily dose reaching the highest PTA for the different target pathogens considered according to PDT values (AUC/MIC).*

PDI	Bacteriostactic = 28			Bactericidal = 45		
Daily dose	10 mg/kg	20 mg/kg	40 mg/kg	10 mg/kg	20 mg/kg	40 mg/kg
P. multocida	27%	69%	95%	9%	39%	81%
A. pleuropneumoniae	31%	61%	88%	18%	39%	70%
H. parasuis	78%	91%	98%	65%	83%	94%
S. suis	94%	97%	99%	92%	94%	97%

Table 10. Merged amoxicillin MIC distribution frequencies of swine respiratory target pathogens isolates from the EU (de Jong et al., 2014; El Garch et al., 2016)

MIC (µg/mL)	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128
P. multocida **(n=382)**		1	56	290	26	2		1				2	4
A. pleuropneumoniae **(n=378)**		54	36	145	113	2	1	1	2	2	7	3	12
H. parasuis **(n=68)**	23	21	10	10	3						1		
B. bronchiseptica **(n=118)**								1	14	64	21	9	9
S. suis **(n=333)**	226	92	4	7	3						1		

* ECOFF values are determined using the tool ECOFFinder to calculate the 99.9th percentile of ECOFF (Turnidge et al., 2006). In the context of this pilot project, all the requested criteria may not be fulfilled to use these tools with confidence, however in order to follow the methodology defined in section 3.3, the ECOFF of the different target pathogens was calculated. ECOFF value is for *P. multocida* 0.5 µg/mL, for *A. pleuropneumoniae* 2 µg/mL, for *B. bronchiseptica* 64 µg/mL and for *S. suis* 0.06 µg/mL. For *H. parasuis* an ECOFF of 0.0625 µg/mL can be calculated but the value is given only as an example in the context of this pilot project as the minimal number of strains is not reached.

To perform a modelling for dose calculation, two different values for the PD parameters can be selected, (i) a single MIC values corresponding as for example to CBP, ECOFF or MIC_{90} or (ii) a distribution of MICs of the target pathogens. The impact of the PD value on the dose calculated was previously investigated in an ANSES report. The result indicates that the dose values calculated using the MIC distribution were always lower than those obtained with the selected MIC point values (CBP, ECOFF or MIC_{90}). Indeed, when we use a single MIC, we assume that 100% of the strains have the same MIC leading to an overestimate of the dose needed to reach the strains with a lower MIC and underestimate the dose needed for strains with a higher MIC. In this pilot project, according to the observations made in the ANSES report, the whole distribution of MICs for each species was used to estimate the dose covering 90% of the AUC/MIC target (ANSES, 2017). They were investigated to estimate the highest dose required to reach a probability of target attainment of 90 % for the susceptible wild type distribution.

Table 11. Dose (in mg/kg bw per day) required to reach the different value of AUC/MIC according the expected antibacterial effect.

	P multocida	*A. pleuropneumoniae*	*H. parasuis*	*S. suis*
Bacteriostatic	26	35	17	4
Bactericidal 2-log	43	55	26	7
Bactericidal 4-log	57	73	35	9

According this review, *A. pleuropneumoniae* is considered as the least susceptible target pathogen which can be reached with a daily dose ranged between 35 and 55 mg/kg bw. So for the next step of this case study, a mean daily dose of 40 mg/kg bw will be used.

11.2.5.2. T>MIC

Monte Carlo simulations using the PK parameters of one formulation (Formulation M2, Table 5) described in Rey et al. (2014) were performed using simulX of R software implemented with the package mlxR. For this case study, the model/formulation M2 was selected as the worst case in exposure (lowest AUC_{24h}, lowest T above 0.1 µg/ml) representative to a short duration of a zero order absorption of amoxicillin by pigs after a bolus administration. The % of time over 24 hours to maintain different values of MIC were simulated for 5000 individuals using a time precision of 6 minutes. PTA to maintain concentration above the MIC with the wild type distribution of the susceptible bacterial species were estimated from the simulations of different fractionations of 40 mg/kg bw (5 mg/kg bw per 3 h, 10 mg/kg bw per 6 h, 20 mg/kg bw per 12 h, 40 mg/kg bw per 24 h).

Table 12. Overview of Probability of Target Attainment rate according to target value (9.6h) of the PDI (T>MIC) and the MIC distribution (Table 10) of the susceptible bacterial species for different dosage regimens

	P. multocida	*A. pleuropneumoniae*	*H. parasuis*	*S. suis*
5 mg/kg/3 h	83%	77%	96%	98%
10 mg/kg/6 h	73%	67%	92%	97%
20 mg/kg/12 h	47%	42%	83%	93%
40 mg/24 h	28%	25%	77%	91%

The results of the PK/PD analysis, using T>MIC as a PDI for amoxicillin, show that the PTA increase with dose and dose fractionation (Table 12). A single daily dose of 40 mg/kg bw leads to a T>MIC higher than 40% of 24 h for 28%, 25%, 77% and 92% of simulated PK curves with *Pasteurella multocida, Actinobacillus pleuropneumoniae, Haemophilus parasuis* and *Streptococcus suis*, respectively. The dose of 40 mg/kg bw fractioned as 5 mg every 3 h increases the percentages of animals reaching this target (83%, 77%, 96%, 98%). It should be noted that the latter approach could be compatible with an administration via drinking water and could be viable under field conditions where pigs have *ad libitum* access to water. It can then be concluded that oral administration of amoxicillin by drinking water is a good route of administration allowing a continuous exposure along the day and that an improved daily dose should be set at 40 mg/kg bw to allow an acceptable exposure of the different target pathogens.

- ***Main conclusions on the amoxicillin case study in relation to dose review and adjustment:***

As a reminder to summarise this first case study, by following the different steps, the PK/PD relationship allows to define a dosage regimen taking into account PK and PD variability but also by considering the probability to reach the target value of the selected PK/PD index for a defined drug-bug combination.

For the amoxicillin case study, different conclusions can be drawn:

- **Concerning the dose computed:**
    - Different doses can be computed in function of the therapeutic objective (e.g. Bacteriostatic, Bactericidal 2-log, Bactericidal 4-log);
    - Different doses can be computed in function of the target pathogens MIC distribution. Higher dose should for example, be applied to cover adequately the least susceptible bacterial species.
    - Different doses can be computed for diferent target pathogens. Therefore, it seems that one general dose for all 4 pathogens is not fully relevant (for *S. suis* it seems extra overestimation, for APP it seems underdosing).
- **Concerning the modelling using AUC/MIC or T>MIC as PDI:**
    - When modelling the Probability of Target Attainment (PTA; 90%) according to the selected PDI and MIC, it can be concluded that for T>MIC, the computation of the PDI requires simulation of time-concentrations curves which requires pharmacometric tools. The interest of this approach is to further refine the dosage regime in relation to the way of administration of the treatment. Indeed, the results in Table 12 revealed that fractionation of the dose increases the probability to attain the target value of the PDI. This is mainly due to the short half-life of the active substances.
    - T>MIC provides a better option for defining a precise daily dose for time-dependent antibiotics but it needs then the definition of a frequency of administration by day to guarantee an acceptable exposure.
    - AUC is less precise but allows to define a daily dose allowing a good exposure and thus without taking into account the frequency of administration. The determination of a daily dose reaching the PTA of 90% using T>MIC as a PDI will not be feasible as the computed dose will be too high. The PK/PD analysis using T>MIC as PDI could be used to further refine the interval frequency after the determination of a daily dose using AUC/MIC.
    - The outcome of this pilot exercise, using AUC/MIC, indicates example of the dose calculation for theoretical respiratory disease model in pigs with amoxicillin in drinking water where the dose 40 mg/kg bw seems can cover the bacteriostatic effect on *A. pleuropneumoniae* and bactericidal 2-log for *P. multocida* and *H. parasuis.* For *S. suis* this dose seems to be overestimated. For susceptible strains of *S. suis* dose of 9 mg/kg bw seems to have bactericidal 4-log effect.

A recent paper (Burch and Sperling, 2018) reviewed the use of amoxicillin in swine looking at the various formulations and routes of administrations in regards to clinical efficacy. They considered epidemiological cut-off values in their PK/PD correlation and concluded that an oral dose of 20 mg/kg bw might not be suitable and should be increased. At the same time should be noted that authors of this above cited study also concluded that (cited word for word: "It may be misleading to pursue changes in dose just on PK/PD principles, without taking into account clinical responses,

pharmacovigilance and lack of efficacy reports. A more complete assessment is required, before finite recommendations are implemented regarding dose and indication").

11.2.6. Set a PK/PD breakpoint

The last step of the proposed approach to address doses is the definition of clinical breakpoint, or PK/PD breakpoints when lacking clinical data (cf. chapter 3.3 – step 7). According to the data available for amoxicillin, ECOFFs vary between the targeted bacterial species. In our example, the PK/PD breakpoint could be set at 0.5 µg/mL as the PTA of 90% for strains with MIC above 1 µg/mL is never reached (Figure 16). This value seems compatible with ECOFFs of studied species but with the limitations that our dataset is too small to determine them correctly for all bacterial species (n<300). The highest daily dose tested of 40 mg/kg bw allows to reach a PTA close to 90% when AUC/MIC is used but not with T>MIC for which the PTA value depends on the rate of administration.

11.2.7. Define an improved daily dose

According to the PK/PD modelling done in for this case study, the approved oral daily dose of 20 mg/kg bw is insufficient to sufficiently expose the target pathogens for 24 hours. A recent paper reviewed the use of amoxicillin in swine looking at the various formulations and routes of administrations in regard to clinical efficacy. According to their review, there is no evidence of lack of clinical efficacy with the approved dose of 20 mg/kg (Burch and Sperling, 2018). However, according to the paper of Rey et al. (2014) also mentioned in this review, an oral dose of 20 mg/kg bw might not be suitable as they considered epidemiological cut-off values in their PK/PD correlation and concluded that the dose should be increased (Burch and Sperling, 2018; Rey et al., 2014). Indeed, using AUC/MIC as a PDI, the dose of 20 mg/kg bw is not able to reach a PTA of 90% for the different target pathogens. To achieve this goal, the outcome of this pilot exercise, indicates that the adjusted dose to treat respiratory disease in pigs with amoxicillin in drinking water is 40 mg/kg bw to cover the major pathogens *P. multocida*, *A. pleuropneumoniae*, *S. suis* and *H. parasuis* but can never reach sufficient concentrations to treat *B. bronchiseptica*. However, as amoxicillin is a time dependent antimicrobial where T>MIC is considered as best predictors of clinical efficacy, a second step was applied to refine the daily dose firstly set using AUC/MIC. Using T>MIC, the results show that the PTA increase with dose and dose fractionation (Table 12). Thus, the medication by drinking water represents a good route administration for amoxicillin allowing fractionating the dose of 40 mg/kg bw newly defined, during the day in function of the drinking rhythm and behaviour of the treated animals. Furthermore, when a medicinal product is presented in a solution prior to administration through drinking water, the product's formulation will usually not influence the bioavailability of the active substance (EMA/CVMP, 2000a).

11.3. Withdrawal period

The Withdrawal Periods (WP) of the various products authorised in the EU Member States vary greatly and range from 2 – 28 days (an overview is provided in Annex 5). This overview was generated around 2010 and might not be complete and up to date anymore. However, it is unlikely that major changes have occurred in the meantime. There is no obvious explanation why for some products the WP is rather long or short. In this context it should be noted that most of the products are generics for which no product specific residue depletion studies were usually required[2].

[2] The products are soluble powders which are administered orally via drinking water. For this reason, generic products can make direct reference to the WP of the pioneer product.

Table 13. Selection of amoxicillin products (powder for oral administration via drinking water) for the treatment of respiratory disease in pigs licensed in the EU via the Mutual Recognition procedure for illustration of how the methodology could work

Product	Posology (amoxicillin trihydrate)	Withdrawal Period (WP)
A	16 mg/kg bw per day for 5 days	2 days
B	20 mg/kg bw per day for 5 days	6 days
C	20 mg/kg bw per day for 5 days	14 days
D	20 mg/kg bw per day for 5 days	2 days
E	20 mg/kg bw per day for 5 days	2 days
F	13 mg/kg bw per day for 5 days	2 days

11.3.1. Pharmacokinetics

The pharmacokinetic data described below were derived from literature and data provided by the pharmaceutical industry.

A literature search was done in Scopus(R) (keywords: amoxicillin and pharmacokinetic and pig) which revealed only very few recent studies (> year 2008). For this reason, the pharmacokinetic data were mainly taken from the publication of Schwarz et al. (2008).

Several pharmacokinetic studies were conducted in pigs in which animals were treated with amoxicillin by different routes of administration: intravenous (i.v.), intramuscular (i.m.), or oral. After i.v. administration, amoxicillin is rapidly distributed and eliminated, as suggested by the low values for volume of distribution at steady-state (VDSS) and its low mean residence times (MRT). Different absolute bioavailability percentages were calculated after oral administration, ranging from 11% to 50%, depending on the formulation type and administration under fed or fasting conditions (JECFA, 2011).

A GLP-compliant comparative cross-over trial was performed in pigs treated with amoxicillin by i.v., i.m. and oral routes, in order to investigate the bioavailability of various product formulations. Absorption of amoxicillin after oral administration was slow and incomplete (Agersø and Friis, 1998a). The C_{max} value of 1.6 mg/ml was observed in fasted pigs after 1.9 h., while a lower peak concentration of 0.8 mg/ml was reached after 3.6 h in fed pigs (Agersø and Friis, 1998a). Oral bioavailability was only 31% in fasted animals and 28% in fed animals. The reported differences in bioavailability, C_{max} and the time to maximum serum concentration (t_{max}) were not statistically significant. A comparative overview of the pharmacokinetics of amoxicillin in pigs after administration is presented in Table 14 (Schwarz et al., 2008). In the cited studies differences in PK-parameters were quite large, which might be caused by differences between formulations.

Table 14. Comparative description of amoxicillin pharmacokinetic parameters in pigs after oral administration (in feed or drinking water) of different formulations of amoxicillin at different doses. (copied from Schwarz et al. (2008))

Preoral administration	T_{max} (h)	C_{max} (µg/ml)	AUC (mg/h/l)	Vss (l/kg)	MRT (h)	Cl_B (l/h/kg)	Bioavailability (F)
Anadon et al. (2000)* dose: 20 mg/kg	0.96±0.18	6.76±0.67	25.2±3.6	1.81±0.23	n.d.	0.3±0.03	0.39±0.08
Anfossi et al. (2002) dose: 50 mg/kg microgranular formulation**	2.5±1.37	4.2±2.41	18.9±9.18	n.d.	4.01±0.84	n.d.	n.d.
Anfossi et al. (2002) dose: 50 mg/kg microgranular formulation**	1.78±0.36	3.36±1.36	14.15±5.43	n.d.	4.02±0.75	n.d.	n.d.
Anfossi et al. (2002) dose: 50 mg/kg**	2.06±1.63	2.85±0.74	12.11±2.4	n.d.	3.86±0.81	n.d.	n.d.
Hernandez et al. (2005) dose: 15 mg/kg**	5.8±2.3	0.76±0.05	n.d.	n.d.		n.d.	0.11±0.05
Martinez-Larranaga et al. (2004) dose: 20 mg/kg**	0.97±0.29	7.37±0.42	27.4±4.93	1.35±0.2	4.47±0.30	n.d.	0.41
Morthorst (2002)* dose: 20 mg/kg***	0.55±0.85	21.6±34.5	21.4±12.9	n.d.	n.d.	n.d.	0.98

* Oral administration not defined
** in feed
*** in drinking water

The most recent studies available since 2008 are briefly summarised below. In summary, the pharmacokinetic parameters assessed and evaluated where broadly in line with what has been published before.

Godoy et al. (2011) made a comparative pharmacokinetic assessment of amoxicillin given to healthy pigs and pigs suffering from respiratory disease. After single intravenous bolus administration of amoxicillin to healthy pigs, the VDSS was 0.61 l/kg, total plasma clearance was 0.83 l/h/kg and MRT 0.81 h. After oral bolus administration, the mean absorption time was 1.6 h and the peak plasma concentration of 3.09 µg/ml was reached after 1.2 h. The oral bioavailability was 34%. Pharmacokinetic parameters calculated (C_{maxss}, C_{minss}, C_{avss} and AUC_{24ss}) were significantly lower in healthy pigs in comparison to diseased pigs. This was due to higher bioavailability and longer absorption period observed in diseased pigs. Dose linearity was demonstrated in diseased pigs over a dose range of 4-18 mg/kg bw.

Menotta et al. (2012) compared the bioavailability of a coated amoxicillin to an uncoated formulation in pigs. Oral bioavailability of the formulation with coated amoxicillin was higher than with uncoated amoxicillin, AUC was significant higher and there were statistically significant differences in C_{max}, Time to C_{max} (T_{max}) and MRT. That confirms that the galenics of the formulation may have a significant effect on the pharmacokinetic profile. However, for conventional oral formulations in drinking water (powder

and granules) a difference in oral bioavailability is not expected, because of the good solubility of amoxicillin trihydrate in water.[3]

Dai et al. (2017) conducted a relative bioavailability study of an oral amoxicillin-apramycin combination in pigs. The study was done in a three-way cross-over design comparing the pharmacokinetics of amoxicillin and apramycin either as single components, or as combination product. The test articles were given intra-gastrically to fastened pigs at a dose of 16 mg/kg bw amoxicillin. There was no difference in the pharmacokinetic profile of amoxicillin whether administered alone or in combination with apramycin. Of interest are the basic pharmacokinetics parameters for amoxicillin obtained in this study. The peak plasma concentration was reached after 1.92 h with a C_{max} of 3,25 µg/ml and $AUC_{0-\infty}$ of 8.43 mg/h/l. The MRT was 3.43 and $T_{1/2}$ was 6.33 h in plasma.

In addition, several pharmacokinetic studies were made available from industry. Following, only the key findings are briefly reported.

A pilot study was set up to investigate the plasma pharmacokinetics of amoxicillin-trihydrate in eight 14-week old pigs after single (pulse) oral administration of a soluble powder, first through feed and two weeks later through drinking water. Two dosages, i.e., 14.5 and 29 mg amoxicillin/kg bw were tested. When administered in combination with pelleted feed, absorption of amoxicillin was somewhat delayed as indicated by the T_{max} of about 2.25 h and the terminal half-life in plasma of about 1.1 h for the 14.5 mg/kg bw dose and 1.7 h for the 29 mg/kg bw dose. These values are higher than the corresponding values observed after administration in water. This indicates that absorption is the rate limiting step for elimination. The maximum plasma levels obtained do not linearly increase with the dose, i.e., 1.0 and 1.25 mg amoxicillin per animal. This is also indicated by the observed area under the curve (AUC) for the two dosages, which tend to be somewhat lower for the higher dosage. The plasma-concentration profiles show that amoxicillin is rapidly absorbed after administration in drinking water as indicated by the observed T_{max} of about 0.75 h and the terminal half-life of 0.5 to 1.0 h in plasma, suggesting that rate of absorption is not limiting for elimination. This is also indicated by the observed AUCs for the two dosages, which are proportional and represent more than 99% of the total extrapolated AUC at 7.25 h after consumption of the dose. The maximum plasma levels obtained show a roughly linear increase with the dose, with C_{max} values of 1.5 and 2.7 mg amoxicillin per animal for the 14.5 mg/kg bw and 29 mg/kg bw dose, respectively.

In a second pilot study the pharmacokinetics of amoxicillin was assessed after repeated administration. Eight 14-weeks old pigs were divided into two medicated groups of four animals. Group I received a continuously administered daily dose of 8.0 mg amoxicillin/kg bw, mixed through the daily ration of drinking water for three consecutive days. Group II similarly received an oral dose of 16.0 mg amoxicillin/kg bw mixed through the daily ration of drinking water. Two weeks after the continuous medication, the animals received a single pulse dosage of 10. 0 or 20.0 mg/kg bw per day respectively. The average plateau plasma levels were ranging between 0.2 and 0.4 µg/ml after dosing of 10 mg/kg bw per day and between 0.3 and 0.7 µg/ml after the daily dosage of 20 mg/kg bw. After daily single pulse dosing peak plasma levels ranging from 0.7 to 1 µg/ml for the 10 mg/kg bw dose, and from 1.1 to 2.1 µg/ml for the 20 mg/kg bw dose were obtained.

Further data were provided by Company B (1) which are summarised in the two figures below. Only arithmetic means and standard deviations were available. Since the final elimination phase is assumed to be log-linear, for the determination of half-lives or withdrawal periods geometric means would be more appropriate, as they correspond to arithmetic means of log-transformed data.

[3] Data from a solubility study indicated that amoxicillin trihydrate (product; amoxicillin 80% oral powder) is soluble in water of different qualities (soft / pH=5; hard / pH=8) and temperatures (20 ºC; 5 ºC) in the concentration of 1 g in 600 ml of water (Company A).

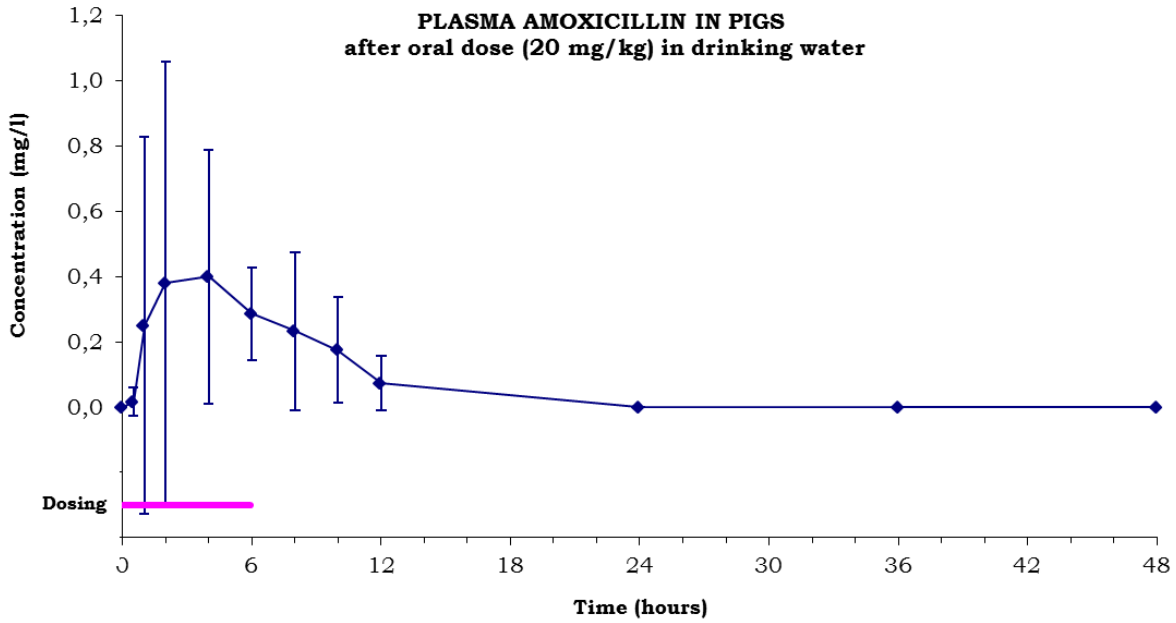

Figure 17. Amoxicillin plasma concentrations in pigs after a single oral dose. Arithmetic mean values and standard deviation (+/-) are shown

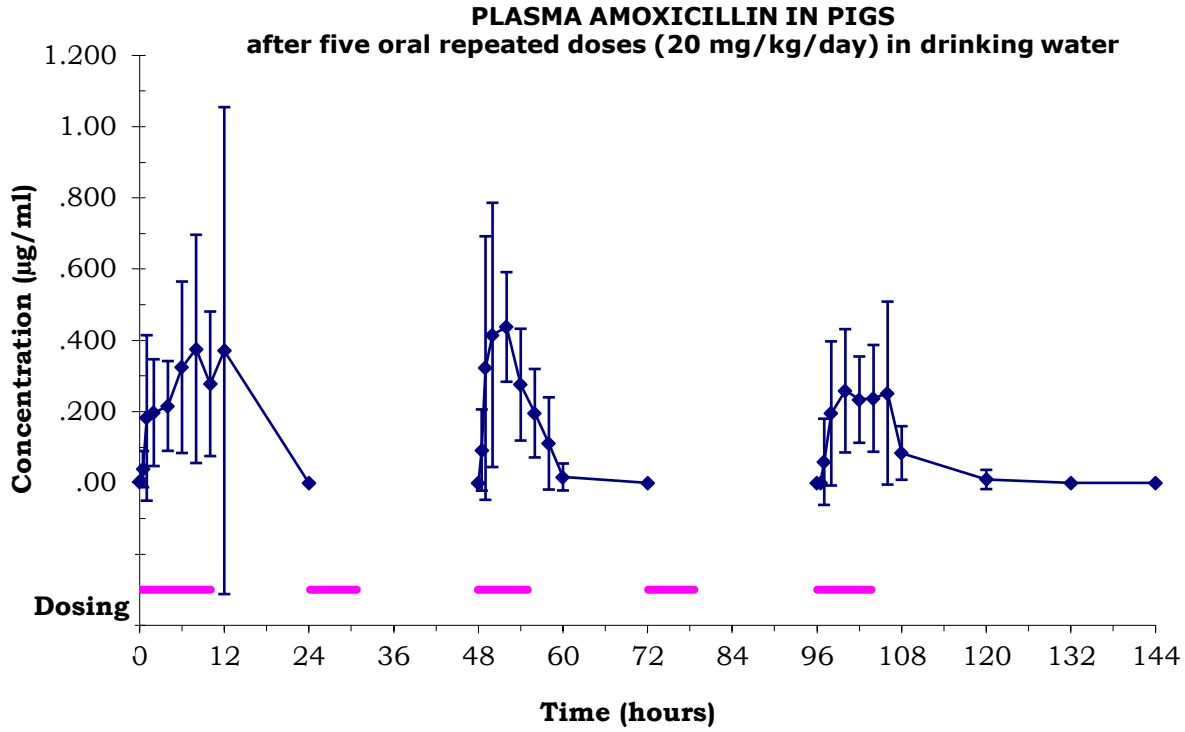

Figure 18. Amoxicillin plasma concentrations in pigs after repeated dosing. Arithmetic mean values and standard deviation (+/-) are shown

11.3.1.1. Dose linearity

One of the limiting conditions for using the proposed extrapolation method to calculate a withdrawal period is that linear kinetics must apply in the WP determining tissue. From studies in pigs and human,

dose linearity was not always seen and it appears that it is limited by a saturated absorption (de Velde et al., 2016).

The various studies assessing dose linearity are briefly described below.

Godoy et al. (2011) established a dose linearity in plasma for amoxicillin in diseased pigs from 4 to 18 mg/kg bw, at steady state (ss) for C_{maxss}, C_{minss} and C_{avss} (average concentration at steady state), as well as linearity of amoxicillin absorption as reflected by a constant AUC/dose ratio. The authors of the paper discussed that the dose-linearity shown is not in agreement with results from other papers, which in their opinion is attributable to nonlinear absorption, and they stated that the dose level which saturates the absorption in pigs is not clear.

Rey et al. (2014) referred in his paper to the study of Godoy et al. (2011) worked under the dose linearity assumption and this is also referred to by ANSES (2017).

A comparative pharmacokinetic study was conducted by Company B(1) in pigs comparing a dose of 5 mg/kg bw, 10 mg/kg bw and 20 mg/kg bw. Dose linearity in plasma across the three dosages was assessed based on the data are depicted below in Figure 19 and Figure 20.

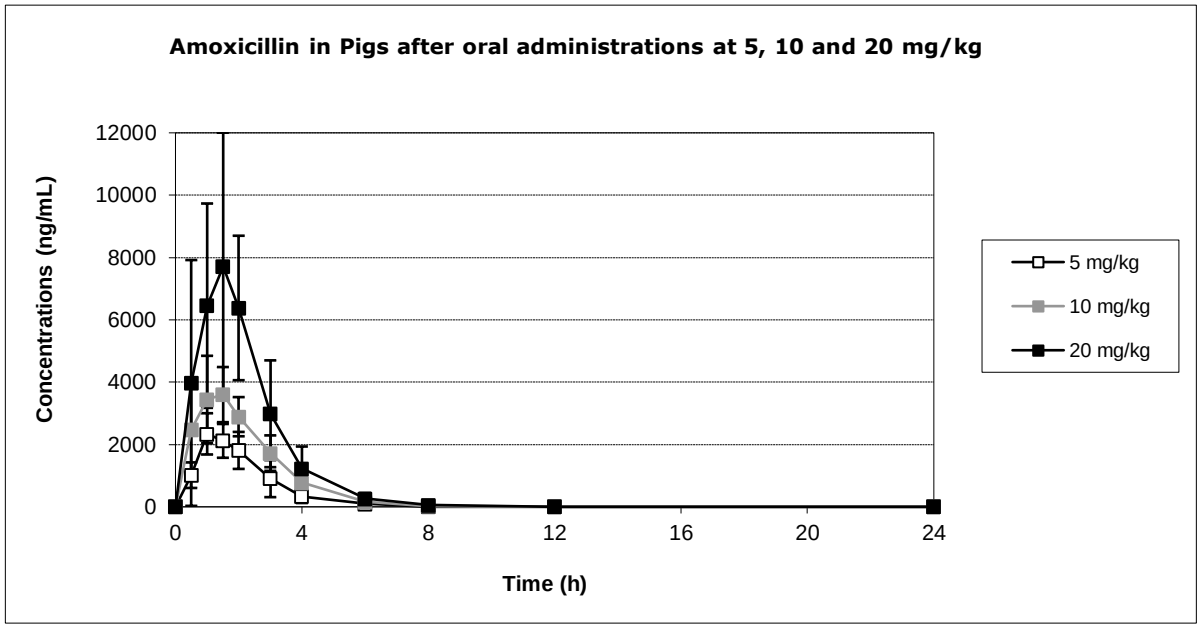

Figure 19. Amoxicillin plasma concentrations at three different dose levels. Arithmetic mean values and standard deviation (+/-) are shown

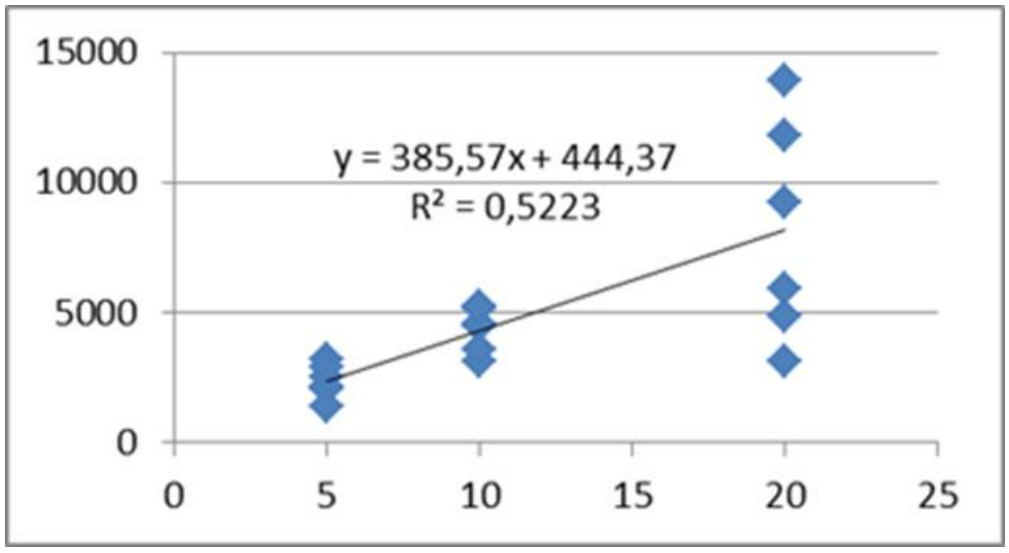

Figure 20. Individual amoxicillin plasma concentrations (C_{max}) at three different dose levels (5 mg/kg bw, 10 mg /kg bw and 20 mg/kg bw). X-axis: dose (mg/kg bw); Y-axis: plasma amoxicillin concentrations (ng/ml)

Visual inspection of **Figure 20** suggest that dose linearity might be given for doses from 5 mg/kg bw up to 20 mg/kg bw.

Data from humans clearly state a dose linearity of amoxicillin 250 mg capsules GP over a range of 250-3000 mg. Data in humans may also be considered because of the very similar gastro-intestinal tract system between the two species[4].

[4] (https://www.medicines.org.uk/emc/medicine/25916)

11.3.1.2. Overall Summary of Pharmacokinetics

Studies have shown that the oral bioavailability of amoxicillin can be quite variable which is associated with different formulations and different methods of oral administration (gavage, fasted vs. non-fasted pigs, food-interaction). Bioavailability in diseased animals is also significantly higher than in healthy animals. No data up to 40 mg/kg are available- for this case study, it is assumed that the bioavailability will not change.

Regarding C_{max}, studies have demonstrated a dose-linearity relationship in plasma between 5 and 20 mg/kg bw. For the purpose of this example it is assumed, that dose-linearity is given for doses up to 40 mg/kg and that it is also given in the various tissues considered. Both are crucial prerequisites for the applicability of Equation 2. Unfortunately, in this example, there is not enough data available to verify this assumption.

Plasma protein binding of amoxicillin has been described to be 28% and can be considered to be low.

Due to lack of respective data it is assumed that the final elimination phase is log-linear in each tissue; generally, this has to be made plausible.

11.3.2. PK/PD Considerations

Using PK/PD modelling methods, within this pilot project, an improved dose of 40 mg/kg bw could be calculated (see above). This dose will be used in the section of this case study that considers the extrapolation of the withdrawal periods.

11.3.3. Metabolism

The two major metabolites of amoxicillin are amoxicilloic acid and amoxicillin piperazine-2,5-dione (diketopiperazine). These metabolites have lost the antibacterial activity of the parent component, but the amoxicilloic acid could have potential allergic properties. The metabolites are of no relevance for the purpose of this case study.

The CVMP did not establish an ADI for penicillins but stated: "In connection with therapeutic use of penicillins hypersensitivity reactions are by far the most commonly encountered side-effects…. At least 6 µg penicillin (equivalent to 10 IU penicillin) seems normally necessary to provoke an allergic reaction."

A microbiological Acceptable Daily Intake (ADI) has been established by JECFA for amoxicillin, and this ADI covers the allergic risk associated with these two metabolites displaying almost nil antibacterial activity.

11.3.4. Radiolabelled residue depletion studies

There were no amoxicillin radiolabel residue depletion studies in pigs available for evaluation.

11.3.5. Maximum Residue Limits

The CVMP did not establish an ADI for penicillins. In order to adequately protect the consumer and secure dairy production, the CVMP recommended the following maximum residue levels for six penicillins:

Table 15. EU Maximum Residue Limits for penicillins

Pharmacologically active substance	Edible Tissues (µg/kg)	Milk (µg/kg)
Benzylpenicillin	50	4
Ampicillin	50	4
Amoxicillin	50	4
Oxacillin	300	30
Cloxacillin	300	30
Dicloxacillin	300	30

JECFA (2011) and JECFA (2017) assessed amoxicillin at their 75th meeting in 2011 and their 85th meeting in 2017 and came to the following conclusions:

- An ADI of 0–0.002 mg/kg bw was established by the Committee based on a microbiological endpoint, equivalent to an upper bound value of 0.12 mg for a 60 kg person.
- The Committee recommended MRLs for amoxicillin in cattle, sheep, pig and finfish tissues of 50 µg/kg and in cattle and sheep milk of 4 µg/kg, determined as amoxicillin parent compound. The Committee determined also an Acute Reference Dose and a Global Estimated Acute and Chronic Dietary Exposure.
- It is assumed, that tissue distribution is complete at MRL-level

11.3.6. Tissue residue studies

Only few residue depletion studies in pigs are available. JECFA (2011) reviewed data from 1979 where amoxicillin was given orally as an oily suspension. Amoxicillin was eliminated very quickly and no residue depletion profile could be established in tissues and organs. It was concluded that for many studies in all species assessed, namely cattle, pigs and poultry, the sampling time intervals were too long to permit a detailed analysis of residue depletion in tissues and, consequently, there are a substantial number of reported findings <LOQ (limit of quantification).

The same conclusions apply to the study published by Reyns et al. (2008). Residue depletion of amoxicillin residues occurred rapidly and residues were below the limit of detection (LOD) already 48 h after last administration of 20 mg/kg bw amoxicillin administered once by gavage (stomach tube).

A non-GLP residue depletion study was conducted in Belgian Landrace stress-negative pigs. Twenty animals received an i.v. bolus of amoxicillin at a dosage of 20 mg/kg bw through a catheter in an ear vein. Animals (n=4) were killed at 12, 48, 60, 72 and 84 h post-dosing. Amoxicillin and its major metabolites, amoxicilloic acid and amoxicillin diketopiperazine, were quantified in kidney, liver, fat and muscle tissues. Similarly, 20 animals received the same dose of amoxicillin by oral administration through a stomach tube. Samples were collected at the same time points (Reyns et al., 2008). Table 16 summarizes the data obtained. Twelve hours after both oral and i.v. administration, amoxicillin concentrations in kidney samples were relatively high, but decreased rapidly, and 36–48 h after treatment, amoxicillin concentrations were below the LOQ of 25 µg/kg in all tissue samples. The amoxicilloic acid metabolite remained much longer in kidney tissue and also in liver, consistent with other *in vivo* residue depletion tissue studies in pigs (De Baere et al., 2002).

Table 16. Mean tissue concentrations (ng/g) (and standard deviations) of amoxicillin (AMO), amoxicilloic acid (AMA) and amoxicillin diketopiperazine (DIKETO) in pig tissue after i.v. and oral administration of amoxicillin at 20 mg/kg bw (from Reyns et al. (2008))

Time and route of administration									
Tissue	**Chemical**	**12h**		**48h**		**60h**		**72h**	**84h**
		oral	**i.v.**	**oral**	**i.v.**	**oral**	**i.v.**		
Kidney	AMO	618 (359)	915 (148)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	AMA	10132(1) (3096)	5575(1) (744)	205(115)	100 (79)	213 (115)	120 (40)	<LOD	<LOD
	DIKETO	88 (61)	47 (23)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Liver	AMO	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	AMA	1 379(2) (201)	546(2) (198)	35 (14)	<LOQ	42 (24)	<LOQ	<LOD	<LOD
	DIKETO	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Fat	AMO	<LOQ	39 (20)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	AMA	127 (68)	118(66)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	DIKETO	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Muscle	AMO	<LOQ	35 (18)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	AMA	30 (17)	32 (22)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	DIKETO	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Notes: LOD= 1.7, 7.1 and 2.0µg/kg for AMO, AMA and DIKETO, respectively, in pig kidney; 3.5, 14.2 and 1.6µg/kg for AMO, AMA and DIKETO, respectively, in liver; 1.5, 11.1 and 0.9µg/kg for AMO, AMA and DIKETO, respectively, in muscle; and 1.7, 10.6 and 0.8 for AMO, AMA and DIKETO, respectively, in fat. LOQ at least 25µg/kg for all components in all tissue matrices. (1) Significant at P= 0.025. (2) Significant at P= 0.0001

Martinez-Larranaga et al. (2004) performed a study in twelve pigs treated with daily oral doses of 20 mg/kg bw amoxicillin for five days. The mean residue concentration (n=4) of amoxicillin in kidneys was 21.4 µg/kg six days after administration of the last dose and in liver residues were 12.3 µg/kg. No amoxicillin could be detected in fat or muscle at that time point. The data are shown in Table 17 and Figure 21.

Table 17. Arithmetic mean (sd) plasma concentrations (µg/ml) and tissue concentrations (µg/kg) of amoxicillin in four pigs given 20 mg/kg amoxycillin orally for five days (copied from Martinez-Larranaga et al. (2004))

Tissue	Time after last dose (days)	Concentration of amoxicillin
Plasma	1	0.048 (0.003)
	2	ND
	4	ND
	6	ND
Muscle	2	23.6 (2.44)
	4	13.6 (1.34)
	6	ND
Kidney	2	559.7 (94.9)
	4	149.2 (41.1)
	6	21.4 (1.49)
Liver	2	49.1 (6.53)
	4	20.7 (2.05)
	6	12.3 (2.15)
Fat	2	24.7 (4.21)
	4	11.9 (1.41)
	6	ND

Limit of quantification= 0.01µg/g, limit of detection= 0.003µg/g ND Not detectable

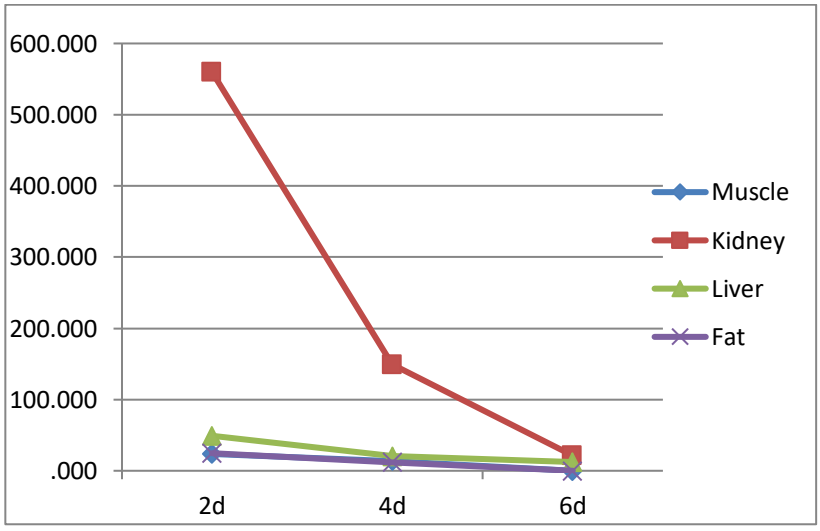

Figure 21. Mean amoxicillin tissue residues (µg/kg) in muscle, liver, kidney and fat from pigs given amoxicillin at a dose of 20 mg/kg bw orally for 3 time points within 5 consecutive days (Martinez-Larranaga et al., 2004)

Elimination half-lives based on the data from Martinez-Larranaga et al. (2004) are given in Table 18. Since no single animal data was available, for the purpose of this example elemination half-lives have been calculated from the mean values instead of the individual data. It is acknowledged that half-lives should ideally not be determined from aggregated data and that half-lifes derived from arithmetic means instead of individual animal data might be biased.

Table 18. Elimination half-life in pig tissues

Commodity	Elimination half-life	Comment
Liver	2.7 days	low fitting of curve with data
Kidney	0.85 days	good fitting of curve with data
Muscle	2 days	Only two slaughter times with residues concentrations above the LOD.
Fat	2 days	Only two slaughter times with residues concentrations above the LOD.

In another residue depletion study, amoxicillin was administered twice daily via drinking water at a dose of 10 mg/kg bw or once daily at a dose of 20 mg/kg bw for 5 consecutive days (Company B (2)). Mean residue data shown below in Figure 22 and Figure 23. Amoxicillin residues were detectable in tissues and organs over a rather long period of time.

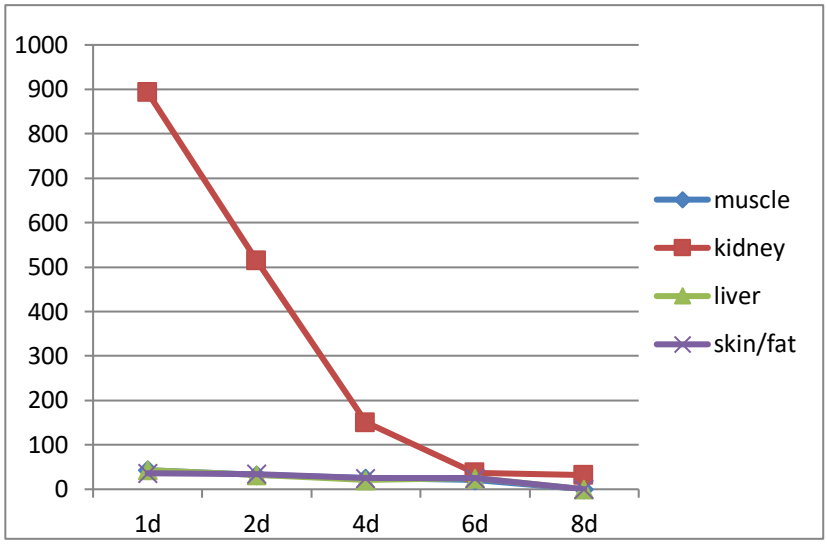

Figure 22. Mean amoxicillin residues (µg/kg) in pigs after oral administration twice daily via drinking water at a dose of 10 mg/kg bw amoxicillin in 4 animals per group; HPLC method, LOQ: 20 µg/kg

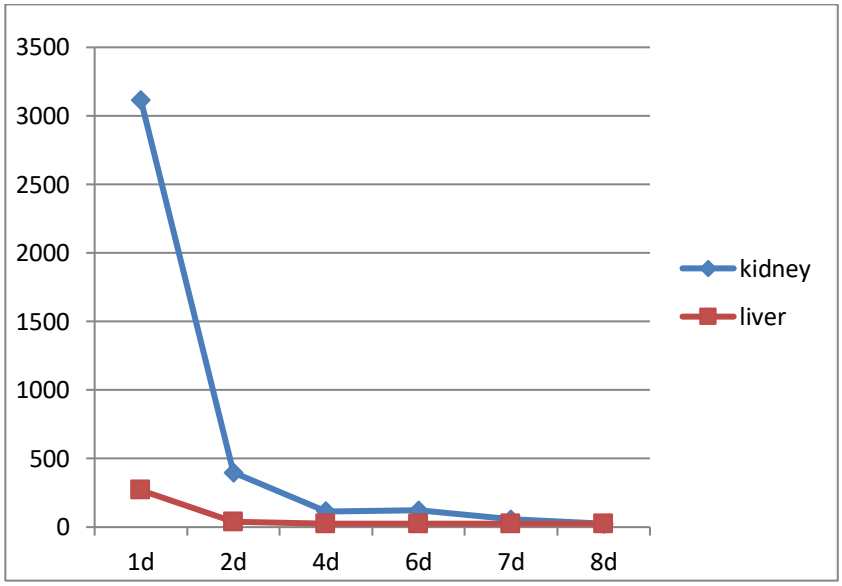

Figure 23. Mean amoxicillin residues (µg/kg) in pigs after oral administration of 20 mg/kg bw amoxicillin, once a day in liquid meal for 5 days, 4 animals per group, HPLC method, LOQ: 20 µg/kg

The elimination half-lives shown below have been calculated from the two tissue residue depletion studies (10 mg/kg bw given twice daily for 5 consecutive days and 20 mg/kg bw given once daily for 5 consecutive days (data from Company B (2)).

Table 19. Elimination half-life: data from pigs after oral administration of amoxicillin twice daily via drinking water at a dose of 10 mg/kg bw (n=4)

Commodity	Elimination half-life	Comment
Liver	1.2 days	Low fitting of curve with data
Kidney	1.8 days	Low fitting of curve with data
Muscle	NC	Cannot be calculated no amoxicillin residue detectable whatever the slaughtering time
Fat	0.45 days	Only two slaughter times with residues concentrations above the LOD. Poor relevance of the calculated half-life

NC = not calculated

Table 20. Elimination half-life: data from pigs after oral administration of amoxicillin at a dose of 20 mg/kg bw, once a day in liquid meal for 5 days (n=4)

Commodity	Elimination half-life	Comment
Liver	0.7 days	Only two slaughter times with residues concentrations above the LOD. Poor relevance of the calculated half-life
Kidney	1.3 days	Low fitting of curve with data
Muscle	NC	Cannot be calculated no amoxicillin residue detectable whatever the slaughtering time
Fat	NC	Cannot be calculated no amoxicillin residue detectable whatever the slaughtering time

NC = not calculated

Three more residue depletion studies were provided by two pharmaceutical companies. The product were given orally via drinking water at different dose levels (11 mg/kg bw, 20 mg/kg bw and 60 mg/kg bw) over a period of 5 consecutive days. Twenty-four hours after the last administration of the respective product, no amoxicillin residues were detectable in liver, kidney, muscle or fat. The samples were assayed by a microbiological method with an LOQ of 0.01 µg/g.

11.3.7. Residue summary

Amoxicillin residues deplete rather rapidly. Residues in muscle and fat or fat/skin are universally very low. Residues are usually found in liver and kidney depending on the product formulation and dose used. Residues are consistently highest in kidney.

11.3.8. Overall conclusions for the extrapolation of a withdrawal period for amoxicillin administered orally to pigs

Amoxicillin is well absorbed and reaches maximum concentrations in the plasma within hours. Residue elimination is also rather fast and dose linearity is given in plasma up to a dose of 20 mg/kg; it is assumed that here this can be transferred to tissues and up to a dose of 40 mg/kg (generally, such an assumption needs sufficient substantiation). Furthermore, it is assumed that distribution is complete at MRL, and the withdrawal period determining tissue remains the same.

Tissue residues are rather low and often not detectable after 24 hours of the last administration of the product. Residues are highest in kidney which should be the target organ for the determination of the withdrawal period. It remains to be discussed, whether the different plasma levels of amoxicillin in diseased animals (higher) should be also considered for the extrapolation of the withdrawal period and the PK/PD analysis. However, this would be not consistent with current regulatory practices and guidelines and is thus not considered at this time.

For the extrapolation of a new withdrawal period considering a higher dose, kidney residue elimination / half-life is considered which is rather short and below 48 hours, expecting that kidney will remain the withdrawal determining tissue. As a worst case approach a half-life of 48 h was used in the extrapolation of the WPs.

As mentioned above, not all conditions outlined in chapter 4 have been checked to be fulfilled. However, for the demonstration of the method within this case study this was assumed.

11.3.9. Withdrawal period calculation

The new withdrawal periods were calculated using Equation 2.

It has been noted that the current withdrawal periods for the amoxicillin products vary considerably between products. There is no obvious reason for this. One explanation could be that the products do differ in their oral bioavailability. However, this may not explain the great differences in all the cases. However in this pilot project it was agreed to extrapolate from the **current** WPs of the products (see 2.2.).

Table 21. Current WPs and the WPs calculated for a dose of 40 mg amoxicillin/kg bw for the products listed in Table 13 applying the elimination half-life for each product

Product	Posology (amoxicillin trihydrate)	Current WP (days)	Extrapolated WP (days)
A	16 mg/kg bw per day for 5 days	2	5
B	20 mg/kg bw per day for 5 days	6	8
C	20 mg/kg bw per day for 5 days	14	16
D	20 mg/kg bw per day for 5 days	2	4
E	20 mg/kg bw per day for 5 days	2	4
F	13 mg/kg bw per day for 5 days	2	6

11.4. Environmental risk assessment

Because there may be different authorised doses for the same or similar products, as a general rule, the situation for the product with the highest authorised (total) dose for the same target animals is used for the comparison, provided that an ERA exists for that product at that dose for the relevant target species. In the case of amoxicillin products for use in drinking water for pigs, ERAs were available addressing the risks at a dose of 20 mg/kg bw per day for 5 days.

11.4.1. Step 1: Determine the assessment situation for amoxicillin

For the products containing amoxicillin for use in drinking water for pigs at doses of 20 mg/kg bw per day for up to 5 days, the existing ERAs went into Phase II because the PEC_{soil}-trigger of Phase I was exceeded. Considering that the adjusted dose of 40 mg/kg bw per day for up to 7 days is higher than the currently authorised dose, it was concluded that the ERA for the adjusted dose would also enter Phase II.

In the available Phase IIA assessments, fate and effect studies were considered, and the RQs were determined for the various test species representing the terrestrial and aquatic environments. The RQs for terrestrial species were in the range of 0.005-0.084, and the RQs for aquatic species were in the range of 0.012-0.43.

When doubling the dose from 20 to 40 mg/kg bw per day for 5 days (the maximum duration for most of the products), the RQs will be increased by a factor of 2, resulting in a maximum RQ of 0.86. This RQ remains below 1. In addition, the dose increase will not result in a (Phase II Tier A) $PEC_{groundwater}$

higher than 0.1 µg/L. However, when the duration is extended to 7 days (as for some authorised products), the highest RQ (for aquatic species) would increase to 1.2. While this is only a slight exceedance of the RQ of 1, it would indicate the need for a Tier B assessment. Within the limited sample of products available for this pilot project, no Tier B data were available. Beyond this pilot project, it should first be investigated if Tier B data are available from any of the MAHs.

However, within the context of this pilot project and in lieu of Tier B data, it was considered that most products have a treatment duration of 3-5 days, and all products have roughly the same PK when given via the drinking water at the same dose. Therefore, it was concluded that 3-5 days could be sufficient for all products concerned and having the same indication. A limitation to 5 days as the maximum treatment duration was considered as a possible Risk Mitigation Measure (RMM), which could be applied to all such products concerned. Overall, it was concluded that the adjusted dose does not give rise to concerns in relation to environmental risks. Further consideration of steps 2-8 of the proposed approach was not necessary.

11.4.2. Conclusion on the ERA

It was concluded that doubling the dose of amoxicillin from 20 mg/kg bw per day to 40 mg/kg bw per day for a maximum duration of 5 days will not present a risk for the environment based on the data available in the pilot project.

11.5. Target animal safety

As noted in the introduction, the approved doses of amoxicillin for administration in drinking water to pigs vary widely between 10 – 20 mg/kg bw, to be given once or twice daily, for 3-7 consecutive days. According to the outcomes of the PK/PD modelling, it is proposed that the dose should be doubled to 40 mg/kg bw for the given swine respiratory disease indication.

11.5.1. Step 1: Determine the target animal safety profile for the active substance and establish the MOS for the active substance according to the revised dose, pharmaceutical form and route of administration

A review of the TAS studies provided by MAHs involved with the pilot project was undertaken.

Margin of safety studies

A GLP TAS study showed that amoxicillin was well tolerated in pigs aged from 12 weeks' age dosed at **25 mg/kg bw x 10 days** (n=3) or **116 mg/kg bw** (n=3) or **264 mg/kg bw** (n=3) x 5 days; although this conclusion was based on physical findings, haematology and biochemistry, only.

A further GLP TAS study showed that amoxicillin when administered via drinking water was well tolerated at doses of **20, 60** or **100 mg/kg bw x 15 days**; however, there were some limitations of the study, e.g. only 4 pigs per dose group, and cardiac lesions in 2 pigs were not followed up.

Reproductive safety studies were not available to the pilot project.

Local safety studies: Not applicable.

Palatability: Specific studies were not provided to the pilot project.

Conclusions: Aqueous oral solutions may be eligible for a biowaiver from bioequivalence studies (EMA/CVMP, 2000a) allowing extrapolation of TAS data between different formulations. A 'no effect level' has been shown for a dose of ≥ 116 mg/kg bw x 5 days in 6 animals, including at 264 mg/kg bw x 5 days in 3 of those animals; although this was based only on clinical findings and

haematology/biochemistry. 'No effect' was shown in a further study up to 100 mg/kg bw x 15 days in 4 healthy pigs. The studies provided did not allow identification of target organs or toxicity profile. Palatability studies were not provided. In both studies, amoxicillin was administered via the drinking water (pulse or ad lib); however, it is not commented if this affected the intake.

11.5.1.1. Step 1a: Review supplementary data from dossiers, if needed e.g. dose-finding studies

Data not available to the pilot project.

11.5.2. Step 2: Safety in the target population

Data not available to the pilot project.

11.5.3. Step 3: Safety based on post-marketing pharmacovigilance

Data not available to the pilot project.

11.5.4. Step 4: Safety based on published literature and authorisations in third countries (if needed)

Peer-reviewed Journals

Mrvos, R., Pummer, T.L., & Krenzelok, E.P. (2013). Amoxicillin renal toxicity: how often does it occur? *Pediatric emergency care*, **29**(5): 641-643. (Mrvos et al., 2013)

Grey literature

CVMP Summary Report Penicillins

Penicillins have a low toxicity in the normal sense of the word; the **therapeutic index is more than 100**, and toxic effects have only been seen after extremely high doses. No teratogenic effects have been recorded.

In connection with therapeutic use of penicillins **hypersensitivity** reactions are by far the most commonly encountered side-effects. The amount of penicillin haptene necessary to sensitize a subject is several orders of magnitude higher than the quantity needed to trigger an allergic reaction

Furthermore, it takes a much higher oral dose to induce an allergic reaction than if the product is administered parenterally.

Information from SPCs of EU-authorised products:

SPC 4.3: Do not use in animals with serious kidney malfunction including anuria and oliguria.

SPC 4.6: Penicillins and cephalosporins may cause hypersensitivity following administration. Allergic reactions to these substances may occasionally be serious.

Rarely, gastro-intestinal tract signs associated with alteration of the intestinal flora (for example, loose stools, diarrhoea) may occur.

SPC 4.7: Studies performed in Laboratory animals (rat, rabbit), did not show a teratogenic, embryotoxic or maternotoxic effect of amoxicillin. Safety of the product in the pregnant and lactating sows was not demonstrated. Use only accordingly to the benefit/risk assessment by the responsible veterinarian

SPC 4.10: No side effects were observed after administration at 5 times the recommended dosage. No problems with overdosage have been reported. Treatment should be symptomatic and no specific antidote is available.

TOXNET

'ANIMAL STUDIES: Reproduction studies have been performed in mice and rats at doses up to 2000 mg/kg. There was **no evidence of harm to the foetus due to amoxicillin**. However, 100 ug/mL amoxicillin altered rat **renal development** *in vitro*. Prolonged use of amoxicillin might have a negative effect on bone formation around implants.'

Human toxicity: SIGNS AND SYMPTOMS - *Clostridium difficile* associated diarrhoea (CDAD) has been reported with use of nearly all antibacterial agents, including amoxicillin, and may range in severity from mild diarrhoea to fatal colitis. Treatment with antibacterial agents alters the normal flora of the colon leading to overgrowth of *C. difficile*.

Toxicological evaluation of certain veterinary drug residues in food (JECFA, 2011) In laboratory animal toxicological studies, NOAELs were largely based on the highest doses tested and were from 250 to 2000 mg/kg bw per day. Dogs receiving doses of 500 mg/kg bw showed **gastrointestinal effects** due to disturbance of the GI flora.

Human toxicity: Gastro-intestinal, allergic effects and hepatotoxicity are reported. In humans the incidence of hepatotoxicity is identified at <0.02 to 3 per 100,000 prescriptions. It was concluded that amoxicillin is unlikely to cause reproductive or developmental toxicity in humans.

Textbooks

Prescott, J.F., & Dowling, P.M. (Eds.). (Giguère et al., 2013). *Antimicrobial therapy in veterinary medicine. John Wiley & Sons.: 'Penicilllins and beta-lactam antibiotics are generally **remarkably free of toxic effects even at doses grossly in excess of those recommended.** The major adverse effects are **acute anaphylaxis and collapse;** milder hypersensitivity reactions…are more common…. Anaphylactic reactions are less common after oral rather than parenteral administration…Less common adverse reactions include **haemolytic anaemia and thrombocytopenia**.' 'One hazard with broad-spectrum penicillins is the potential to **disturb the normal intestinal flora**.'*

Conclusions: Published studies on the toxicity/safety of amoxicillin in pigs were hard to locate on a basic internet search (PubMed, Google scholar). According to grey literature and standard texts, amoxicillin has a wide margin of safety in laboratory and veterinary species, and humans. Hepatotoxicity and renal toxicity may occur rarely. Gastrointestinal disturbances may occur due to disruption of the microbiota. Amoxicillin is unlikely to cause reproductive or developmental toxicity. The adverse event of most concern in humans is anaphylaxis, which is generally regarded as idiosyncratic. Although it takes a higher oral dose to induce an allergic reaction than if the drug is administered parenterally, it is not clear if increasing the dose within the therapeutic range would increase the risk of hypersensitivity developing in pigs.

11.5.5. Step 5: Conclude on the safety of the increased dose of the active substance according to the pharmaceutical form and route of administration

No specific studies are available that would demonstrate a MOS above the approved dose (20 mg/kg bw per day) consistent with current VICH requirements. However, based on two GLP TAS studies, despite some limitations in the studies, it has been demonstrated in 10 healthy pigs that doses of 100 mg/kg or higher administered orally for at least 5 days were well tolerated.

Reproductive safety studies in the pig were not available to the pilot project.

Published literature indicates that amoxicillin is safe in laboratory species at doses well in excess of those used therapeutically. Hepatotoxicity and renal toxicity may occur rarely. Gastrointestinal disturbances may occur due to disruption of the microbiota. Amoxicillin is unlikely to cause reproductive or developmental toxicity, based on human and rodent data. The most common and concerning adverse events are hypersensitivity reactions – it cannot be concluded if these idiosyncratic reactions would increase in frequency in pigs following an increase of the dose regimen.

Overall, it is concluded, based on the limited data available, that the proposed dose of 40 mg amoxicillin/kg bw per day for 5 days in drinking water is likely to be adequately tolerated in pigs.

11.5.6. Step 6: Further considerations for the conclusion on the safety and benefit-risk for individual products

Excipients used in different formulations include:

- Pentasodium triphosphate
- Silica Colloidal anhydrous
- Trisodium phosphate anhydrous
- Na carbonate
- Na citrate
- Lactose monohydrate – lactose intolerance may be dose-dependent.
- Na Glycine carbonate – mildly toxic by ingestion.
- Na hexametaphosphate
- Mannitol – potential for laxative effect, depending on level of intake.

The above excipients are all commonly used in veterinary medicinal products. It seems unlikely that a doubling of intake would have implications for target animal safety, but this would be considered on a product-by-product basis according to the individual composition, since some precautions are identified above.

11.5.7. Step 7: The conclusions above are incorporated into the final benefit-risk for the dose increase for each individual product

Overall, it is concluded, within the context of this pilot project, that VMPs administered at the proposed dose of 40 mg amoxicillin/kg bw per day for 5 days in drinking water are likely to be adequately tolerated in pigs for the treatment of respiratory disease. A final conclusion for individual products cannot be drawn at this moment due to limitations on available data.

11.6. Overall conclusion and recommendations on amoxicillin

The approaches on dose review and adjustment, WP, ERA and TAS as described in chapters 3, 4, 5, and 6, respectively, were tested in the case study on amoxicillin products, orally administered via the drinking water, for the treatment of respiratory infections in pigs. The most common dose currently authorised for this indication is 20 mg/kg bw per day for 5 days.

It should be noted, that the outcome of the dose review was based on a limited amount of data, gathered from public sources or provided by industry. Assumptions necessary for applying the methodology introduced in this reflection paper could not always be checked, at some places not fully correct methods were used for pragmatic reasons. Therefore, the numerical results (e.g. adjusted dose, WP etc.) do not reflect a final outcome (e.g. after a referral in which all related VMP authorised in the EU are included). In addition, the case studies, including the calculations, should not be regarded as reflecting the only possible or definitive methodology.

In order to review and adjust the dose, the following pathogens were considered to be relevant: *Actinobacillus pleuropneumoniae, Bordetella bronchiseptica, Haemophilus parasuis, Pasteurella multocida* and *Streptococcus suis*. The adjusted dose was determined as 40 mg/kg bw per day. It was noted that, due to the low susceptibility, it was not possible to establish a dose for *B. bronchiseptica*, and therefore pigs infected by this pathogen should not be treated with amoxicillin via the drinking water.

For the establishment of the WP, only a limited number of studies were available for this pilot project. Since the depletion of residues of amoxicillin after oral administration to pigs is very rapid, most of the older residue studies confirmed that residues are already below LOD after a few days. However, this challenge could be overcome by the use of the hour glass approach. Data and insights from multiple sources (e.g. FARAD, literature, published thesis's, registration dossiers) were combined to find the relevant PK parameters and eventually terminal half-lives of the depletion of residues in various tissues could be gathered. Dose proportionality in all tissues across the relevant dosing range was assumed without sufficient data for its demonstration. As kidney is the WP-determining tissue, the half-life of kidney was used for the WP extrapolation; without further check it was assumed that with increasing dose kidney remains WP-determining. In view of the limitations in data availability, a "worst-case" half-life of 2 days was chosen. Based on the application of Equation 2, increasing the dose to 40 mg/kg thus only leads to relatively low increases in the WPs.

For addressing the environmental risks, adequate Phase I and Phase II ERA data were available for the authorised dose of 20 mg/kg bw per day for 5 days. For the adjusted dose, the RQs remained below 1 when the duration is maximally 5 days, and above 1 when the duration is 7 days. It was considered that the duration of 3-5 days may be sufficient for products with the same indication, which would justify the limitation of the duration to maximally 5 days, in order to limit the exposure to the environment. Overall, the adjusted dose for amoxicillin does not give rise to concerns for the environment.

In relation to TAS, no specific safety issues were identified after consideration of all provided data from the registration dossiers and other relevant sources. It was concluded that amoxicillin administered at the adjusted dose is likely to be adequately tolerated in pigs.

12. Annex 2: Case study oxytetracycline

The case studies were conducted simultaneously with, and helped the development of the non-experimental approaches. Consultation processes have led to further improvements of the proposed methodologies. The consequence of this is that the case studies may not be completely compatible with the proposed revised methodologies in all aspects. Therefore, the case studies should be seen as an illustration only. Also, the case studies were based on a limited amount of data, gathered from public literature and provided by industry, and consequently sometimes assumptions (e.g. dose-linearity, half-lives, completeness of distribution at MRL level) were accepted that would normally require a more robust scientific foundation. In conclusion, the case studies only illustrate how non-experimental approaches could work and that these may be helpful in addressing the problem statement explained in Chapter 1.

12.1. Introduction

Oxytetracycline (OTC) is a commonly used broad spectrum tetracycline antibiotic in veterinary medicine. In the EU oxytetracycline is licensed in various formulations (powders, solution for injection, suspension for spray, premix and tablets), for a variety of animals (food producing and non-food producing).

This case study will be limited to the solution for injection formulation to be used for respiratory infections in cattle.

The reason for selecting oxytetracycine for dose review is based on the fact that there are different approved dosage recommendations for the same or similar products in the EU. However, the CVMP did not look for and therefore does not have and is not aware of any evidence to suggest that the the currently approved doses are not effective under conditions of field use.

Oxytetracycline is a broad spectrum antibiotic effective against both Gram-positive and Gram-negative bacteria with a bacteriostatic effect. OTC binds to 70S and 80S ribosomes blocking the attachment of aminoacyl-transfer RNA to the ribosomal messenger RNA thereby blocking the ability of bacteria to produce proteins. This prevents the bacteria from growing and multiplying.

Oxytetracycline is normally available as the dihydrate or hydrochloride salt.

The solution for injection is available in 10% ("short acting") and 20% and 30% ("long acting") formulations. The approved doses are:

- 20% and 30% formulations: 20 or 30 mg/kg bw, single injection; in some approved labels: repeated after 48 or 72 hours in severe cases.
- 10% formulations: between 4–20 mg/kg bw per day, daily injection for between 1 and 5 days

Licensed products are indicated for a wide variety of infections primarily septicaemia, respiratory and gastro-intestinal infections, as well as foot rot, soft tissue infections and furunculosis and enteric redmouth disease in aquaculture.

This case study will focus on the indication for respiratory disease caused by *Pasteurella multocida*, *Mannheima haemolytica* and *Haemophilus somni*.

12.2. Dose review and adjustment

12.2.1. Pharmacokinetics

One of the challenges of the case study for oxytetracycline injectable products is the possibility that the pharmacokinetics differ between the various formulations. Depending on how much products differ in their pharmacokinetic profile, there may be a need for a product-by-product PK/PD analysis which might result in different outcomes for the adjusted dose. Therefore, the possible existence of formulation-specific pharmacokinetics was investigated.

First, the composition was considered for a range of products (i.e. the OTC injectables for cattle authorised in The Netherlands), including 20% ("long acting"; LA) and 10% ("short acting"; SA) formulations (an overview is given in Annex 7). As it turned out, all formulations have a comparable composition / similar composition / similar galenics, namely containing water and other solvents, chelators, complexing agents, preservatives, and substances for adjusting the pH. The organic solvents and complexing agents in particular, can have the ability to delay / influence the release of the active ingredient from the site of injection and thus influence the (absorption) pharmacokinetics of the formulation. These substances were quite similar across formulations. Therefore, it appears that no major differences in the PK would be expected from the design of the composition of the product. Indeed, Nouws et al. (1985) tested a range of LA (long acting) and SA (short acting) OTC formulations in dairy cows and found that the pharmacokinetics were roughly the same. In addition, OTC half-lives in tissues were similar for LA and SA formulations (see 12.3.).

Whereas the compositions of the formulations are similar in terms of the inactive ingredients, it has to be noted that there is a 2-fold difference in strength between the LA and SA formulations, and that these products have different patterns of use. Therefore, under field conditions, there will be differences in the volume and the number of injections, and these differences may influence the absorption from the injection sites and thus the PK profile. In an unpublished study report provided by the industry, pharmacokinetic profiles were shown to be different between an LA and SA formulation. It was considered that the difference in the number of injections given could well explain the difference in pharmacokinetics.

In view of the above, it was decided to analyse two datasets separately, one representative for an LA formulation and another one representative for a SA formulation.

In this case study, raw PK data from different sources (Marketing Authorisation Holders) were used for the computation of a daily dose. The pharmacokinetics for different concentrations of oxytetracycline formulations (20% and 10%) were determined using old datasets provided by different pharmaceutical companies for doses ranging from 5 to 20 mg/kg bw administered intramuscularly to calves, young cattle and cows. The OTC plasma concentrations for different sampling times were analysed using a non-linear mixed effect model using Monolix® (Lixoft) and simulations of different dosage regimen were performed in R using mlxR package. The PK model choose according the Akaike Information Criteria was a mono-compartmental model using an extravascular administration route. The PK parameters of the two main OTC type pf formulations present in the EU market are reported in the following table and used for our investigation, taking into account the differences of bioavailability associated to the number of injections.

Table 22. Comparison of PK parameters for LA-OTC and SA-OTC for cattle

Parameter	Unit	20 %	10 %
Ka pop	h-1	0.0303	0.057
V/F_pop	$L.kg^{-1}$	0.263	0.203
Cl_pop	$L.kg^{-1}.h^{-1}$	0.0954	0.13
Omega_Ka	h^{-1}	0.252	0.19
Omega_V/F	$L.kg^{-1}$	0.265	0.342
Omega_Cl	$L.kg^{-1}.h^{-1}$	0.269	0.332

The next figure is the graph of observed data and percentiles of distribution of the Population PK model with the 90th percentiles for the two tested formulations.

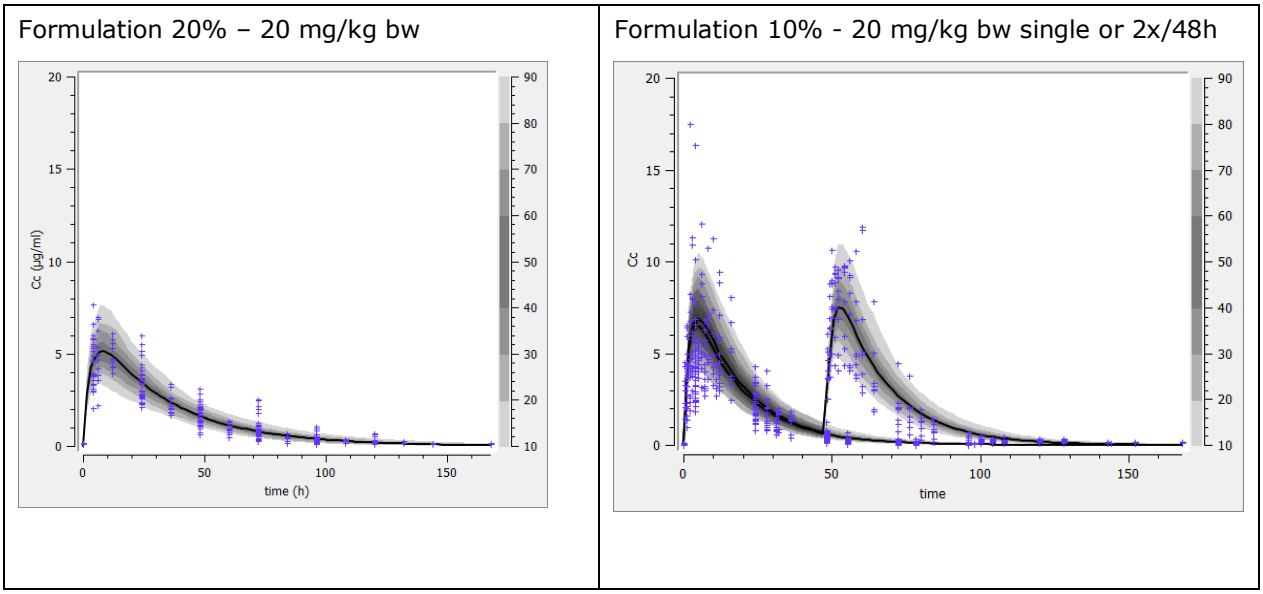

Figure 24. Representation of the distribution of plasmatic concentration in function of time obtained by population PK model for a long acting formulation dose (20% formulation) and a short acting formulation dose (10% formulation)

12.2.2. Target bacteria

The therapeutic indication is the bovine respiratory disease. The targeted pathogens are:

- *Pasteurella multocida*
- *Mannheima haemolytica*
- *Haemophilus somni*

Table 23. Merged tetracycline MIC distribution frequencies of bovine respiratory target pathogens isolates (de Jong et al., 2014; El Garch et al., 2016)

MIC (µg/mL)	0.12	0.25	0.5	1	2	4	8	16	32	64	128
***P. multocida* (n=239)**	3	20	143	24	27	1	5	7	9		
***M. haemolytica* (n=231)**		4	65	129	2	3	6	7	13	1	1
***H. somni* (n=66)**	2	33	27		1	1	2				

*ECOFF values are determined using the tool ECOFFinder to calculate the 99.9th percentile of ECOFF (Turnidge et al., 2006). In the context of this pilot project, all the criteria requested by EUCAST may not be fulfilled to use these tools with confidence, however in order to follow the methodology define in the section 3.3, the ECOFF of the different target pathogens were calculated. ECOFF value is 1 µg/mL for *P. multocida* and 2 µg/mL for *M. haemolytica*. For *H. somni* an ECOFF of 1 µg/mL is calculated but the minimal number of strains is not reached and the value is given only as an example in the context of this pilot project.

12.2.3. PK/PD index

The recommended PDI for tetracyclines is the AUC/MIC as they are time dependent antibiotics acting on the ribosome with a post antibiotic effect (Barbour et al., 2010). Contrary to the amoxicillin case study, there is no need to investigate other PDI for OTC.

12.2.4. Target value for the PDI (PDT)

Studies on the pharmacodynamic activity of oxytetracycline are limited. One PK/PD integration study reported the AUC_{24h}/MIC ratios required for four levels of inhibition for a strain of *M. haemolytica* (Brentnall et al., 2013). MIC was determined in cation adjusted Mueller Hinton Broth (CAMHB) and three calf fluids (serum, exudate, transudate). Bacterial time-kill curves were established *in vitro* in the same matrices. The MICs of the tested strain were 0.8, 14.8, 12.8, and 11.2 in CMHB, serum, exudate, and transudate, respectively. The authors proposed different AUC_{24h}/MIC ratios for bacteriostatic action, 50% reduction in count, bactericidal action and bactericidal eradication. For this pilot study, we used two PDT values (bacteriostatic action = 42, bactericidal action = 59) determined for CAMHB. The PDT is based on *in vitro* data and is not validated on clinical efficacy basis.

12.2.5. Model of the relationship between dose and PDI target attainment

Based on the PK profile of the two tested formulation and the defined PD parameters, the Monte Carlo Simulation was performed with SimulX implement in R with the package mxlR using 5000 random values.

Seven different dosage regimens were tested for each formulation (20% vs 10%):

- 4 x IM administration of 10 mg/kg bw at a 24 h interval
- 1 x IM administration of 20 mg/kg bw
- 1 x IM administration of 30 mg/kg bw
- 1 x IM administration of 80 mg/kg bw
- 2 x IM administrations of 20 mg/kg bw at a 48 h interval
- 2 x IM administrations of 30 mg/kg bw at a 48 h interval
- 2 x IM administrations of 20 mg/kg bw at a 36 h interval

The probability of target attainment for the bacteriostatic and bactericidal activities is estimated for the different interval period between 0-24 h, 24-48 h, 48-72 h and 72-96 h. The range of MIC distribution in table 23 used to calculate the PTA is below or equal to ECOFF. The results of the modelling are provided in Table 24 and Table 25.

Table 24. Probability of target attainment (PTA) in function of AUC/MIC according the dosage regimen of a 20% formulation for the three bacterial species. *Values underlined in grey are below the objective of 90 % for the PTA.*

	Interval	*P. multocida*		*M. haemolytica*		*H. somni*	
Target (bacteriostatic = 42 / bactericidal = 59)		42	59	42	59	42	59
4 doses of 10 mg/kg/24 h	0-24 h	95,9%	90,7%	80,0%	52,1%	99,9%	100,0%
	24-48 h	99,8%	97,9%	98,9%	89,0%	100,0%	100,0%
	48-72 h	100,0%	99,3%	99,8%	96,4%	100,0%	100,0%
	72-96 h	100,0%	99,6%	99,9%	98,1%	100,0%	100,0%
Single dose 20 mg/kg	0-24 h	100,0%	99,4%	99,8%	97,0%	100,0%	100,0%
	24-48 h	97,8%	91,4%	88,9%	61,5%	100,0%	99,3%
	48-72 h	69,9%	40,8%	32,8%	15,2%	88,8%	70,2%
	72-96 h	18,5%	7,2%	5,8%	1,5%	44,8%	25,0%
Single dose 30 mg/kg	0-24 h	100,0%	100,0%	100,0%	99,9%	100,0%	100,0%
	24-48 h	99,9%	98,5%	99,4%	92,4%	100,0%	100,0%
	48-72 h	89,6%	74,0%	62,7%	36,6%	97,9%	91,1%
	72-96 h	44,7%	21,8%	18,2%	7,1%	69,1%	48,8%
Single dose 80 mg/kg	0-24 h	100,0%	100,0%	100,0%	100,0%	100,0%	100,0%
	24-48 h	100,0%	100,0%	100,0%	100,0%	100,0%	100,0%
	48-72 h	99,9%	99,2%	99,4%	96,1%	100,0%	100,0%
	72-96 h	92,0%	81,5%	77,3%	55,9%	97,5%	93,0%
2 doses of 20 mg/kg at 48 h	0-24 h	100,0%	99,4%	99,8%	97,0%	100,0%	100,0%
	24-48 h	97,8%	91,4%	88,9%	61,5%	100,0%	99,3%
	48-72 h	100,0%	100,0%	100,0%	99,8%	100,0%	100,0%
	72-96 h	99,3%	95,9%	96,6%	80,3%	100,0%	99,9%
2 doses of 30 mg/kg at 48 h	0-24 h	100,0%	100,0%	100,0%	99,9%	100,0%	100,0%
	24-48 h	99,9%	98,5%	99,4%	92,4%	100,0%	100,0%
	48-72 h	100,0%	100,0%	100,0%	100,0%	100,0%	100,0%
	72-96 h	100,0%	99,6%	99,9%	97,9%	100,0%	100,0%
2 doses of 20 mg/kg at 36 h	0-24 h	100,0%	99,4%	99,8%	97,0%	100,0%	100,0%

	Interval	*P. multocida*		*M. haemolytica*		*H. somni*	
	24-48 h	100,0%	99,8%	100,0%	98,9%	100,0%	100,0%
	48-72 h	100,0%	99,8%	100,0%	98,8%	100,0%	100,0%
	72-96 h	96,0%	87,2%	81,9%	55,0%	99,7%	97,4%

Table 25. Probability of target attainment (PTA) in function of AUC/MIC according the dosage regimen of a 10 % formulation for the three bacterial species. *Values underlined in grey are below the objective of 90 % for the PTA.*

	Interval	P. multocida		M. haemolytica		H. somni	
Target (bacteriostatic = 42 / bactericidal = 59)		**42**	59	42	59	42	59
4 doses of 10 mg/kg/24 h	0-24 h	97,1%	92,5%	86,1%	61,3%	99,9%	100,0%
	24-48 h	99,3%	96,0%	96,6%	80,9%	100,0%	99,8%
	48-72 h	99,5%	96,8%	97,6%	84,5%	100,0%	99,9%
	72-96 h	99,6%	97,0%	97,8%	85,4%	100,0%	99,9%
Single dose 20 mg/kg	0-24 h	100,0%	99,7%	99,8%	98,2%	100,0%	100,0%
	24-48 h	78,3%	55,4%	44,9%	24,9%	92,9%	79,5%
	48-72 h	6,7%	2,6%	1,6%	0,4%	20,4%	8,9%
	72-96 h	0,2%	0,2%	0,0%	0,0%	0,7%	0,4%
Single dose 30 mg/kg	0-24 h	100,0%	100,0%	100,0%	99,9%	100,0%	100,0%
	24-48 h	93,4%	81,4%	74,7%	49,4%	99,0%	94,3%
	48-72 h	19,4%	8,2%	6,7%	2,1%	41,6%	23,5%
	72-96 h	0,8%	0,7%	0,1%	0,0%	2,5%	1,6%
Single dose 80 mg/kg	0-24 h	100,0%	100,0%	100,0%	100,0%	100,0%	100,0%
	24-48 h	100,0%	99,6%	99,8%	97,8%	100,0%	100,0%
	48-72 h	73,5%	55,3%	47,5%	28,8%	88,0%	75,7%
	72-96 h	12,2%	5,7%	4,3%	1,3%	26,3%	15,4%
2 doses of 20 mg/kg at 48 h	0-24 h	100,0%	99,7%	99,8%	98,2%	100,0%	100,0%
	24-48 h	78,3%	55,4%	44,9%	24,9%	92,9%	79,5%
	48-72 h	100,0%	99,8%	99,9%	99,0%	100,0%	100,0%
	72-96 h	80,8%	60,6%	49,5%	28,7%	93,9%	82,6%
2 doses of 30 mg/kg at 48 h	0-24 h	100,0%	100,0%	100,0%	99,9%	100,0%	100,0%

	Interval	P. multocida		M. haemolytica		H. somni	
	24-48 h	93,4%	81,4%	74,7%	49,4%	99,0%	94,3%
	48-72 h	100,0%	100,0%	100,0%	100,0%	100,0%	100,0%
	72-96 h	94,6%	83,8%	78,6%	54,4%	99,2%	95,3%
2 doses of 20 mg/kg at 36 h	0-24 h	100,0%	99,7%	99,8%	98,2%	100,0%	100,0%
	24-48 h	99,9%	99,4%	99,7%	97,1%	100,0%	100,0%
	48-72 h	99,0%	95,6%	94,9%	80,0%	100,0%	99,6%
	72-96 h	39,5%	20,3%	16,5%	6,9%	64,7%	45,0%

The result of the modelling shows that a daily dose of 10 mg/kg bw during 4 days for both formulations (10% and 20%) leads to a PTA higher than 90% for two pathogens but not for *M. haemolytica* the 1st day. A sufficient exposure was obtained for the two PK/PD target (bacteriostatic or bactericidal) for the three pathogens during the last three days. The single administration of a 10% or a 20% formulation at a dose of 20 mg/kg bw leads to a sufficient AUC/MIC ratio for the first 24 h for the three target pathogens. However, the PTA falls below 90% for *M. haemolytica* during the second day (24-48 h) with the 20% formulation and also for *P. multocida* and *H. somni* (bactericidal effect) with the 10% formulation. For both formulations, PTAs are below 90% for the three pathogens the 3rd day. To reach a PTA higher than 90% for the three bacterial species and for the two PK/PD target during three days with a single injection, the dose of a 20% formulation must be increased to a value close to 80 mg/kg bw (Table 25). With a 10% formulation, the exposure is sufficient only for two days even at a dose of 80 mg/kg bw. Two administrations at 48 h apart of a 20% formulation leads to a sufficient exposure from the 1st to the 3rd day and allow maintaining at least a PTA above 90% for a bacteriostatic activity for the three target pathogens during the four days. This is sub-optimal for *M. haemolytica* during the 2nd day where the PTA is below 90% but very close to this value for a bacteriostatic activity (88,9%). An increase of the administered dose from 20 to 30 mg/kg bw improves the PTA for *M. haemolytica* which leads to PTA of 90% for both PDIs during the four days for all the target pathogens. With a 10% formulation, two administrations of 20 mg/kg bw or 30 mg/kg bw at 48 h are not able to reach the PTA of 90% for the 2nd and the 4th day for *P. multocida* and *M. haemolytica*.

Another approach to improve the PTA of the 2nd day for *M. haemolytica* without modifying the authorised dose is to reduce from 48 to 36 h the interval between the two administrations of dose of 20 mg/kg bw. With this dosage regimen, the PTA is higher than 90% for the bacteriostatic and bactericidal activity against the three bacterial species during three days with a 20% formulation and a 10% formulation.

12.2.6. Main conclusions on the OTC-LA case study

Based on the available data, different conclusions can be drawn from the OTC case study:

- Four administrations of 10 mg/kg bw of a 10% or a 20% formulation leads to a PTA greater of 90% for *P. multocida* and *H. somni* during four days but for *M. haemolityca* the PTA is below 90% the first day (bacteriostatic effect).
- A single administration of 20 mg/kg bw of a 10 and 20% formulation leads to a PTA of 90% for the three target pathogens at least for the first 24 h. Then, PTA decline in function of time and in function of target pathogens MIC distribution.

- For the time period between 24-48 h, the single administration of 20 mg/kg bw of a 20% formulation sufficiently exposes *P. multocida* and *H. somni* but not *M. haemolytica*, the least susceptible pathogen. From the second to the fourth days, PTAs of a 20% formulation are higher than those obtained with a 10% formulation
- After 48 h, the single administration of 20 mg/kg bw of 20% formulation leads to a PTA below 90% for all the target pathogens which justifies the second administration.
- According the PK/PD modelling, PTA can be improved by increasing the administrated dose of a formulation or by repeating the administration with a shorter time interval.

By defining an improved frequency of administration (48 h versus 36 h), PTA can also be improved, especially in this case study for *M. haemolytica*. For this target pathogen, using an administration of 20 mg/kg bw 36 h apart of a 20% formulation, the PTA is above 90% for 3 days.

12.2.7. Set a PK/PD breakpoint

As for the amoxicillin case study, the next step of the proposed approach to address doses is the definition of clinical breakpoint, or PK/PD breakpoints when lacking clinical data (see 3.3.7.). According to the data available for oxytetracycline, in our example, the PK/PD breakpoint should be set at 2 µg/mL. It is compatible with values of estimated ECOFF of bacterial species targeted with the caution that the PD data was not optimal. *Mannheima haemolytica* has the highest ECOFF and is the less susceptible species.

12.2.8. Define an improved daily dose

For the oxytetracycline case study, it was decided to analyse two datasets separately, one representative for a LA formulation (20% formulation) and another one representative for a SA formulation (10% formulation). According to the chapter 12.3. of this report, no or slight differences where identified between SA and LA formulation regarding PK profiles. However, the 2-fold difference in strength between the LA and SA formulations will have an impact on in the volume and the number of injections, and these differences may influence the absorption from the injection sites and thus the PK profile. Then this difference in the rate of absorption could influence the daily dose defined by a PK/PD approach.

= For the SA – 10% formulation, according to the PK/PD modelling with the provided data, the dose of 10 mg/kg bw administered each 24h allows reaching a PTA of 90% for bacteriostatic activity for all the target pathogens, except during the first 24h for *M. haemolytica* where the PTA is close to this target value (86.1%).

It can then be concluded that, for the SA – 10% formulation, there is no need to increase the daily dose and that the dosage regimen 10 mg/kg bw each 24h provided a sufficient exposure for all the target pathogens tested.

= For the LA - 20% formulation, the modelling showed that the exposure is sufficient to reach the PTA target value only for the two periods 0-24h and 24-48h. According to the SPC of approved product, the dosage regime of the LA formulation is a single injection with repetition after 48 or 72 hours in severe cases. Thus, it can be concluded that the current dose of 20 mg/kg bw reach the PTA of 90% only for the two first days. Then, to improve the PTA for the next days, a second injection should be realised 48h apart or ideally 36h apart for the least

susceptible pathogens and not 72h as suggested. Based on the PK/PD modelling, to reach a PTA of 90% up to 72h with a single injection, the daily dose should be increased to 80 mg/kg bw. However, another approach to improve the PTA is to further refine the interval between the two administrations. Indeed, with the approved dose of 20 mg/kg bw, the PTA is higher than 90% for the bacteriostatic and bactericidal activity against the three bacterial species during three days with a 20% and a 10% formulation when a second injection is administered 48h or 36h respectively. However, in field conditions, the 20% formulation is more adapted than the 10% formulation due to the limitation of the volume that needs to be injected. According to the PK/PD modelling and the rational principles of use of antibiotics, it is not necessary to increase the dose of the LA formulation (up to 80 mg/kg bw) to artificially increase the duration of activity and rather refine the interval frequency of administration.

It can then be concluded that, for the LA – 20% formulation, there is no need to increase the daily dose but further refine the interval between two injection and that the dosage regimen of 20 mg/kg bw with a second injection between 36 to 48h provided a sufficient exposure for all the target pathogens tested.

Considering this pilot study, the reader must read this conclusion with the caution that distributions of MIC used for the simulation were suboptimal as the number of tested strains is limited so the MIC distribution of the wild type population used may not reflect correctly the reality.

12.3. Withdrawal period

12.3.1. Introduction

After systernic absorption, oxytetracycline (OTC) distributes rapidly into the extracellular spaces of animal tissues. It also can cross the placental and the blood-brain barriers. OTC undergoes little or no metabolic degradation in cattle and is eliminated mainly unchanged in the urine. Tubular secretion and passive reabsorption mechanisms are reported to be the mechanisms involved (Mevius et al., 1986). In bovine some (2-10%) epimerisation of OTC into 4-epi-OTC takes place. The marker residue used for determination of the withdrawal periods is defined as the sum of both compounds.

After parenteral administration the WP determining tissue is known to be the site of injection

Different OTC injectable formulations are authorised in the EU. For example, in the Netherlands there are some 25 OTC injectables authorised for use in bovine. A number of their particulars are listed in Table **26**.

Table **26** shows that there is hardly a correlation present between withdrawal periods (WPs) for tissues and offal and the dose of OTC administered.

Possible explanations:

1. The WP for tissues is determined by the depletion rate of residues of OTC from the site of injection. The amount of OTC deposited per injection site is more or less comparable for the various products.
2. Different formulations might have an impact on absorption
3. Where the statistical method could not be used, the WP was determined by the alternative approach. The WP determined according to latter method is highly dependent on the choice of time points in the design of the residue depletion study.

4. Relatively large safety factors have been applied (to account for inadequacies in the (older) residue studies), masking a possible effect between dose and WP.

5. Inadequate sampling of the injection site leading to unspecific spreading of the WPs

6. Influence of injection site location on residual OTC concentrations on the site of injection.

Since the residues on the injection site determine the WP for tissues, increasing the dose (within limits) by simply increasing the number of injections would have no effect on the WP for tissues. This would continue to be the case until, due to the increase of the overall dose, residues in one of the other tissues become WP determining. It should, however, be noted that the animal welfare situation should be considered when applying this method. It could be argued that, in field conditions, 2-3 injections per animal/dosing would be a maximum.

Table 26. OTC injectables authorised in the Netherlands for bovine

VMP no	MA Type	WP tissue (days)	WP milk (days)	Dose (mg/kg)	Duration (days)	max inj vol (ml)	Adm. route
1	30%	35	10	20, 30	1	7,5 and 10	im
2	30%	35	10	20, 30	1	7,5 and 10	im
3	10%	17	6	5, 10	3 to 4	20	im
4	20%	35	8	20	1 to 2	7 and 15	im
5	10%	23	5	10	5	10	im
6	10%	18	5	5, 8	5	5 to 10	im
7	10%	21	5	5,10, 20	3 to 5	15, 5-10	im
8	10%	23	7	10	3	20	Im
9	10%	35	4	4	3	20	im
10	10%	35	4	4	3	20	im
11	10%	35	10	4	3 to 5	10	im
12	20%	35	9	20	1	10	im
13	10%	35	10	4	3 to 5	10	im
14	20%	35	13	20	1	10	im
15	10%	23	7	10	3	20	im
16	10%	28	x	20	1	10	im
17	10%	21	x	10 to 20	3 to 5	5 to10	im
18	20%	35	x	10	3	10	iv/im
19	10%	21	5	5,10-20	3 to 5	15, 5-10	im
20	10%	23	7	10	3	20	im
21	10%	35	4	4	3	20	im
22	10%	35	10	4	3 to 5	10	im
23	20%	27	13	20	1	10	im
24	20%	44	18	20	1 and 3	5	im
25*	20%	31	10	20	1	20	im

* no Respiratory Infection claim

As an example, Table 27 shows the max weight that could be treated, based on a maximum of 3 injection sites per dosing.

Table 27. Theoretical max weight (kg) to be treated for 10% OTC , 20% OTC (in parenthesis) and 30% OTC (in brackets) preparations, based on max 3 inj/day

Dose (mg/day.kg)	Max 5 ml/inj	Max 10 ml/inj	Max 20 ml/inj
5	300 (600) [900]	600 (1200) [1800]	1200 (2400) [3600]
10	150 (300) [450]	300 (600) [900]	600 (1200) [1800]
20	75 (150) [225]	150 (300) [450]	300 (600) [900]
40	38 (75) [113]	75 (150) [225]	150 (300) [450]

12.3.2. Plasma kinetics

In most of the studies reported in public literature, e.g. (Mevius et al., 1986; Nouws et al., 1985; Toutain and Raynaud, 1983), the plasma curve of OTC was followed only for the first 72-120 hours. Meijer et al. (1993) however, using a sensitive method of analysis, followed the plasma levels of OTC over approximately 300 hours, after an i.v. dose of 40 mg/kg bw and an i.m. dose of 20 mg/kg bw. The study revealed a slow terminal elimination phase with a half-life of approximately 95 hours (see figures and tables below). The authors concluded that, since this phase was present after i.v. as well as after i.m. administration, it could not be caused by a prolonged absorption from the site of injection.

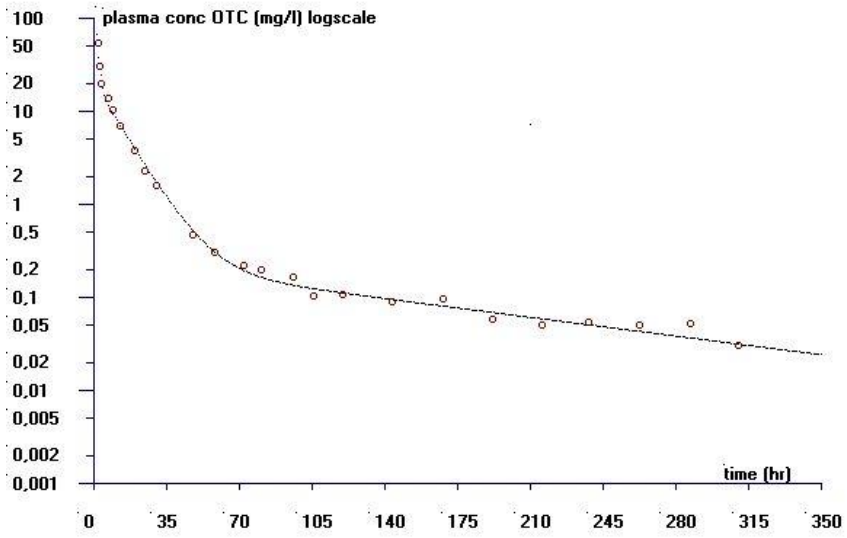

Figure 25. Measured concentration (mean) and mean fitted plasma-concentration time curve for oxytetracycline after single i.v. administration of 40 mg/kg bw to veal calves (n=5); based on Meijer et al. (1993)

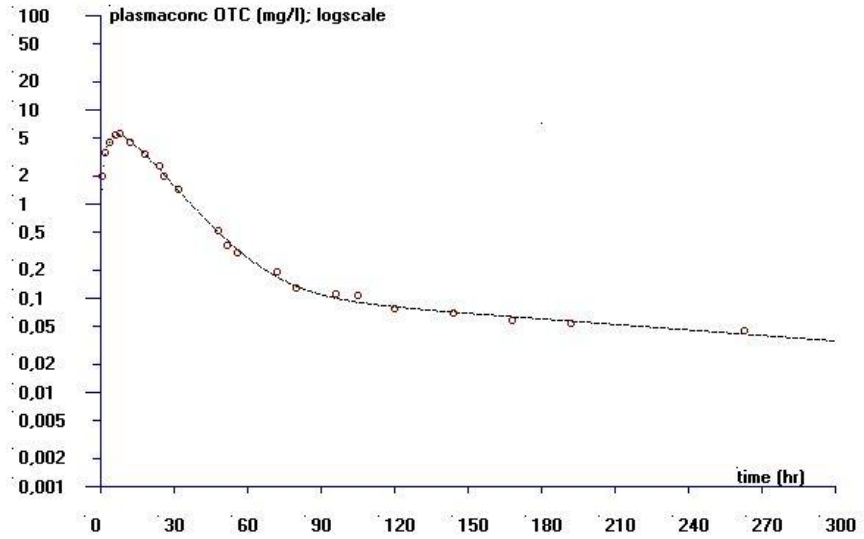

Figure 26. Measured concentration (mean) and mean plasma-concentration time curve for oxytetracycline after single i.m. administration of 20 mg/kg bw to veal calves (n=5); based on Meijer et al. (1993)

Table 28. Individual pharmacokinetic parameters for oxytetracycline after single i.v. administration of 40 mg/kg bw to veal calves (n=5, SD = Standard Deviation); from Meijer et al. (1993)

	Calf						
	86	88	90	92	93	Mean	SD
Dose (mg/kg)	39.88	39.92	39.90	39.90	39.90	39.90	0.01
AUC (µg*h/l)	331.36	301.91	247.67	326.01	289.44	299.28	30.04
Cl (ml/h*kg)	120.35	132.22	161.10	122.39	137.85	134.78	14.63
$V_{d(area)}$ (ml/kg)	17125.48	11072.16	24513.92	21136.37	16872.50	18144.09	4520.96
A (µg/ml)	128.08	100.76	37.09	155.05	135.69	111.33	41.01
$T_{1/2\alpha}$ (h)	0.19	0.16	0.11	0.18	0.16	0.16	0.03
B (µg/ml)	27.51	20.05	13.01	26.27	25.59	22.49	5.38
$t_{1/2\beta}$ (h)	6.46	7.64	10.44	6.19	5.95	7.34	1.66
C (µg/ml)	0.23	0.64	0.26	0.26	0.28	0.33	0.15
$T_{1/2\gamma}$ (h)	98.61	58.03	105.45	119.68	84.82	93.32	20.92

Table 29. Individual pharmacokinetic parameters for oxytetracycline after single i.m. administration of 20 mg/kg bw to veal calves (n=5, SD = Standard Deviation); from on Meijer et al. (1993)

	Calf						
	86	88	90	92	93	Mean	SD
Dose (mg/kg)	19.95	19.95	19.95	19.91	19.97	19.95	0.02
C_{max} (µg/ml)	5.56	6.61	5.09	5.71	6.64	5.92	0.61
t_{max} (h)	5.47	5.47	7.43	5.50	7.45	6.26	0.96
AUC (µg*h/ml)	157.98	150.58	142.89	150.23	163.89	153.11	7.20
Cl (ml/(h*kg)	126.28	132.49	139.62	132.53	121.85	130.55	6.06
$V_{d(area)}$(ml/kg)	23512.47	24251.18	17076.50	14149.93	13716.48	18541.31	4517.16
A (µg/ml)	11.04	10.89	9.29	13.94	15.51	12.13	2.26
$T_{1/2\alpha}$(h)	9.88	8.85	10.18	8.86	8.64	9.28	0.62
B (µg/ml)	0.17	0.16	0.29	0.23	0.17	0.20	0.05
$t_{1/2\beta}$ (h)	129.03	126.85	84.76	73.99	78.01	98.53	24.27
$T_{1/2abc}$ (h)	1.96	1.12	2.29	2.51	1.43	1.86	0.52
F (%)	95.31	99.80	115.38	92.35	113.13	103.19	9.37

Table 29 shows that an absolute bioavailability (F%) of approximately 100% for OTC could be calculated from the data after i.m. administration of 20 mg/kg bw to calves.

Studies covering only the first 120 h after administration all show a bi-phasic elimination. This pattern is roughly the same for the 10% and 20% products (see figures below).

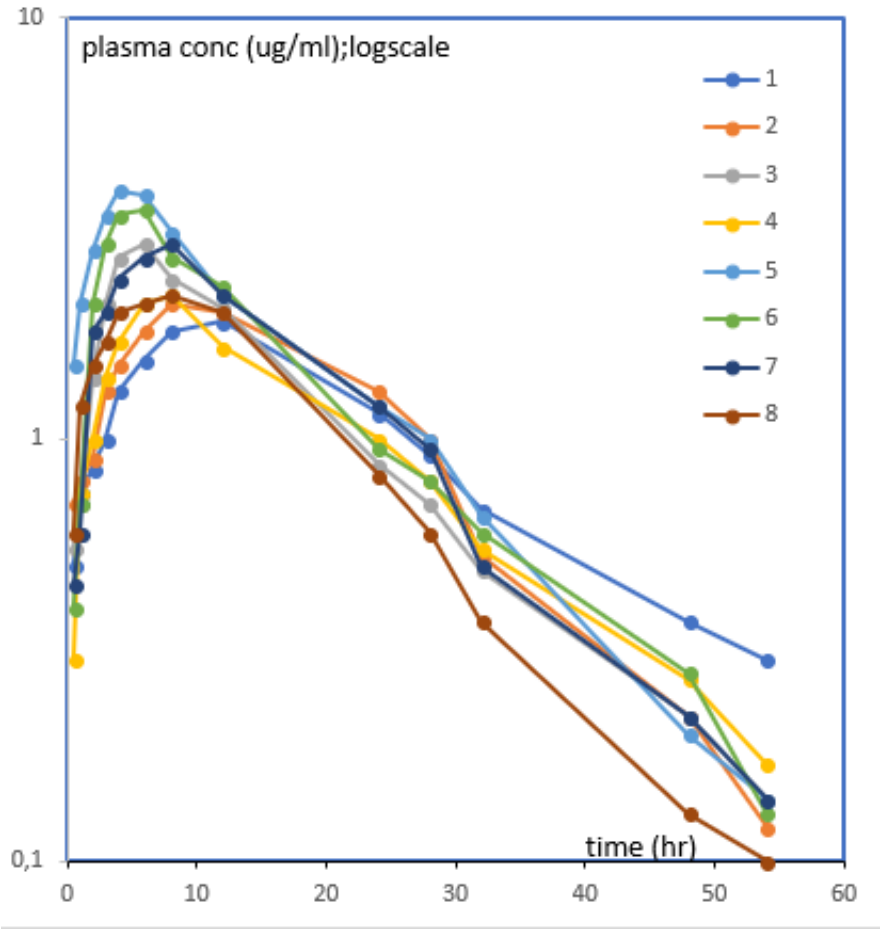

Figure 27. Mean plasma OTC concentration following intramuscular administration of eight different Oxytetracycline-10% formulations to dairy cows at a dose level of 5 mg/kg; based on Nouws et al. (1985)

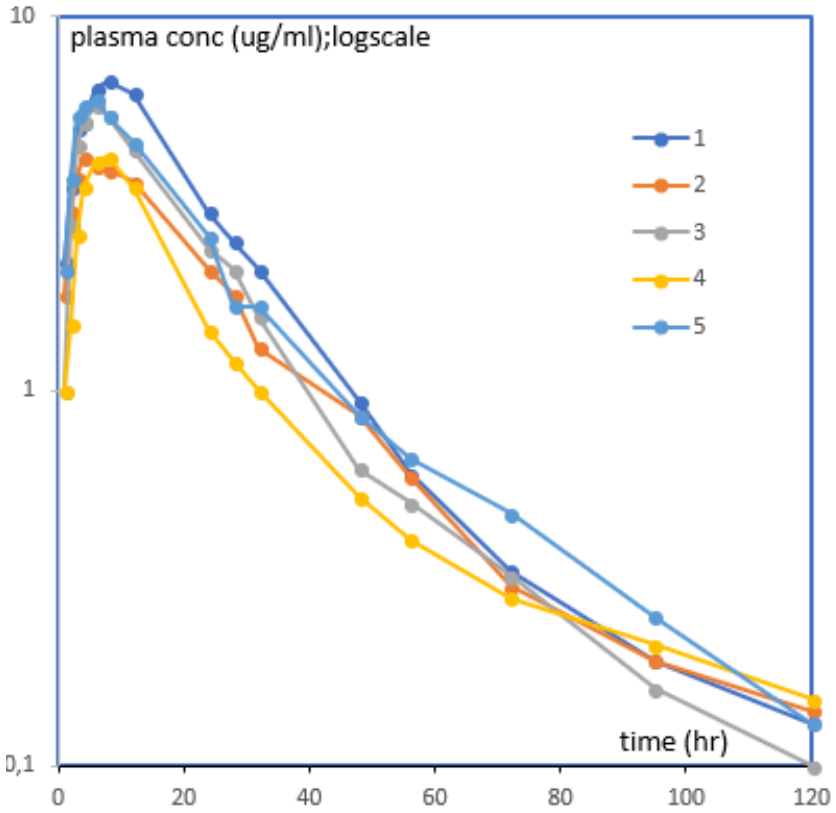

Figure 28. Mean plasma OTC concentrations following intramuscular administrations of five Oxytetracycline-20% formulations to dairy cows at a dose level of approximately 11 mg/kg bw; based on Mevius et al. (1986)

For the eight 10% formulations (i.m.) in Figure 27 the $T_{1/2}$ of first the elimination phase was 9-14 h during the first 60 h period (Nouws et al., 1985).

For the five 20% formulations (i.m.) in the $T_{1/2}$ of first elimination phase was 9–12 h when using data points <48 h. When the plasma concentrations were followed over a longer period of time (up to 120 h), a second phase could be detected ($T_{1/2}$= 25-44 h). It was noted that this phase probably was the result of the change-over situation from the first elimination phase to the final phase of 5-6 days (see Figure 26).

12.3.3. Intramuscular vs Subcutaneous administration

Studies (Clarke et al. (1999); study with product 20) comparing i.m. versus s.c. administration (see Figure 29 and Figure 30) show that the plasma kinetics for both routes of administration are highly comparable.

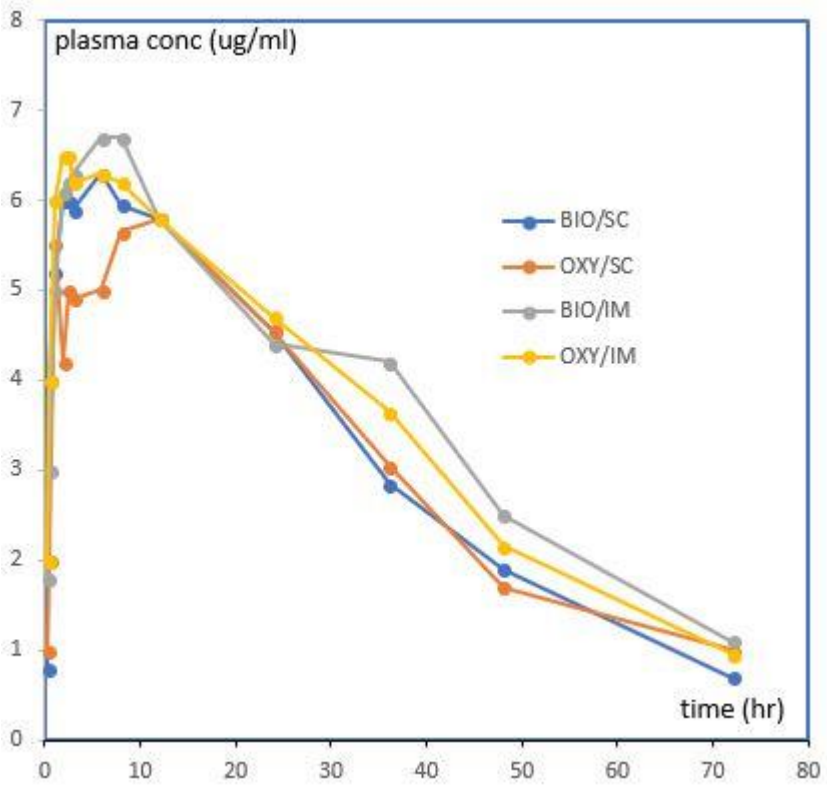

Figure 29. Serum concentrations of oxytetracycline after subcutaneous (s.c.) or intramuscular (i.m.) administration (20 mg/kg bw) of BioMycin 200 (BIO) or OXY shot LA (OXY) formulations to cattle. Data represent mean concentrations; based on (Clarke et al., 1999).

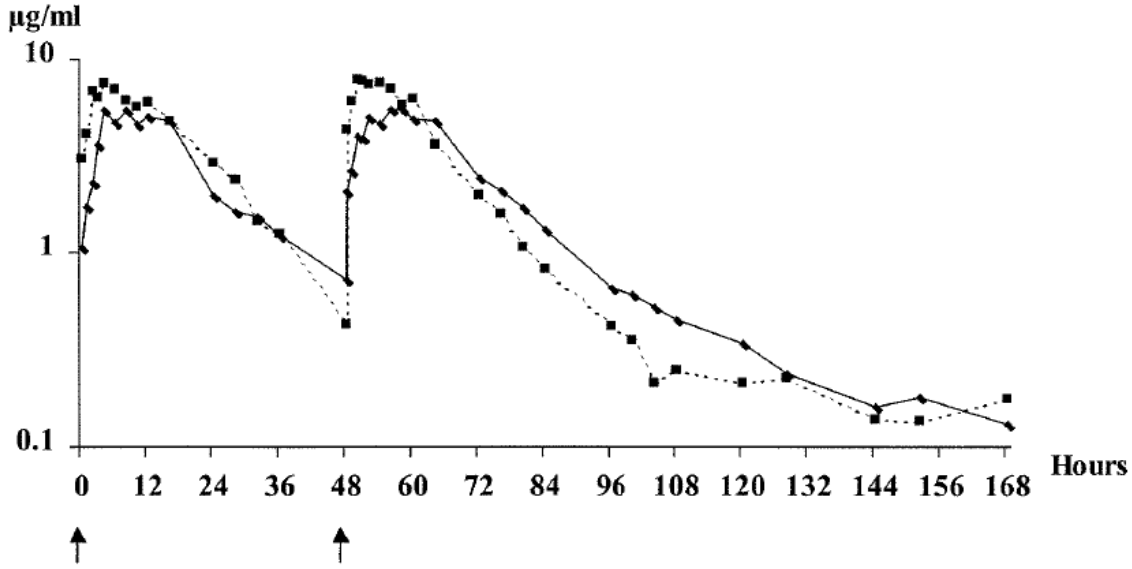

Figure 30. Plasma kinetics after s.c. (solid line) and i.m. (dashed line) administration of a 10% product to calves (study product 20) at a dose of 20 mg/kg bw

12.3.4. Dose linearity

One of the limiting conditions for using the extrapolation method is that linear kinetics must apply.

OTC is mainly excreted via the urine. Since the renal clearance shows signs of an active transport mechanism (tubular secretion) (Mevius et al., 1986) that potentially could lead to non-linear kinetical behaviour at higher plasma concentrations, the influence of the dose on the total body clearance had to be investigated (See Table 30).

Table 30. Listing of calculated total body clearances for OTC in the various studies

Dose (mg/kg)	Administration	CL (ml/kg.hr)	Bovine	Mean bw (kg)	reference
40	Iv	135*	calf	105	Meijer et al. (1993)
20	Im	130*	calf	105	Meijer et al. (1993)
20	Iv	66	calf	212-275	Toutain and Raynaud (1983)
20	Im	78	calf	372-420	Achenbach (2000)
20	Im	83	calf	372-420	Achenbach (2000)
20	Sc	90	calf	372-420	Achenbach (2000)
20	Sc	86	calf	372-420	Achenbach (2000)
5	Iv	43	cow	474-733	Nouws et al. (1985)
5	Iv	76	cow	415-665	Mevius et al. (1986)
11	Im	103*	calf	203-234	FARAD (1997b)
11	Sc	102*	calf	203-234	FARAD (1997b)
20	Im	77	steer	295-377	Clarke et al. (1999)
20	Im	79	steer	295-377	Clarke et al. (1999)
20	Sc	84	steer	295-377	Clarke et al. (1999)
20	Sc	87	steer	295-377	Clarke et al. (1999)

* From literature (Nouws et al., 1983) it is known that the total body clearance in young calves is significantly higher than in older animals.

These data suggest that both, dose and route of administration, have only a limited effect on total body clearance (mean clearance = 88 ± 23 ml/kg.hr). It should be noted that there were only data for few doses and only low numbers of animals per dose (especially given that the total body clearance might differ between young calves and older animals). Furthermore, unfortunately no information is available on the kinetic behaviour in the target tissues. However, in the case of OTC it is not expected that there will be dose-dependent changes in plasma/tissue ratios, and therefore for the purpose of this illustration, it is assumed that linear kinetics apply in all target tissues if the dose is increased moderately within the range of doses administered in these studies.

12.3.5. Maximum Residue Limits

The following EU MRLs were established for the marker residue oxytetracycline and its 4-epimer:

- Muscle: 100 µg/kg
- Liver: 300 µg/kg
- Kidney: 600 µg/kg
- Milk: 100 µg/kg

12.3.6. Residues in tissues

After first absorption the terminal depletion of residues in tissues runs parallel to the plasma curve. The highest concentrations of residues (apart from injection site) are found in kidney and liver.

As an example, the figure below shows the depletion curves as measured in the residue study of Product B. Only data points t>5 days are taken into account.

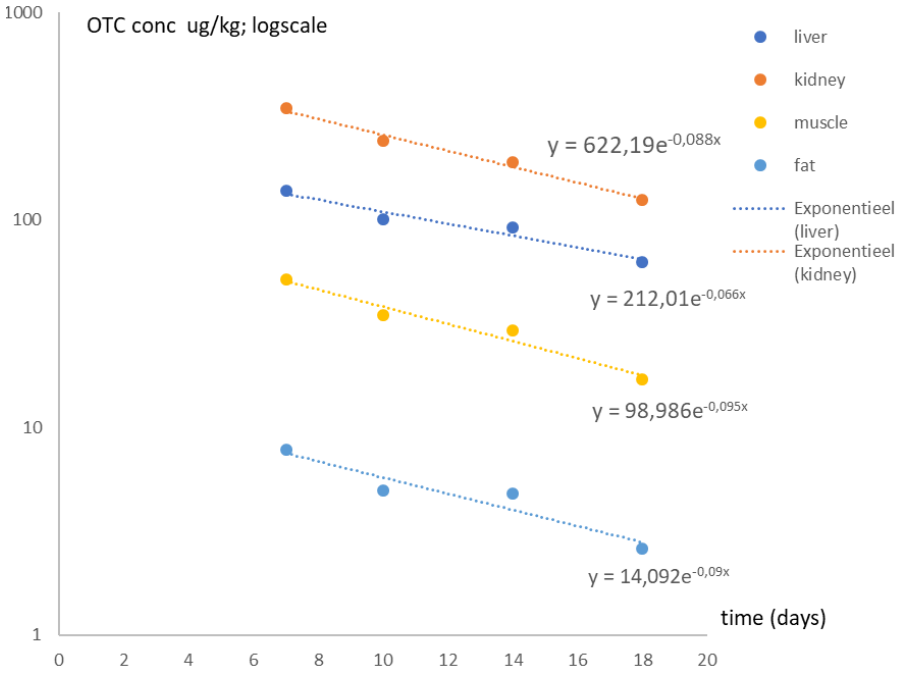

Figure 31. Residue depletion in cattle tissue following the last of 5 i.m. administrations with a 10% OTC injectable formulation at a dose of 10 mg/kg bw per day

Table 31 shows the estimated terminal $T_{1/2}$ values in the tissues from the analysed studies.

Table 31. Estimated $T_{1/2}$ values in the various tissues after administration of OTC for a number of products.

Product type	Adm	Total dose (mg/kg)	Tissue	$T_{1/2}$ (days)	Data source
10%	i.m.	50 (5x10)	Liver	10.5	Product B
			Kidney	7.9	
			Muscle	7.3	
			Fat	7.7	
20%	i.m.	20 (once)	Liver	4.5	Product A
			Kidney	3.9	
			Muscle	4.0	
			Fat	3.1	
20%	s.c	20 (once)	Kidney	5.4	Achenbach (2000)
			Liver	6.0	
20%	s.c.	20 (once)	Kidney	6.9	FARAD (1997a)
			Liver	6.9	
			Muscle	10.9	
20%	s.c.	20 (once)	Liver	4.2	FARAD (1999)
			Kidney	3.6	
20%	i.m.	36 (18 on day 1 and 3)	Kidney	5.5	Study Product 4
			Muscle	4.6	
			Fat	3.5	

A mean tissue half-life of 5.9 ± 2.3 days could be calculated.

Table 31 shows different half-lives for each tissue, which might result from:

- different slaughter points/time spans used in the studies
- incomplete distribution
- effects of different application routes
- different values (e.g. arithmetic means versus geometric means versus individual data) used to calculate half-lives
- combination of some of the points mentioned above

So **if** the withdrawal period for tissues would be determined by the depletion of OTC from the regular tissues and not by the depletion from the injection site, then a half-life of 6 days corresponding to the mean half-life of the various tissues listed in Table 31 is used in the extrapolation equation (Equation 2). It should be noted that a worst case half-life corresponding to the longest half-life of all tissues might be a more cautious choice as outlined in 4.4, especially if the withdrawal period determining tissue is not known.

12.3.7. Residues in the injection site(s)

Figure 32 shows the depletion of OTC from the injection site as measured in one of the studies (Achenbach, 2000), following the s.c. administration of a 20% product at a single dose of 20 mg/kg bw and with a maximum injection volume of 10 ml per injection site.

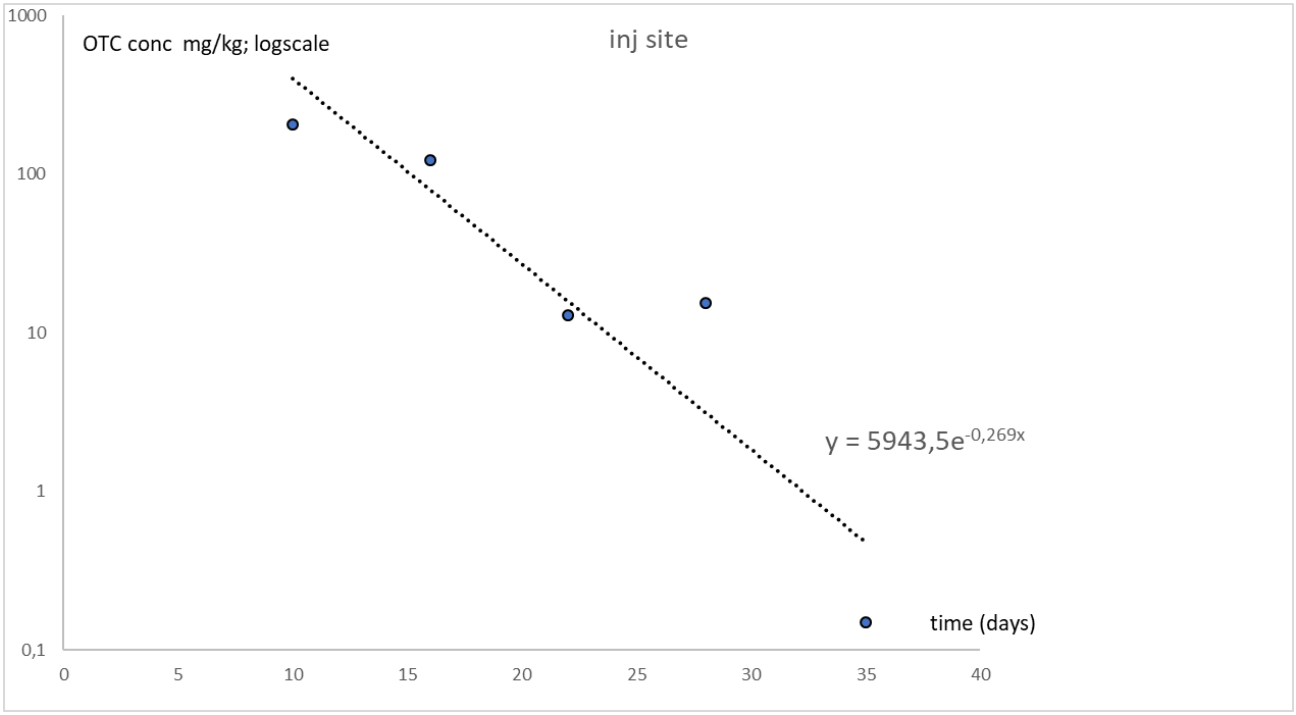

Figure 32. Mean OTC concentration (mg/kg) in injection site following the s.c. administration of a 20% product at a single dose of 20 mg/kg bw; from Achenbach (2000)

Table 32. $T_{1/2}$ values in the injection site for a number of products after kinetic analysis

Type product	Route of adm	ml/inj	$T_{1/2}$ (days)	reference
10%	im	15-20	1.1 and 1.9**	Product B
20%	im	10	1.2 and 1,6**	Product A
20%	sc	10	2.6	Achenbach (2000)
20%	sc	10	3.1	FARAD (1997a)
20%	sc	-	Not possible	FARAD (1999)
20%	im	10	1.1 and 2.9**	Study Product 4

** Inj sites Left and right side of the neck measured separately

Table 32 shows the estimates of the $T_{1/2}$ from the injection site for a number of products.

In calves 10 days after injection (10-20 ml) some 0-0.72% of the amount injected was left at the site of injection (Nouws et al., 1990).

Three theoretical scenarios could be considered as far as increasing the dose of OTC is concerned:

1. When dose increase can be performed by increasing the number of injection sites, maybe no change in WP for tissues would be necessary, but animal welfare could be at stake. As there is a huge difference in the half-lives of different tissues, it has to be checked carefully that no other tissue becomes WP determining. If the WP-determining tissue changes or is not known, the largest half-life amongst all tissues and the injection site should be taken and a new WP has to be determined.
2. When increasing the dose would be performed by increasing the injection volume then an alternative approach would be necessary (see below). In this situation animal welfare (too large injection volumes, irritation) could also be at stake.
3. Dose increase could also be achieved by limiting the maximal weight of the animal to be treated. In that case (if the max volume remains unaltered) no change in WP would be needed, as long as the increase in dose does not result in a change of the withdrawal period determining tissue.

12.3.7.1. Proposed approach of WP extrapolation in case of an increase of injection volume/injection site

Figure 33 shows the relation between max dosing volume and withdrawal period for tissues for the originator products listed in Table **26** (the generics were not taken into account).

The influence of the injection volume on the WP seems to be marginal. This would seem to be a rather controversial conclusion. For example, injecting twice the amount on the site of injection, theoretically would lead to a higher WP. The explanation for the WP-data not showing this probably lies in the fact that in many cases the WP was established using a large safety factor to account for deficiencies in the studies. It might also arise due to differences in formulations or differences in the study population. All these factors could obviously mask the effect of an increase in injection volume.

Although the influence of the injection volume on the WP seems to be marginal in the present dataset, it is proposed that in case of a increased injection volume, in the extrapolation equation (Equation 2) the half-life of 6 days from the tissue depletion data are used since $T_{1/2}$ for injection site was found to

be significantly smaller than this (see Table 32), and bioavailability new dose/old dose is assumed to be equivalent.

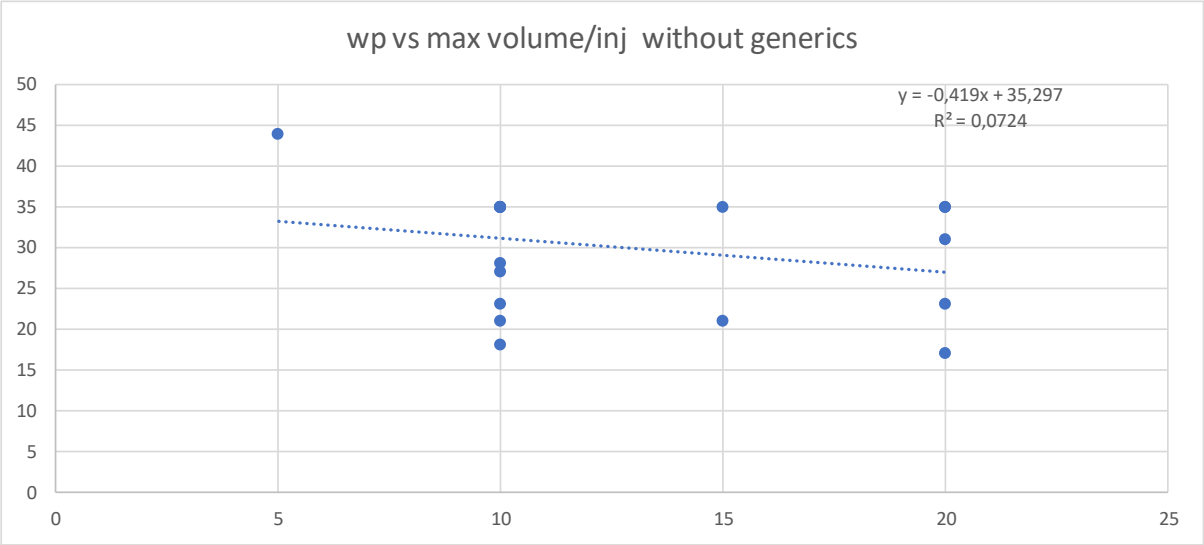

Figure 33. The withdrawal period (y-axis, in days) for cattle of various oxytetracycline injectable VMPs as a function of the injection volume per injection site (x-axis, in ml)

12.3.8. Residues in milk

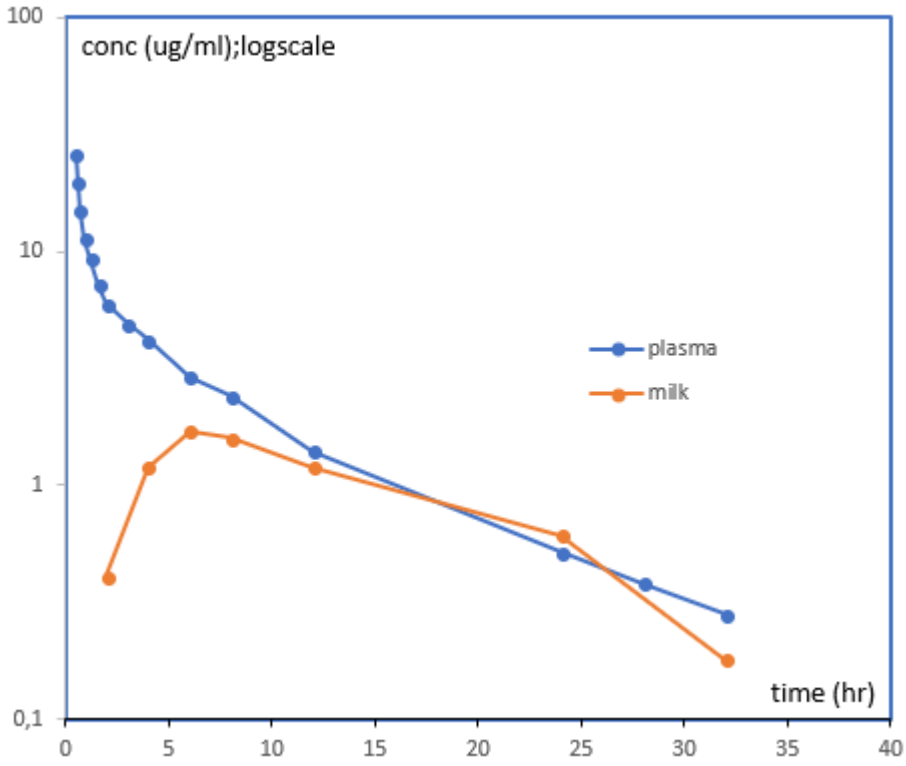

Figure 34. Oxytetracycline concentrations in plasma and milk (mean.) following intravenous administration of Engemycine-10% and Oxysentil® at a dose of ~5 mg/kg bw; based on Nouws et al. (1985)

In Figure 34, after an initial rise, the time dependent course of the concentration of OTC in milk evolves similarly to the concentration in plasma. This pattern was confirmed by other data from Nouws

(see Figure 35 and Figure 36). The ratio milk/plasma was reported to be in the range of 1 to 2 (Nouws et al., 1985).

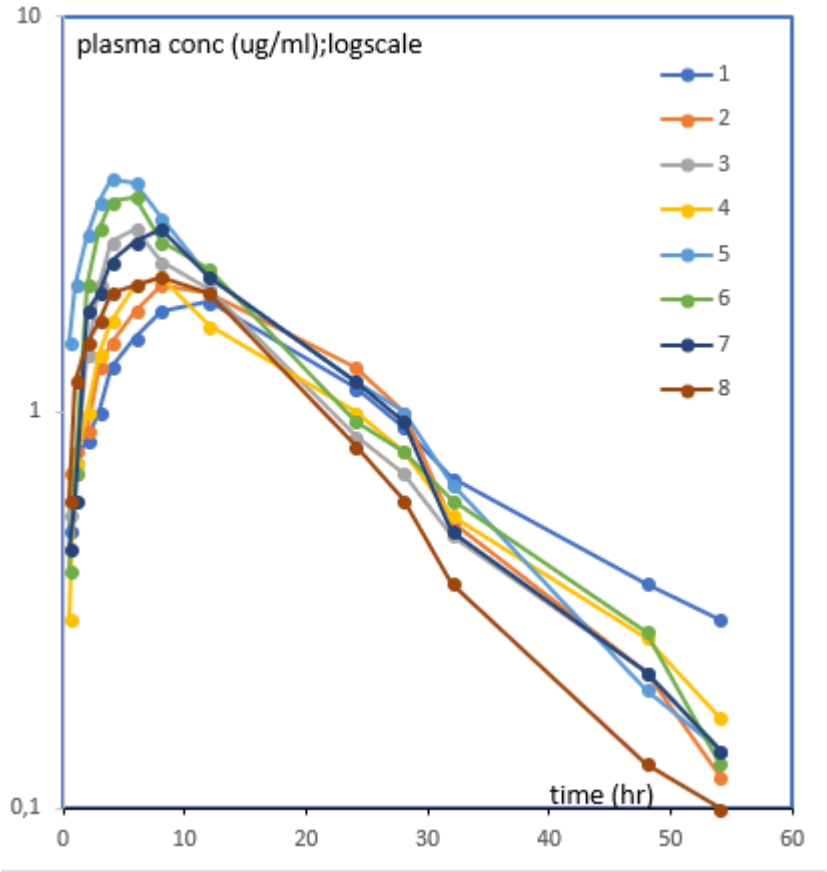

Figure 35. Mean plasma OTC concentrations following muscular administrations of eight different Oxytetracycline-10% formulations to dairy cows at a dose level of 5 mg/kg bw; based on Nouws et al. (1985)

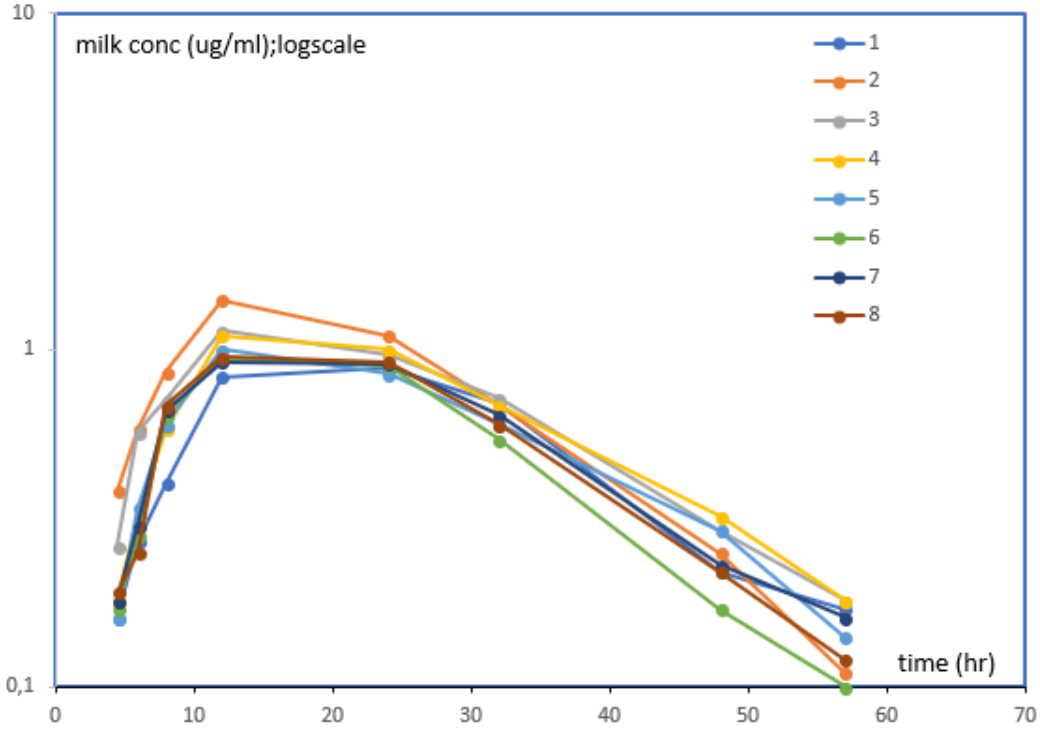

Figure 36. Mean milk OTC concentrations following muscular administrations of eight different Oxytetracycline-10% formulations to dairy cows at a dose level of 5 mg/kg bw; based on Nouws et al. (1985)

In the figure below it is shown that when the milk concentration curve is monitored for a longer period of time, again (as expected) a long (approx. 6 days) terminal depletion phase can be observed (study 6), comparable to the one seen in plasma.

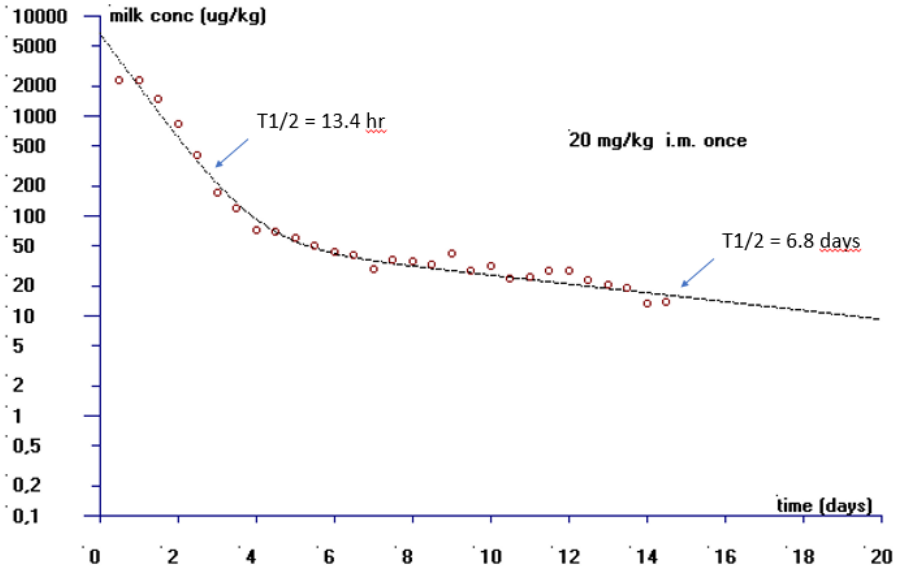

Figure 37. Depletion of mean OTC concentrations in a cow's milk over time after a single intramuscular injection of OTC at a dose of 20 mg/kg bw; data from animal no.6 in Study 6

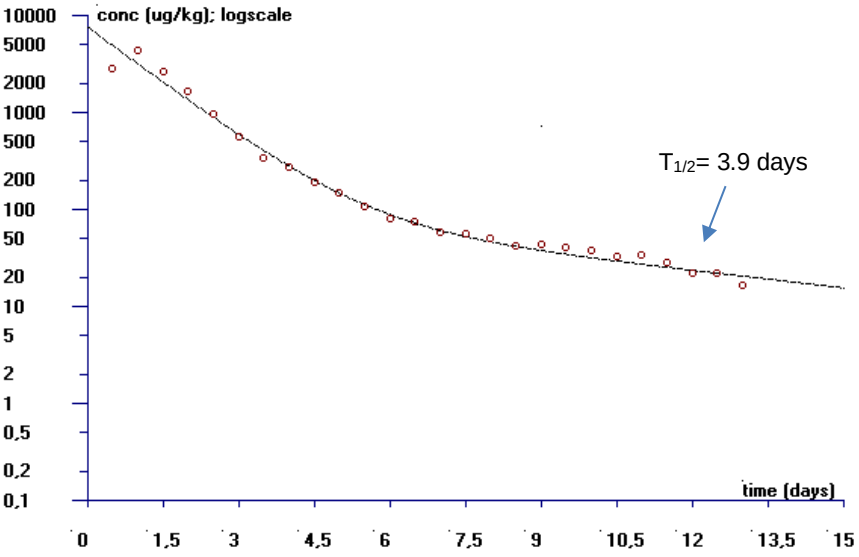

Figure 38. Depletion of OTC mean concentrations in cow's milk over time after a single intramuscular injection of OTC at a dose of 20 mg/kg bw; data from all 10 animals in Study 6

Since the depletion curve of OTC residues in milk, runs parallel with the plasma and tissue concentrations and due to a lack of data, as a pragmatic approach the half-life of 6 days (mean calculated from the tissue depletion data of various tissues) is used in the extrapolation equation (Equation 2).

12.3.9. Withdrawal time calculation

The new withdrawal periods were calculated using Equation 2. It is assumed that linear kinetics are given for the considered dose ranges (see Section 12.3.4. for more details) and that distribution is complete at MRL level.

Using PK/PD methods for the 10% formulations an adjusted dosing schedule of 10 mg/kg bw daily during 3-5 days was set, for the treatment of Bovine Respiratory Infection.
For the 20-30% formulations (long acting) an adjusted dosing schedule 20 mg/kg bw administered twice with an interval of 36-48 h was set. Table 33 and Table 34 list the products that need an adjustment of their current dosing schedule.

Table 33. OTC injectables (10% formulations) authorised in NL for bovine respiratory disease having a dose below 10 mg/kg bw per day

VMP no	MA Type	WP tissue (days)	WP milk (days)	Dose (mg/kg)	Duration (days)	max inj vol (ml)	Adm, route
6	10%	18	5	8	5	10	im
9	10%	35	4	4	3	20	im
10	10%	35	4	4	3	20	im
11	10%	35	10	4	3 to 5	10	im
13	10%	35	10	4	3 to 5	10	im
21	10%	35	4	4	3	20	im
22	10%	35	10	4	3 to 5	10	im

Table 34. OTC injectables (20%-30% formulations) authorised in NL for bovine respiratory disease having a single dose schedule that has to be extended to a second dose 36-48 h after first dose

VMP no	MA Type	WP tissue (days)	WP milk (days)	Dose (mg/kg)	Duration (days)	max inj vol (ml)	Adm, route
1	LA 30%	35	10	20, 30	1	7,5 and 10	im
2	LA 30%	35	10	20, 30	1	7,5 and 10	im
12	LA 30%	35	9	20	1	10	im
14	LA 20%	35	13	20	1	10	im
23	LA 20%	27	13	20	1	10	im

For the 10% formulations, increasing the OTC dose from 4 to 10 mg/kg bw by an increase of the number of injections would lead to no changes in withdrawal periods for tissues of these products, assuming that the injection site is still the withdrawal period determining tissue and the diffusion from injection sites does not significantly decrease as plasma concentrations increase. For milk a $T_{1/2}$ of 6 days (corresponding to the mean $T_{1/2}$ in tissue) would be used in Equation 2 and assuming, that

bioavailability of different formulations is the same, leading to an addition of 6 days for each doubling of the dose.

The two other possible scenarios for increasing the dose that could be considered are specified below. The $T_{1/2}$ value was set to 6 days in case of scenario 1. In both scenarios a maximum of 3 injections per day was used for animal welfare reasons.

1. Increasing the dose could be performed by increasing the injection volume. In this situation animal welfare (too large injection volumes, irritation) could also be at stake, so the maximum injection volume was set to 20 ml per injection site. The results are listed in Table 35.
2. Dose increase could also be achieved by using a maximum number of injections of 3 and subsequently limiting the maximal weight of the animal to be treated. In that case (if the max injection volume would remain unaltered) no change in WP would be needed for tissues as long as the injection site remains the WP determining tissue (assumed for this case study) and since residues at the IS are unchanged. For milk Equation 2 can be used. The results are listed in Table 36.

Table 35. Extrapolated WPs for the 10% formulations for a dose of 10 mg/kg bw, using a maximum number of injections of 3 and adjusting the maximum injection volume to 20 ml when possible

VMP No	MA Type	Dose (mg/kg)	WP tissue old (days)	WP milk old (days)	WP tissue new (days)	WP milk new (days)	Max, weight (kg)
6	10%	8	18	5	24	7	600
10	10%	4	35	4	35	12	600
11,13	10%	4	35	10	41	18	600
21,9	10%	4	35	4	35	12	600
22	10%	4	35	10	41	18	600

Table 36. Extrapolated WPs for the 10% formulations for a dose of 10 mg/kg bw, using a maximum number of injections of 3 without altering the maximum injection volume, and the resulting introduction of a change of maximum bodyweight

VMP No	MA type	Dose (mg/kg)	WP tissue remains (days)	WP milk old(days)	WP milk new (days)	max inj vol (ml)	Max weight (kg)
6	100%	8	18	5	7	10	300
10	100%	4	35	4	12	20	600
11,13	100%	4	35	10	18	10	300
21,9	100%	4	35	4	12	20	600
22	100%	4	35	10	18	10	300

For the 20-30% formulations the repeated injection to different injection sites would lead to no changes in withdrawal periods for tissues of these products, assuming that the injection site remains the withdrawal determining tissue and the diffusion from injection sites does not significantly decrease as plasma concentrations increases due to a change in the concentration gradient between injection site and blood plasma. For milk a $T_{1/2}$ of 6 days would be used in Equation 2, leading to an addition of 6 days to the withdrawal period for each doubling of the dose. Taking into account the interval of 36-48 hours between the two doses, where a certain fraction if the first dose is already eliminated at the time the second dose is given i.e. at that time point the residues are less than doubled, it could be calculated that as a worst case it still would lead to an increase of 6 days. Table 37 shows the resulting withdrawal periods.

Table 37. Extrapolated WPs for the 20%-30% formulations for a dosing schedule that was extended to a second dose 48 h after first dose

VMP no	MA Type	Old WP tissue (days)	Old WP milk (days)	Dose (mg/kg)	Old schedule (days)	New schedule (days)	New WP tissues (days)	New WP Milk (days)	max inj vol (ml)
1	30%	35	10	20,30	1	1 and 3	35	16	10
2	30%	35	10	20,30	1	1 and 3	35	16	10
12	20%	35	9	20	1	1 and 3	35	15	10
14	20%	35	13	20	1	1 and 3	35	19	10
23	20%	27	13	20	1	1 and 3	27	19	10

12.4. Environmental risk assessment

Because there may be different authorised doses for the same or similar products, as a general rule, the situation for the product with the highest authorised (total) dose for the same target animals is used for the comparison, provided that an ERA exists for that product at that dose for the relevant target species. In the case of oxytetracycline injectable products for cattle, ERAs are available addressing the risks at a single dose of 20 mg/kg bw.

12.4.1. Step 1: Determine the assessment situation for oxytetracycline

In accordance with the PK/PD modelling (see 12.1.), the adjusted dose for LA oxytetracycline injectable products for the treatment of respiratory disease in cattle is a single dose of 20 mg/kg bw, to be repeated after 48 hours. For SA formulations, the adjusted dose is 10 mg/kg bw per day for 3-5 days. The SA formulations have the highest total dose (5 times 10 mg/kg bw = 50 mg/kg bw), so the use of SA formulations would lead to the highest environmental exposure.

In the available Phase IIA assessments (based on a single dose of 20 mg/kg bw), fate and effect studies were considered, and the RQs were determined for the various test species representing the terrestrial and aquatic environments. The RQs for terrestrial species were in the range of 0.002-0.17, and the RQs for aquatic species were in the range of 0.00003-0.01.

In view of the information given above, in was concluded that dose increases up to a total dose of 100 mg/kg bw would still result in RQs lower than 1. In addition, this dose level would not result in a PEC_{gw} higher than 0.1 µg/L. This means that the two optimised dosing regimes of 2 x 20 mg/kg bw for the LA formulations and of 5 x 10 mg/kg bw for the SA formulations will not give rise to concerns in relation to environmental risks. Further consideration of steps 2-8 of the proposed approach was not necessary.

It was concluded that the dose review and adjustment for oxytetracycline does not lead to additional environmental risks.

12.4.2. Conclusion on the ERA for oxytetracycline

The dose adjustment for oxytetracycline does not lead to additional environmental risks.

12.5. Target animal safety

The dosing regimens for oxytetracycline injections for cattle are variable, with 10% formulations being administered at lower doses, generally 4–20 mg/kg, for 1 to 5 days, and 20% formulations mostly being administered on a single occasion at a dose of 20 or 30 mg/kg, but with the possibility to repeat after 48 or 72 h. According to the outcomes of the PK/PD modelling, the following dosing regimens are suggested:

10% formulations: 10 mg/kg, every 24h for 5 days

20% formulations: 20 mg/kg repeated once after 36-48 h

Products may be administered by either SC or IM injection. According to residues studies (see 12.3.3.) plasma kinetics are similar for IM and SC administration, therefore studies using either route can be considered together for review of the systemic safety.

12.5.1. Step 1: Determine the target animal safety profile for the active substance and establish the MOS for the active substance according to the revised dose, pharmaceutical form and route of administration

Review of the TAS studies provided by MAHs

Margin of Safety studies

Studies not available to the pilot project

Reproductive safety studies were not available to the pilot project.

Local injection site safety studies

'Product OTC1' is a long acting (LA) formulation containing 200 mg OTC per ml.

Study reports (n=27) were provided for investigations of local (injection site) tolerance. In the first series of studies, >2000 cattle received either a control product (immediate release formulation containing either 50 mg or 100 mg OTC/ml) at 10 mg/kg bw, intramuscularly, or Product OTC1 at the recommended dose of 20 mg/kg bw, intramuscularly, except for 25 animals which received OTC 1 at 44 mg/kg bw in error. Observations related to clinical signs and histopathology of injection site (IS) lesions, only.

The signs observed in 2389 animals treated with either OTC1 or control included: Pain on injection, injection site swellings that in some cases were still visible at 24 h, but reduced at 48 h; salivation, trembling (and 2 cases of collapse with immediate recovery). There was no increase in adverse events in animals administered OTC1 at 44 mg/kg bw.

A second series of studies focused on histopathological findings at the IS 28 days after administration of 'Product OTC1' at the RTD (20 mg/kg bw IM) to 74 animals in total. Either 10 ml or 20 ml was administered at each IS. For the 20 ml injection volume, there were 56% of sites that were sub-optimal, whereas for 10 ml volume, only 5% of sites were sub-optimal. The 10 ml volume was also tolerated by calves (>100 kg weight).

'Product OTC2' is a formulation containing 200 mg OTC per ml, administered as a singleinjection. A single study was provided for which one of the aims was to investigate injection site safety of two slightly different formulations. The product was administered to 10 animals by IM injection at a dose of 20 mg/kg bw with a maximum injection volume of 10 ml per site.

There were local reactions which varied from slight to severe in all 10 animals after injection but had mostly resolved clinically after 1 week; although it is not clear, these reactions may have caused the

animals to appear lethargic for approximately 2 days after injection. Inflammatory IS reactions were still present in most animals at necropsy after 2 to 3 weeks.

Palatability studies: Not applicable.

Conclusions: Full study reports of TAS studies investigating dose multiples for MOS and reproductive safety for the products were not available to the pilot project. In one proprietary study, OTC was administered in error at a dose of 44 mg/kg bw to 25 animals. Although there was no increase in adverse events, this study evaluated clinical signs only. Further conclusions on the target organs and toxicity profile or the MOS cannot be made based on the studies available.

Multiple proprietary intramuscular IS safety studies were provided for one 20% formulation (including other OTC formulations as controls) and a single study investigated IS safety of two versions of another 20% formulation.The studies were not fully compliant with VICH GL43 in that a negative control group was not included. Histopathology demonstrated inflammatory lesions that persisted for up to 28 days. No IS studies were available from MAHs to the pilot project investigating the SC administration route.

It is apparent that OTC injections (regardless of strength) are irritating and there is a rationale to restrict the IS volume. It seems plausible that oxytetracycline itself is an irritant, although tolerability to individual formulations may be affected by their excipient composition.

12.5.1.1. Step 1a: Review supplementary data from dossiers, if needed e.g. dose-finding studies

Data not available to the pilot project.

12.5.2. Step 2: Safety in the target population

Data not available to the pilot project.

12.5.3. Step 3: Safety based on post-marketing pharmacovigilance

Data not available to the pilot project.

12.5.4. Step 4: Safety based on published literature and authorisations in third countries

Literature review – A review was conducted using PubMed and the terms <oxytetracycline> <cattle> and <toxicity> or <safety>.

In a study from Terhune and Upson (1989), 30 healthy calves were administered OTC LA formulation at 40 mg/kg bw IM. Reactions and toxicosis were limited to anaphylaxis (n=1) and IS swellings (n=2).

Textbooks

Prescott & Dowling states that in animals tetracyclines are irritants and may cause damage at injection sites (Giguère et al., 2013). Calcium-binding may cause acute cardiac toxicity. Anhydrotetracyclines damage plasma membranes and bind to serum albumin.

Plumb's Veterinary Drug Handbook (6th Ed) (Plumb, 2008) indicates that tetracyclines are excreted in milk in a ratio of milk:plasma of 0.25 to 1.5.

Grey literature

Information available from SPCs of EU-authorised products

SPC 4.3 – Contraindications: Several products include contraindications from use in animals suffering from renal or hepatic damage or with known hypersensitivity to oxytetracycline.

SPC 4.9 – Dosing and administration: Several products include restrictions on the injections volume at any one site from between 10 to 20 ml.

SPC - warnings for the target spp.

Warnings relate to possible occurrence of gastrointestinal disorders, allergic reactions, photosensitivity, hepatotoxicity, nephrotoxicity, tooth discolouration and injection site reactions. The incidence of adverse events is not clear from the SPCs of these long-authorised products.

Concerns also relate to use during pregnancy and effects on foetal development. For one product it is advised that although oxytetracycline is excreted in the milk, concentrations are generally low and the product 'can be safely administered to lactating animals'.

OTC is reputed to have 'low general toxicity' although the MOS is not available from SPCs.

CVM FOIA reports

NADA 113-232 Liquamycin LA 200

In the USA Liquamycin LA-200 is authorised for treatment of pneumonia in cattle at a single dose of 20 mg/kg bw, or for other indications at 6.6–11 mg/kg bw for 4 days.

Study 2532D-60-96-164 investigated the local safety of SC injection at 20 mg/kg bw as part of a residues depletion study in 26 calves with average weight 253 kg. SC injections resulted in transient swellings from as early as D1. These peaked at D7 but resolved clinically without intervention. The SC route resulted in smaller lesions than IM. Histopathological exam revealed that lesions did not completely resolve within the 28 day WP.

NADA 141-312 Hexasol injection (OTC 300 mg/ml + flunixin meglumine 20 mg/ml)

P-FLO-020 investigated the safety of Hexasol when administered at 0, 1x, 3x and 5x the RTD of 29.9 mg OTC + 2 mg flunixin/kg for 3 administrations at 72 h apart to 24 M/F calves (6/group) aged 3 to 5 months and weighing 100 to 147 kg. There was a dose-dependent increase in AST to 5x ULN until D7; no evidence of hepatotoxicity was found and this was considered to be related to muscle inflammation. Creatinine and urea increased in the 5x group and peaked at the high ULN at D4. 2 calves in the 5x group had much higher levels and were euthanised on D7; examination of the kidneys detected cortical tubular necrosis consistent with mild renal toxicity.

Conclusion - This study showed that a dose of 90 mg oxytetracycine/kg bw (n=6), repeated on 3 occasions at 72 h apart, was a 'no effect level' for renal toxicity; pathology was present at 150 mg/kg bw. A dose of 150 mg/kg bw was a no effect level for liver toxicity. The combination with flunixin may have impacted the systemic and local safety profile.

NADA 141-143, 2003 Tetradure 300 and Oxytetracycline Injection containing oxytetracycline 300 mg/ml

Published data from Griffin et al. (1979), Lairmore et al. (1984), Riond and Riviere (1989), Terhune and Upson (1989), Vaala et al. (1987), were considered.

079/96: A GLP TAS study to investigate the safety of Oxytet 30 following IM injection to cattle. OTC was administered at 1x, 2x and 4x the RTD of 30 mg/kg bw on 3 occasions at 72 h apart to 24 cattle aged 6 to 9 months and weighing 214 to 286 kg. A maximum injection volume was 10 ml per IS.

Localised IS reactions were noted in all groups and reflected the total dose administered with the highest incidence of lameness in the 4x group.

Anorexia was observed in the 4x group after the 3rd injection and lasted 8 days.

The most notable findings were increased urea and creatinine in the 4x group which was accompanied by histopathological changes indicating renal dysfunction detected at necropsy at D21. No post-mortem changes were noted in the 1x and 2x groups. No hepatic pathology was noted.

Conclusion - This study showed a no effect level for renal toxicity up to 60 mg/kg bw (n=8), repeated on 3 occasions at 72 h apart; renal pathology was seen at 120 mg/kg bw.

041/95: GLP PK study to support safety of IV and IM administration of Oxytet 30 at 30 mg/kg bw dose. The study involved 12 cattle weighing from 409 to 441 kg. No evidence of collapse, neurological effects or changes in gait were observed. Hardness and swelling were noted to varying degree at IS for both routes, but resolved by D 28.

089/96: GLP IS safety study. A dose of 30 mg/kg bw and 60 mg/kg bw was administered IM at a max of 10 ml/site on 3 occasions at 72 h apart in the neck, rump and leg. IS were monitored and examined by histopath at 15 days after the final injection. No IS reactions were noted at the neck sites, although some localised tissue necrosis may still be present at 21 days.

Overall conclusions – In regards to extrapolation of safety data from formulations used in literature studies, it has to be considered that the exact formulation of these products is not be available; however, the PK of several oxyteracycline 'solution for injection' formulations was reviewed in chapter 12.2.1. , with the conclusion that no major differences in the PK would be expected. In chapter 12.3.3. it was noted that PK profiles are similar after IM and SC administration. Based on the TAS studies available, there appears to be a 'no effect level' up to 60 mg oxytetracycline/kg bw after IM injection repeated on 3 occasions at 72h intervals, above which there may be impacts on renal function. It should be considered that this conclusion is based on findings in small numbers of animals.

Consistent with the studies provided by MAHs, literature studies with Liquamycin 200 LA and Tetradure 300 formulations identified inflammatory injection site reactions at recommended treatment doses that persisted on histopathology for up to 28 days. In a study investigating the Liquamycin LA 200 formulation, the SC route resulted in smaller injection site lesions compared with the IM route.

12.5.5. Step 5: Conclude on the safety of the increased dose of the active substance according to the pharmaceutical form and route of administration

The data available indicate that OTC has renal toxic effects with a NOEL at 60 mg/kg bw by intramuscular administration. As the pharmacokinetics are similar for the SC and IM route of administration, this NOEL can be extrapolated to SC administration.

No studies were available to the project addressing the reproductive safety of oxytetracycline.

Irritant effects limit the volume that can be administered at each IS, and this may vary with the formulation and route (SC vs. IM) of administration. For some 200 mg/ml formulations, the maximum IS volume stated in the SPC is 10 ml. Where this is based on local safety reasons, this should be taken into account if there is a dose increase that might lead to a need for multiple injections.

12.5.6. Step 6: Further considerations for the conclusion on the safety and benefit-risk for individual products

The following excipients have been included in different EU-authorised formulations:

- 2-Pyrrolidone
- Benzylalcohol
- Citric acid monohydrate
- Dimethylacetamide
- Disodium Edetate Dihydrate Ethanolamine
- Glycerolformal
- Hydrochloric Acid
- Macrogol 1500
- Magnesium Chloride Hexahydrate
- Magnesium Oxide
- Methyl-4-hydroxybenzoaat (E218)
- Monoethanolamine
- N-methyl-2-pyrrolidone
- Polyethylene Glycol 200
- Povidone K 17
- Propyl-4-hydroxybenzoaat (E216)
- Sodium formaldehyde sulphoxylate dihydrate

The excipients may impact on local tolerance and this should be taken into account on a product-by-product basis if a dose change is needed.

12.5.7. Step 7: The conclusions above are incorporated into the final benefit-risk for the dose increase for each individual product

For oxytetracycline injections, the adjusted doses suggested by the PK/PD modelling for the treatment of bovine respiratory disease fell within the range of doses already approved for different EU 10% and 20% formulations, with the only modification being a reduction in the interval for repeat injections of the 20% formulations from 48-72 h to 36–48 h.

The data available indicate that oxytetracycline has renal toxic effects which manifest above a dose of 60 mg/kg bw administered IM (repeated on 3 occasions) – this would impact on the scope for any dose increase. No specific MOS studies were provided to address repeated administration at an interval of 36 h for the 20% formulations. This could be a limitation; however, the suggested dose of 20 mg/kg bw repeated once after 36 h (total 40 mg/kg bw) for 20% formulations is expected to give a C_{max} and overall exposure below the threshold for renal toxicity, and therefore is likely to be adequately tolerated in cattle for the treatment of the indication for respiratory disease. In addition, some 10% formulations are already approved at doses up to 20 mg/kg bw daily for 5 days, supporting any risk for cumulative toxicity from the single repeated injection at 36 h of the 20% products at the same dose.

No data were available to support reproductive safety for the reduced dosing interval for the 20% formulations. This may need to be mitigated by additional warnings in SPC 4.7.

In terms of those 10% formulations for which the dose of 10 mg/kg bw represents a dose increase, it may be of more practical significance that local irritant effects can limit the volume that can be administered at each injection site. The maximum tolerated injection volume may vary with the formulation and route (SC or IM). It is suggested that the maximum dose volume at any site should not exceed that already stated in the SPC for individual products, or where not stated should be based on a review of the TAS data for the individual product. The number of injections that can practically be administered would have to be taken into account and could result in a restriction on the maximum bodyweight of animal for which a product could be used.

12.6. Overall conclusion on oxytetracycline

The approaches on dose review and adjustment, WP, ERA and TAS as described in chapters 3, 4, 5, and 6, respectively, were tested in the case study on oxytetracycline products, administered by injection, for the treatment of respiratory infections in cattle, including lactating cattle. The solution for injection is available in 10% ("short acting") and 20% ("long acting") formulations. The approved doses are 4–20 mg/kg bw per day, daily injection for between 1 and 5 days for the 10% formulations, and 20 or 30 mg/kg bw, single injection, repeated after 48 or 72 hours in severe cases for the 20% formulations.

It should be noted, that the outcome of the dose review was based on a limited amount of data, gathered from public sources or provided by industry. Assumptions necessary for applying the methodology introduced in this reflection paper could not always be checked, at some places not fully correct methods were used for pragmatic reasons. Therefore, the numerical results (e.g. adjusted dose, WP etc.) are merely indicative, and do not reflect a final outcome (e.g. after a referral in which all related VMP authorised in the EU are included). In addition, the case studies, including the calculations, should not be regarded as reflecting the only possible or definitive methodology.

In order to review and adjust the dose, the following pathogens were considered to be relevant: *Pasteurella multocida*, *Mannheima haemolytica* and *Haemophilus somni*.

Because formulation-specific differences in PK may exist, the compositions and the PK of various products were analysed, revealing no significant differences in PK. However, the difference in strength will require different injection volumes which may impact on the absorption kinetics. Therefore, the PK/PD analysis was done for the 10% and 20% formulations separately.

The adjusted calculated doses for the 10% and 20% formulations were 10 mg/kg bw and 20 mg/kg bw, respectively. These doses fell within the range of doses already approved for authorised products in the EU, with the only modification being a reduction in the interval for repeat injections of the 20% formulations from 48-72 h to 36–48 h.

For the establishment of the WP, a half-life of 6 days was used for the extrapolation of WPs for both tissues and milk, resulting in low to moderate increases of the WPs.

For addressing the environmental risks, adequate Phase I and Phase II ERA data were available for the authorised dose of 20 mg/kg bw. For the adjusted doses (5x10 mg/kg bw or 2x20 mg/kg bw), the RQs remained below 1. Therefore, the adjusted doses for oxytetracycline do not give rise to any additional concerns for the environment.

In relation to TAS, the data available indicate that oxytetracycline has renal toxic effects which manifest above a dose of 60 mg/kg bw (repeated on 3 occasions) – this would impact on the scope for any dose increase. The suggested dose of 20 mg/kg bw repeated once after 36 h (total 40 mg/kg bw)

for 20% formulations is expected to give a C_{max} and overall exposure below this threshold for renal toxicity and 10% formulations are already approved for administration at 20 mg/kg with a 24 h interval between doses. Therefore, the revised dose interval is likely to be adequately tolerated in cattle for the treatment of respiratory disease.

In terms of those 10% formulations for which the dose of 10 mg/kg bw represents a dose increase, it may be of more practical significance that local irritant effects may limit the volume that can be administered at each injection site. The maximum tolerated injection volume may vary with the formulation and route (SC or IM). It is suggested that the maximum dose volume at any site should not exceed that already stated in the SPC for individual products, or where not stated should be based on a review of the TAS data for the individual product. The number of injections that can practically be administered would have to be taken into account and could result in a restriction on the maximum bodyweight of animal for which a product could be used.

13. Annex 3: Data available for PK/PD analysis

From MAA applications, extensions, variations			
Study type	Main objective	Design	Further objectives
Pharmacodynamic studies	Mode of action MIC distribution by pathogens	Time-kill curves MIC	MBC MIC50, MIC90, %R
Pharmacokinetic studies	*Characterize the pharmacokinetics of the active substance (**products with different formulations might have different PK profiles and therefore might need specific PK/PD approaches)* Characterize the bioavailability of the active substances according the route and mode of administration and the drug formulation	Healthy animals Intravenous route Route of administration Final formulation (or close) Plasma kinetics	Dose determination
Bioequivalence study	Comparison with reference product	Healthy animals	Cmax, AUC
Post-marketing experience			
Data source	Content	Considerations	
Literature search			
Antimicrobial susceptibility survey	MIC distribution	By region, period Sample origin Method	
Time-kill curves	Antimicrobial effect along time	Design Inoculum size Culture conditions (media, O2/C02)	
Pharmacokinetic studies	Animal species Population pharmacokinetics	Products may be used at different doses. Sampling scheme Analytical method PK analysis	
PK/PD studies	Animal species Bacterial species Experimental model	Products may be used at different doses Animal characteristics Mode of administration Sampling scheme Analytical method PK/PD analysis	

14. Annex 4: Definition of PK, PD and PK/PD indices

Source: Ahmad et al. (2016)

PK/PD index	Definition	Unit	References
Pharmacodynamics			
MIC	The minimal inhibitory concentration is defined as the lowest concentration of antibiotic that inhibits completely the growth of the specific organism being tested.	mg/L or μg/mL	Mouton et al. (2005)
MBC	MBC is the lowest concentration at which 99.9% reduction in bacterial count is achieved	mg/L or μg/mL	Taylor et al. (1983)
MPC	MPC (mutant prevention concentration): the lowest concentration that prevents the emergence of mutants after 120 hours of incubation	mg/L or μg/mL	Shimizu et al. (2013)
PAE	Postantibiotic effect is the time of suppression of bacterial growth after the bacteria are exposed to antibacterial for a short time	Time (h)	Mouton et al. (2005)
Pharmacokinetics			
AUC	The area under the concentration time curve over 24 h at steady state unless otherwise stated. It is equivalent to a single dose AUC0−∞	μg·h/mL	Mouton et al. (2005)
f	Prefix indicating that the pharmacokinetic parameter values or PK/PD index values used are unbound (free) fractions of the drug		
CMax	The highest concentration of drug reached or estimated in the compartment of reference	mg/L or μg/mL	Mouton et al. (2005)
PK/PD integration			
$T > \mathrm{MIC}$	The cumulative percentage of 24 h period in which the drug concentration exceeds the MIC at steady state pharmacokinetic condition	%	Mouton et al. (2005)
$\mathrm{AUC/MIC}$	The area under the concentration time curve divided by MIC	No unit	Mouton et al. (2005)
$C\mathrm{Max/MIC}$	The peak concentration of drug divided by MIC	No unit	Mouton et al. (2005)

15. Annex 5: Withdrawal periods of amoxicillin products authorised in the EU Member States

Trade name	Country	Posology for pigs	WP for pigs (days)
Amoxi-Mix 10%-lösliches Pulver zum Eingebne für Tiere	AT	20 mg amoxicillin/kg day about 5-7 days	14
Suramox 50 % - lösliches Pulver zum Eingeben für Schweine XL	AT	20 mg/kg (400mg powder/10 kg)	14
Tamox - Granulat für Tiere XL	AT	10 g Tamox-granules / 50 kg = 10 mg Amoxicillin/kg 2 times per day about 2-5 days	14

Trade name	Country	Posology for pigs	WP for pigs (days)
Moxapulvis 15%	BE	20 mg amoxicillin/kg, 2 times/day	1
Amoxycilline 70%	BE	10-20 mg/kg/d for 4-5 d	2
Dokamox 80% ecuphar	BE	10 mg/kg 2 times/d or 20 mg /kg once a day for 3-5 d	5

Trade name	Country	Posology for pigs	WP for pigs (days)
Aciphen Kompaktat	DE	10 mg/kg KGW über 2-5 Tage	1
Amoxanil 200 F	DE	10 mg/kg KGW 2x tgl. über 3-5 Tage	3
Amoxanil 200 F-AMV	DE	10 mg/kg KGW 2x tgl. über 3-5 Tage	3
Amoxicillin 10%	DE	2x tgl. 10 mg kg KGW mind. 3 Tage	3
Amoxicillin 100%	DE	2-10 mg/kg KGW 2 x tgl. über 5-7 Tage	3
Amoxicillin-Trihydrat	DE	2-20 mg kg/KGW 2 x tgl. über 2-5 Tage	3

Trade name	Country	Posology for pigs	WP for pigs (days)
Amoxinsol vet.	DK	10 mg/kg 2 times daily for up to 5 days	6
Clamoxyl vet.	DK	5-10 mg amoxicillin/kg bodyweight 2 times daily in 3-5 days	6
Stabox vet.	DK	20 mg amoxicillin (as trihydrate) pr. kg body weight pr. day and night (q.s. 400 mg drug pr. 10 kg bodyweight pr. day and night)	14

Trade name	Country	Posology for pigs	WP for pigs (days)
		for 5 following days orally in wetfeed.	

Trade name	Country	Posology for pigs	WP for pigs (days)
Moxadin	ES	100 g/1,5 L warm water, twice per day, during 2 days	3
Hipramox-P	ES	0.6-1 g/L drinking water during 3-5 days. In general: 0.1 g/kg bw/day	7
Vetrimoxin polvo	ES	5-10 mg amoxicillin/kg bw, i.e. 0.5-1 g Vetrimoxin Polvo/10 kg bw each 12 hours during 3-4 consecutive days.	10
Neudiavall polvo	ES	2 sachets/1000 L, during 5 days	10
Stabox 50% pos cerdos	ES	20 mg amoxicillin (as trihydrate)/ kg bw and day, i.e. 400 g Stabox/10 kg bw and day, during 5 consecutive days	14
Eupensol porcino	ES	143 mg/10 kg bw/12 h during 5 days. 286 g Eupensol/1000 l water twice per day during 5 days	14

Trade name	Country	Posology for pigs	WP for pigs (days)
AMOXIVAL 10	FR	10 mg amoxy / kg b.w.x 5 days if necessary: 20 mg / kg.	2
BIOTORNIS	FR	10 mg amoxy / kg b.w x 5 days if necessary: 20 mg / kg	2
COFAMOX 10	FR	10 mg amoxy / kg b.w.x 5 days if necessary: 20 mg / kg.	2
SURAMOX 10 Poudre Orale	FR	10 mg amoxy / kg b.w.x 5 days if necessary: 20 mg / kg.	2

Trade name	Country	Posology for pigs	WP for pigs (days)
VETRIMOXIN P.O.	FR	10 mg amoxy / kg b.w.x5 days if necessary: 20 mg / kg.	2
AXILLIN Poudre Orale	FR	10 mg amoxy / kg b.w.x 5 days if necessary: 20 mg / kg.	2
SURAMOX 50 Poudre Orale Porc	FR	20 mg amoxy / kg b.w. x 5 days.	14

Trade name	Country	Posology for pigs	WP for pigs (days)
Tadamox granulate	GR	10 mg amoxicillin/kg BW (10 g Tadamox per 50 kg BW), twice daily for 2-5 days	3
Amoxicillin 15%	GR	younger than 6 months old): 250 g/100 lt drinking water for 3-5 consecutive days (i.e. 40 mg amoxicillin/kg BW/24 h), older than 6 months old): 500 g/100 lt drinking water for 3-5 consecutive days (i.e. 40 mg amoxicillin/kg BW/24 h)	28
Bremamox	GR	suckling piglets: 2 g powder twice daily, weaned piglets (20-40 kg BW): 2-4 g powder twice daily, pigs (60-200 kg BW): 6-20 g powder twice daily	28

Trade name	Country	Posology for pigs	WP for pigs (days)
OCTACILLINE	NL	Pigs less than 6 months: 10-20 g/100 l drinking water (5.6-11.2 mg amoxicillin/kg bw) per day, during 3-5 days. Pigs more than 6 months: 15-30 g/100 l drinking water (5.6-11.2 mg amoxycillin/kg bw) per day, during 3-5 days. P	2

Trade name	Country	Posology for pigs	WP for pigs (days)
Amoxindox 50	IT	40 mg product/kg b.w./day (corresponding to 20 mg	1

Trade name	Country	Posology for pigs	WP for pigs (days)
		amoxycillin trihydrate/kg b.w./day) for 5 days.	
Amoxid	IT	20-30 mg amoxicillin/kg bw	2
Supramox S.P.	IT	0.1-0.2 g/10 kg bw/day (corresponding to 8-16 mg amoxicillin/kg bw) for 3-5 days	2
Vet-Cillin 80	IT	0.25 g of product/10 kg bw (corresponding to 10.5 mg amoxicillin/kg bw) in severe cases the dose can be doubled	3
Amoxicillina Triidrato 80% Ascor Chimici	IT	1.72-2.87 g of Amoxicillin Tridrate 80%/100 kg bw (corresponding to 12-20 mg amoxicillin/kg bw)	7
Amossicillina Triidrato 25% Adisseo Filozoo	IT	6-12 g of product/100 kg b.w./day (corresponding to 1.5-3 g amoxycillin trihydrate/ 100 kg b.w./day) for 6 days.	14

Trade name	Country	Posology for pigs	WP for pigs (days)
STABOX 50%	PT	20 mg/kg b.w. during 5 consecutive days	14

16. Annex 6: Overview of the data available regarding target animal safety

From MA applications, extensions, variations			
Study type	**Main objective**	**Design**	**Further objectives**
Target Animal Safety studies preferably according to principles of VICH GL 43:			
(a) **Margin of Safety Studies**	Characterise the toxicity profile and target organs Identify the margin of safety (MOS) based on the occurrence of adverse events.	Healthy animals Final formulation (or close) 0, 1x, 3x, 5x ORTD, for 3x dose duration Physical examinations and observations, clinical pathology, necropsy, histopathology	Local tolerance Formulation-specific AEs Palatability issues at higher dose
(b) Injection Site Safety Studies	Evaluate local tolerance according to dose, duration, route(s), vehicle and volume of injection. Time to resolution.	Healthy animals Final formulation 0 & 1x ORTD Clinical observations, clinical pathology, gross pathology and histopathology	
(c) Reproductive Safety Studies	Identify adverse effects on male or female reproduction and viability of offspring	Healthy animals Males: 0 & 3x ORTD x one spermatogenic cycle Females: 0 & 3x ORTD from prior to breeding to end of post-natal period	
Dose-determination studies/ Dose confirmation studies	To determine the optimal dose by investigating efficacy in a range of doses.	Limited numbers of uniform animals, often in challenge model, controlled conditions. Final formulation (or close) Efficacy endpoints Dose determination studies: Variable dose range, e.g. 0x, 0.5x, 1x, 2x ORTD Dose confirmation studies: usually 1x ORTD, possibly natural	May also report safety outcomes

From MA applications, extensions, variations			
		disease outbreak, larger animal numbers	
Clinical field studies preferably according to principles of GCP	Identify safety issues in the target (diseased) population at the RTD	Final formulation 1x ORTD for proposed duration Target/diseased population AEs reported as: serious/non-serious causality incidence reversibility	Relationship of AEs to dose, evidence for safety in sensitive sub-populations
Safety studies in non-target laboratory animals (GLP or GLP-like)	To establish user safety and safety of residues in food (ADIs) Identification of target organs and toxicological end-points Establishment of NO(A)ELs	Single and repeat-dose toxicity Reproductive & developmental toxicity Not always final formulation	
Post-marketing experience			
Data source	Content	Considerations	
Pharmacovigilance – **PSURs including signal detection**	Serious and non-serious AEs AEs following off-label use AEs in mother/ offspring Causality Incidence of AEs	Further investigations carried out Updates to safety warnings in the SPC Evidence of previously unidentified toxicity Drug interactions AEs associated with off-label use, especially at overdose Urgent safety issues Evidence from use in 3rd countries (possibly at higher dose)	Lack of efficacy at RTD, Validity of withdrawal periods, Environmental incidents
Publically available data			
Literature searches: Data from peer-reviewed journals, official reports, textbooks	According to study design. Toxicity data		

From MA applications, extensions, variations			
Information on excipients – e.g. MRL summary reports, Codex reports, GRAS list Authorisations from VICH participant countries	Published SPCs and assessment reports where available, to provide information on higher dosing regimens.	May provide evidence of use at different doses.	

17. Annex 7: Overview of compositions of OTC formulations

Overview of compositions of OTC formulations authorised in The Netherlands

Product		Alamycin LA	Alamycin LA 300	Cyclosol LA	Oxy LA inj	Tridox Pro Inj	Vetroxy LA	Alamycin 10	Cyclosol 10%	Duphacycline 100	Engemycine 10%	Geomycine -ject	Oxyject 10%	Oxymax	Oxytetra	Oxytetracycline HCl 10%	Oxytetracycline 10% + PVP Pro Inj	Oxytetracycline 10% Pro Inj
"LA or SA"		LA	LA	LA	LA	LA	LA	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA
Dose (mg/kg), treatment schedule		20, 1x	20-30, 1x	20, 1x	20, 1x	20, 1x	20, 1x	4, 3-5 days	5, 3-4 days	10, 5 days	4-5, 5 days	5-20, 5 days	10-20, 3-5 days	5-20, 3-5 days	10, 3 days	4, 3-5 days	4, 3 days	4, 3 days
OTC concentration		20%	30%	20%	20%	20%	20%	10%	10%	10%	10%	10%	10%	10%	10%	10%	10%	10%
2-Pyrrolidone	solvent	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Povidone	solubiliser	-	-	+	-	-	-	-	+	+	+	+	-	+	+	-	+	+
Dimethylacetamide	solvent	+	+	-	-	-	+	+	-	-	-	-	-	-	-	+	-	-
Glycerolformal	solvent	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+
Macrogol 1500	viscosity	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
N-methyl-2-pyrrolidone	solvent / effect on viscosity	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Magnesium chloride	complexing agent	-	-	-	-	-	-	+	+	+	-	+	+	+	+	+	+	+
Magnesium Oxide	complexing agent	+	+	+	+	+	+	-	-	-	+	-	-	-	-	-	-	-
Disodium edetate	chelating agent	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Citric acid	pH-adjustment	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-
Hydrochloric Acid	pH-adjustment	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Monoethanolamine	buffering agent	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sodium formaldehyde-sulphoxylate-dihydra	antioxidant	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Methyl-4-hydroxybenzoaat (E218)	preservative	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+
Propyl-4-hydroxybenzoaat (E216)	preservative	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+
Benzylalcohol	preservative	-	-	-	-	-	-	-	-	+	-	+	+	+	-	-	-	-
Water for Injection	solvent	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

The orange shaded cells represent ingredients that can have the ability to inhibit the release of the active ingredient from the site of injection