1 19 July 2018
2 EMA/CVMP/849775/2017
3 Committee for Medicinal Products for Veterinary Use (CVMP)

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5 Reflection paper on dose optimisation of established
6 veterinary antibiotics in the context of SPC harmonisation

7 Draft

Adopted by CVMP for release for consultation	19 July 2018
Start of public consultation	27 July 2018
End of consultation (deadline for comments)	31 January 2019

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Keywords	antimicrobial resistance (AMR), summary of product characteristics (SPC), Dose optimisation, pharmacokinetic/pharmacodynamic (PK/PD) modelling, target animal safety (TAS), withdrawal periods (WP) and the environmental risk assessment (ERA)

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Executive summary

The Committee for Medicinal Products for Veterinary Use (CVMP) has conducted a pilot project on dose optimisation of established veterinary antibiotics. Established veterinary antibiotics are not always used at the authorised dose. Doses may need to be reviewed in order to maintain effectiveness and to limit the development of antimicrobial resistance (AMR). However, a change in dose may have implications for target animal safety (TAS), withdrawal periods (WP) and the environmental risk assessment (ERA). 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 the antimicrobial resistance problem. The project aimed at developing and testing non-experimental approaches for dose optimisation and evaluating the consequences on WP, TAS, and ERA, with the final objective to improve the Summary of Product Characteristics of veterinary antibiotics authorised in the EU.

Dose optimisation of products or groups of products also could be helpful in the process of the harmonisation of authorisation of VMPs throughout the EU. The desired minimum level of harmonisation would obviously be a harmonisation of individual products authorised across different Member States (i.e. at product level). However, because of the group-wise analysis (i.e. grouping of products with the same animal species, disease, route of administration, and pharmaceutical form), some aspects such as the optimised dose, may also be applied to different products within the same group, as was done in this pilot project for the case studies with amoxicillin and oxytetracycline.

Non-experimental approaches based on well-established scientific principles, were used, namely PK/PD integration for dose optimisation, PK modelling for WP adjustment, and scientific review approaches to address the safety of both target animals and the environment, using data from the registration dossiers and published literature. Where needed, the group 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 methods developed and used were applicable to both case studies and a comprehensive and scientifically sound review of the approved doses was possible. PK/PD analysis clearly showed that the dose for AMO should be 40 mg/kg bw, which is twice the dose for most of the currently authorised products. For OTC, different optimised doses had to be calculated for the 10% vs 20% formulations, due to different pharmacokinetics. For the 10% formulations, the optimised 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 optimised dose was two doses of 20 mg/kg bw, given 36-48 hours apart. This dose was the same as for most authorised products; however the addition of a second dose is currently not part of most of the authorisations. The calculation of new WPs 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.

While a non-experimental dose review appears possible, its implementation depends very much on the support of all interested parties, including the Heads of Medicines Agencies, the Federation of Veterinarians of Europe, and industry.

56 This Pilot Project was performed to test the feasibility of the various non experimental methods. It
57 should be noted, that the outcome of the dose review was based on a limited amount of data, gathered
58 from public sources or provided by industry. Therefore the numerical results (e.g. optimised dose, WT
59 etc.) are merely indicative, and may not reflect a final outcome (e.g. after a referral in which all related
60 VMP authorised in the EU are included).

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1. Introduction

The Committee for Medicinal Products for Veterinary Use (CVMP) started a pilot project on dose optimisation of established veterinary antibiotics to which AnimalhealthEurope (formerly IFAH Europe), and the European Group for Generic Veterinary Products (EGGVP) were invited to provide anonymised data. The results of this project are for consideration by the CVMP 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 development of AMR, under modern animal production conditions.

Indications that a review of the posology could be needed can be based on the use of the product in the field, susceptibility patterns of the target pathogens, pharmacokinetic and clinical data. Should there be a need to optimise 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) and the environmental risk assessment (ERA). If the optimisation 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 the resistance problem.

The CVMP recognised that the current regulatory environment does not stimulate the realisation of the desired dose optimisations. CVMP wished therefore to explore if non-experimental approaches to improve the SPCs of old veterinary antibiotics could be identified in lieu of new clinical, safety and residue data. 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).

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

1.2. Scope

169 This pilot project comprises the development and testing of non-experimental scientific approaches for
170 dose optimisation, and for assessments of safety for consumers, target animals and the environment;
171 these approaches can be used as tools for improving the label instructions of established veterinary
172 antibiotics authorised in the EU, in the context of SPC harmonisation. Proposals for selection and
173 prioritisation of candidate antibiotics for dose optimisation will be made. Whilst recommendations for
174 future implementation of dose optimisation can be made, the selection of regulatory procedures for
175 SPC harmonisation and the legal implications are outside the scope of the pilot project.

1.3. Aim of the project

177 The general aim of the pilot project is to obtain knowledge on the feasibility of the use of modelling or
178 other approaches as a substitute for clinical data, residue depletion data, ERA data, and TAS data, as a
179 tool for the optimisation of the posology for established veterinary antibiotics in the context of
180 harmonisation of product literature of individual products.

181 Specific objectives included:

182 • to agree on the rationale/objectives for the optimisation of the posology for established veterinary
183   antibiotics;

184 • to establish criteria for selection of products for which doses should be optimised/reviewed;

185 • to obtain a common understanding of the applicability of PK/PD modelling and other sources of
186   information for posology optimisation;

187 • to obtain an agreement on the PK/PD techniques and applicability to be used for dose optimisation
188   in the context of harmonisation of established veterinary antibiotics;

189 • to obtain an agreement on the acceptability and applicability of PK techniques for withdrawal
190   period extrapolation in the context of harmonisation of established veterinary antibiotics;

191 • to obtain an agreement on the approach to be used for the evaluation of the impact of posology
192   optimisation on target animal safety in the context of harmonisation of established veterinary
193   antibiotics;

194 • to obtain an agreement on the approach to be used for the evaluation of the impact of posology
195   optimisation on environmental safety in the context of harmonisation of established veterinary
196   antibiotics;

197 • to discuss the possible approaches for the regulatory processes to effectuate the harmonisation of
198   the product literature, and consider the impact and implications on the future product development
199   and improvements.

200 • to explore possibilities for funding under Horizon 2020 or other funding sources, for studies to fill
201   gaps in data for off-patent veterinary antibiotics related to optimising dosing with respect to
202   minimising risks from AMR where progress is not possible without generation of additional data.

1.4. Development and testing of the approaches

The non-experimental approaches developed were based on scientific considerations, and on well-established modelling techniques. Where needed, the group consulted additional experts from academia, regulators, and industry. A PK/PD modelling approach for the dose optimisation, 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. These approaches are described in chapters 3, 4, 5, and 6, respectively.

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 chapters 7 and 8, respectively.

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. optimised 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).

1.5. Acknowledgements

2. General considerations

2.1. Criteria for selection of products for which doses should be optimised

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 optimisation may be needed, the following criteria is proposed:

- the existence of different dosage recommendations for the products in the SPCs,
    - within a product between MSs; different doses within a product from the same MAH are a clear indicator of the need to optimise the dose.
    - or between similar products without obvious reasons (such as differences in formulation)
- evidence of lack of efficacy from pharmacovigilance data, formularies, literature
- evidence of decreased susceptibility or increased resistance of target pathogens.

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

244 **Table 1.** Scoring table for prioritisation of selected candidates for dose optimisation

AMEG categorisation	OIE categorisation	Administration route	Antibiotic consumption (in accordance with ESVAC data)*	Specific evidence of AMR risk
Category 2 ++	VCIA ++	Group oral ++		Expert judgement
Category 1 +	VHIA +	Parenteral or individual oral +		
No category /	VIA /	Topical/local** /		

245 * Stratification to be further developed
246 **The PKPD approach has not been considered for topical/locally applied products within this project
247 The scores are graded as "/" (nil), "+"and "++".

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

250 This pilot project, was aimed at the dose optimisation and harmonisation at the level of the veterinary
251 medicinal product, not at the level of the pharmacologically active substance. The decision was based
252 on the following scientific and practical considerations.

253 1. Although products with the same active ingredient may be indicated for the same condition in the
254   same target animal, the difference in formulation and route or method of administration may result
255   in different absorption characteristics and therefore a different pharmacokinetic profile.
256   Consequently, in some cases a different posology may be needed to attain a similar plasma
257   concentration of the active ingredient.

258 2. A product-by-product approach will result in safe and effective posologies, with a minimal market
259   disturbance.

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

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275
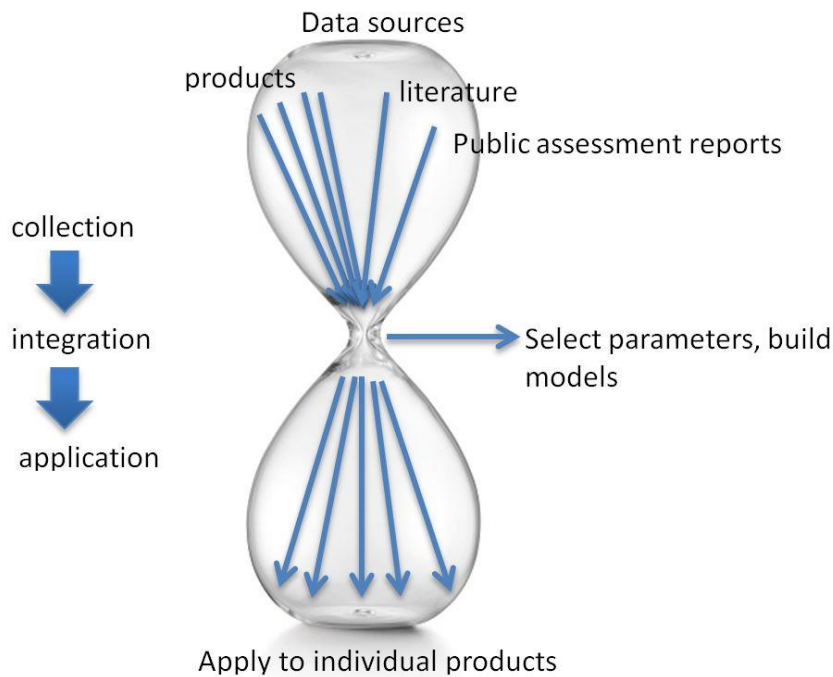
Figure 1. The hour glass approach

3. PK/PD approach for dose optimisation

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/627/2001-Rev.1) 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 susceptibility of the target bacteria. Therefore, for all compounds with systemic activity, the *in vitro* susceptibility data (Minimal Inhibitory Concentration, MIC) (Pharmacodynamic or PD) collected should be compared with the concentration of the compound at the relevant biophase (Pharmacokinetic or PK) following administration at the assumed therapeutic dose as recorded in the pharmacokinetic studies. Based on MIC data, and target animal PK data, an analysis for the PK/PD relationship may be used to support dose regimen selection and interpretation criteria for resistance. The overall assessment of the PK/PD relationship should be sufficiently comprehensive to assess with reasonable confidence whether or not the investigational antibacterial agent, 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 or experimentally infected animals.

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

In the last 20 years, the PK/PD approach has been recognised as an important tool for the development of new antibiotics as a way to integrate different data about antibacterial efficacy, pharmacology and bacteriology during product development (Drusano, 2016). According to guideline EMA/CVMP/627/2001-Rev.1, 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 a very few dose regimens in clinical trials (dose confirmation and clinical field studies). In human health, the PK/PD approach is also used in the process of definition of a clinical breakpoint 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 was recommended to review available data to investigate the dosage regimen of established veterinary antibiotics and to assess their potency against target pathogens.

The PK/PD approach combines information about the PK of the molecule and the PD which describe the effect of the molecule on the target bacteria. Mathematical models have been developed to describe the evolution of concentration-time curve and to assess the effect on bacteria using parameters observed *in vivo* or extrapolated from *in vitro* or *ex vivo* studies. These approaches are currently used to analyse data obtained from different experimental studies and to simulate different exposure conditions (Nielsen & 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 parameter and apharmacodynamic parameter to predict clinical efficacy is labelled as a PK/PD index (PDI). Minimal inhibitory concentration (MIC) is the most used pharmacodynamic parameter. It 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 2):

- the total concentration integrated over a given time interval (area under the curve, AUC),

- the highest concentration (C_{max}) observed at the peak,

- the time during which the concentration exceeds a specific threshold (time above MIC, $T_{C>MIC}$).

PK/PD assessments are based upon the MIC for the target pathogen and the unbound antibiotic concentration in the host plasma, because only the free fraction has an 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 classes of antibiotics, a consensus was reached to propose the definition of PK/PD target (PDT) predicting a high level of cure (>80-90 %).

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342 - Betalactams (penicillins, cephalosporins) exhibit time-dependent microbiological effects, meaning
343   that maximizing $fT>MIC$ will enhance bacterial killing. In general, betalactams require 40-80%
344     $fT>MIC$ of the dosage interval to achieve bactericidal activity depending on the individual class
345   and the target bacterial species (Ambrose, Bhavnani et al., 2007).

346 - For fluoroquinolones which are concentration-dependent, $fAUC_{24h}/MIC$ predicts efficacy against
347   gram-negative bacteria if a target value from 70 to 125 is reached. A target value of 125 hours,
348   corresponds to mean concentrations over 24 hours equal to 5 times the MIC (i.e. 125/24)
349   (Ambrose et al., 2007; Schentag, et al., 2000).

350 - For aminoglycosides, the fC_{max}/MIC is used as best predicator of therapeutic efficacy. It is
351   generally agreed that to obtain a clinical response of >90% in patients and reduce the risk of
352   emergence of resistance, C_{max}/MIC needs to be 8-12 (Moore et al., 1984; Craig et al., 1998).

353 It is important to note that all three PK/PD indices are correlated in the sense that C_{max}/MIC describes
354 an intensity, T>MIC describes a duration, and AUC/MIC is a combination of intensity/duration. The
355 calculation of the three PK/PD indices is always tested as derived from the same PK data. The best
356 PK/PD index for a certain antibiotic-bacteria combination is determined by plotting the value of a
357 specific endpoint (typically log_{10} CFU/ml after 24 hours of treatment) versus the magnitude of each of
358 the three PK/PD indices. The PK/PD index should ideally be used in combination with clinical
359 information to determine an optimal dose and dosing regimens. It must be considered as a
360 simplification when it is used in isolation. Several points should be kept in mind for its use. To note
361 that, different dosing regimens could result in the same PK/PD index value. All indices are based on an
362 MIC which is a measure of the net effect on growth and antibiotic-induced bacterial killing over the
363 incubation period. MIC is determined at a fixed time and at a fixed concentration using standardized
364 medium and growth conditions. MIC testing has been highly standardized (e.g. CLSI, EUCAST) to avoid
365 potential errors due to different testing methodologies. However, MIC values may differ if they are
366 tested in other conditions. Also, MIC testing requires a 2-fold dilution approach which provides only an
367 approximate inhibitory value.

368 It should be noted that recently, some scientific evidence has established that the AUC_{24h}/MIC index
369 could also be used for time-dependent antibiotics, as for example for phenicols (Manning et al., 2011)
370 or beta-lactams (Nielsen et al., 2011; Kristoffersson et al., 2016). These recent updates to the
371 knowledge of PK/PD relationships have shown, using mathematical physiological models, that when the
372 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
373 the T>MIC index for predicting antibacterial activity. These new insights in PK/PD relationships could
374 be of importance for those veterinary medicines which are long-acting formulations. Thus, the use of
375 AUC/MIC as a universal PK/PD index would facilitate the finding of an optimal dosage regimen of most
376 long-acting formulations (Toutain et al., 2017).

3.3. Proposed approach to address doses

378 It is assumed that in regards to dose improvement, products will be harmonised in groups dependent
379 on:

380 • Active substance

381 • Target animal species

382 • Disease

383 • Route of administration

384   •   Pharmaceutical form

385 Refer to Annex 1 for an overview of the PK and PD data available for the proposed modelling approach
386 to address doses.

387 Refer to Annex 2 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

390 Most pathogens of clinical interest are located extracellularly and the biophase for antibiotics is the
391 extracellular fluid (Schentag *et al*., 1990). Extracellular fluids are difficult to sample but if there is no
392 barrier to impede drug diffusion, the concentration of free antibiotic in plasma approximates its free
393 concentration in the extracellular space (Toutain & Bousquet-Melou, 2002). So the PK/PD integration is
394 appropriate for acute infections in vascularized tissue.

395 The PK/PD integration approach allows the calculation of a dose by taking into account the combined
396 PK and PD properties of an antibiotic. The simplest relationship between the dose and the PK/PD
397 parameters is given by the following equation:

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

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

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

409   -   Is there a dose linearity?

410   -   Is there a difference in bioavailability between products?

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

412

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

414 The pharmacodynamic effects of the active substance against the target pathogen bacteria must be
415 defined. Two types of information are required.

416   1) The mode of action of the active substance and the relationship between concentration and
417   bacterial killing rate must be defined. According the pharmacological class of the active
418   substance, the mode of action can be defined as time-dependent or concentration-dependent.

419   2) Determine the MIC distribution for the wild type (WT) population of the active substance
420   against the target bacteria and establish the epidemiological cut-off value (ECOFF), which is
421   the MIC value identifying the upper limit of the WT population.

422

423   **MIC$_{50}$**   **MIC$_{90}$ / ECOFF**

424   Wild Type population   non Wild Type population
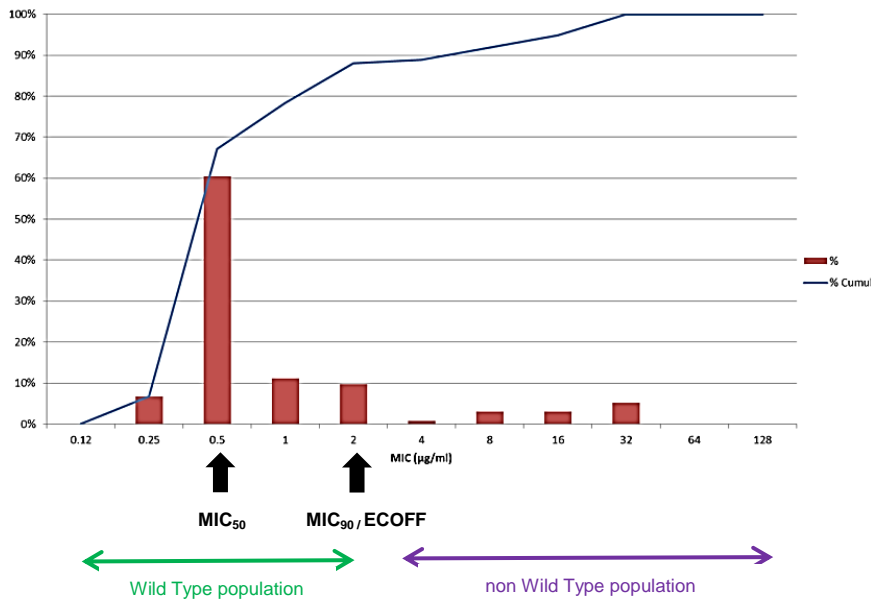
425 **Figure 2.** Oxytetracycline MIC distribution for *P. multocida* and comparison of MIC$_{50}$, MIC$_{90}$ and ECOFF

426 values. ECOFF definition from EUCAST: MIC value identifying the upper limit of the WT population.

427 MIC$_{90}$ stands for Minimum Inhibitory Concentration required to inhibit the growth of 90% of susceptible

428 organisms. MIC$_{50}$ stands for Minimum Inhibitory Concentration required to inhibit the growth of 50% of

429 susceptible organisms.

430

431 In regards to the PD component, to address the dose using PK/PD integration, a review of the PD data

432 and scientific papers to support the choice of a mode of action and to provide the MIC distribution will

433 have to be done. The following points should be considered:

434   -   What is the available information on the pharmacodynamics of the active substance, and of

435   other compounds belonging in the same pharmacological class, against the targeted bacterial

436   species?

437   -   What are the data available to describe the MIC distribution?

438   -   Is the MIC determination based on standardised method?

439   -   Are they any available time-kill curves obtained on strains representative of the targeted

440   bacterial species?

441   -   Which is the least susceptible target pathogen, i.e. the dose-limiting bacterial target species?

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

443 The PK/PD index is the key parameter in the modelling of dose (Annex 2). Three PDI are commonly

444 used (Mouton *et al.*, 2012):

445   •   AUC/MIC : the ratio between the total concentration integrated over a given time interval (area

446   under the curve, AUC) and MIC,

447   • C_{max}/MIC : the ratio between the highest concentration (C_{max}) observed at the peak and MIC

448   •   T>MIC : time above MIC, the period of time when the concentration exceeds the MIC.

Concerning the definition of the PDI, a review of the scientific literature to support the choice according to the pharmacological class of the antibiotic, the pharmacokinetics of the active substance in the target animal species in that class and the chosen target pathogen will have to be done. The following points should be considered:

- What is the mode of action of the active substances against the targeted bacteria (time or concentration dependent)?

- What is the pharmacokinetic profile of the active substance?

- What is the protein binding of the active substance?

- Which PK/PD index is considered best predictive for clinical efficacy in the target animal species for the indication?

In the context of this pilot project, an approach based on two steps is proposed to model an optimal dosing. The point of departure for the PK/PD analysis will be the AUC/MIC for all antibiotic classes to define a daily dose and then, the analysis would be refined with the T>MIC or the C_{max}/MIC in function of the antibiotic class.

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

After selecting the index appropriate to 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), 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 according the pharmacological class of the antibiotics, the clinical indications and the targeted bacteria. In this case, 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 clinical expected outcome (risk of relapse)?

- What is the risk of mutant selection for the pathogen?

- What is the therapeutic objective of the treatment (bacteriostatic, bactericidal, magnitude of the reduction e.g. 2-4log)?

In case of a lack of available information from veterinary pharmacology, the PDT can be derived from available data from experimental or pre-clinical trials in the target animal species or supported by pharmacological and clinical data obtained in human medicine.

3.3.5. Step 5: Set a Probability of target attainment for the PDI value (PTA)

The next step consists in the determination of the percentage of animals, in the treated population, for a particular dosage regimen, likely to attain the target value of the selected PDI, across a range of relevant MIC values. According to the disease to be treated, the mode of usage (individual, group treatment) a Probability of Target Attainment (PTA also historically termed Target Attainment Rate or

488 TAR) for the PDI value must be defined. The acceptable level of PTA is still under debate. Values of
489 99%, 95% or 90% have all been used. Based on expert considerations (Toutain *et al.*, 2017), it was
490 considered that in the context of this project of dose optimisation of VMPs a PTA of 90% is acceptable
491 when a population PK/PD model takes into account simultaneously the population PK and the MIC
492 distribution of the wild type population with a MIC below or equal to the ECOFF.

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

495 According to the PK and PD data available, the relationship between dose and PDI can be defined using
496 two of approaches.

497 -   The first approach is based on a summary of PK parameters (AUC, clearance, fraction
498   unbound, etc.). If they are available, a meta-analysis can be performed to derive an overall
499   mean and standard deviations of each parameter from the pool. A model of the relation
500   between dose and PDI can be used to estimate distribution of the PDI (equation 1) and
501   calculate the PTA of the PDT. This approach can be used to define a daily dose based in
502   relation with the point of departure as PDI, the AUC/MIC and estimate a range of dose.

503 -   The second approach requires the use of pharmacokinetic raw data (time, concentration) for
504   different dosage regimen, different formulations and different individual characteristics (age,
505   weight, sex). A population pharmacokinetic analysis based on non-linear mixed effect
506   algorithm can be performed to estimate distribution of the PDI and calculate the PTA for a
507   PDT. This approach is applied to analyse the other PDI (T>MIC, C_{max}/MIC) chosen in function
508   of the antibiotic class, because it requires to estimate the distribution of their values in
509   function of the population distribution of key pharmacokinetic parameters (bioavailability,
510   volume of distribution, clearance).

511 In both cases, a Monte Carlo Simulation (MCS) of 5000 cycles should be performed. The range of
512 doses tested must be based on good veterinary practices and pragmatic approaches of the feasibility of
513 treatment in field conditions. The number of daily doses and interval between doses must be justified.

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

515 The definition of a new CBP first needs the determination of three critical MIC values; which allow a
516 decision to be made on the CBP.

517 The three critical concentrations are:

518 (i)   Wild type cut-off: ECOFF. An ECOFF is defined for each bacterial species targeted by
519   the treatment.

520 (ii)   PK/PD cut-off: is the maximal MIC value reaching the PTA of the selected PDI

521 (iii)   Clinical cut-off: MIC value reflecting clinical outcomes and able to discriminate
522   between clinical failure and success. It requires data able to discriminate clinical
523   case outcomes according the MIC of isolates and the level of exposure.

524 The CBP is the final concentration value determined by considering all three critical MIC values. To
525 ensure that a dose leads to an optimal exposure, a CBP does not cut the wild type distribution of
526 targeted pathogens. If a dose is defined, a CBP can be set in relation with the PTA for different values
527 of MIC (Mouton *et al.*, 2012). However, within the context of this pilot project, and in the absence of

528 clinical data reflecting the clinical outcomes according the MIC of isolates and the level of exposure,
529 only a PK/PD breakpoint could be established.

3.3.8. Step 8: Define an optimal daily dose

531 After complying with all the previous steps, the results of the PK/PD integration approaches should
532 allow to define an optimal daily dose based on the available PK and PD data used for the computation.
533 For each case, the new daily dose will be defined as the one able to reach a PTA of 90 % for the least
534 susceptible target pathogen.

4. PK approach for withdrawal period adjustment

4.1. General considerations on the calculation of withdrawal periods

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

540 • ensure consumer safety;

541 • guarantee a level playing field for MAHs regarding the estimation of WPs.

542 It is acknowledged that these methods can be considered a pragmatic compromise between science
543 and feasibility. From a scientific point of view, a large amount of residues data would be needed to
544 cover all aspects and variables involved. Therefore, multiple residue depletion studies would be needed
545 in order to cover the large variation under field conditions, such as different breeds, different animal
546 life stages with different ages and body weights, different housing and feeding conditions, and different
547 health status. However, in view of the costs involved and the number of experimental animals needed,
548 such data requirements are considered not practicable, and therefore, as a pragmatic approach, only
549 one standardised residue depletion study is normally required. Although this approach may have
550 scientific limitations in terms of predictability under field conditions, it is considered that the resulting
551 WPs are adequately protective for consumers in view of the many safety margins that already exist in
552 the consumer safety assessment (ADI/MRLs).

4.2. Current situation regarding withdrawal periods for established antibiotics

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

557 • Dossiers of established veterinary antibiotics often contain old residue studies. These studies may
558   be non-GLP, using old analytical methods, but often represent field conditions.

559 • Even when the same residue depletion data were available, the same products may have different
560   WPs in the different Member States.

561 • Although there are many generic products for a number of VMPs, there may be only few residue
562   depletion studies available (e.g. in an article 35 referral on ivermectin there were only 11 residue
563   depletion studies covering 287 authorisations of VMPs).

564 • Residue studies often failed to meet the statistical demands of the required first order kinetical
565 decay (e.g. due to low numbers of time points in the elimination phase), which led to the use of
566 the so-called alternative method, applying chosen safety margins.

567 • Most of the more recent residue depletion studies do comply with required statistical criteria.
568 However, they are often designed to minimise inter-animal variance, although this may have the
569 consequence that they are less representative of field conditions.

570 ### 4.3. Proposed algorithm to address the extrapolation of withdrawal periods

571 The proposed method for the calculation of WPs in this project is similar to the algorithm used by
572 FARAD (Food Animal Residue Avoidance Databank) since 2002. Both make use of long established and
573 validated pharmacokinetic principles. The Extrapolated Withdrawal-Interval Estimator (EWE) algorithm
574 from FARAD provides a tool for calculating withdrawal periods in case of off-label use (Martin-Jimenez
575 *et al.*, 2002). After calculation of the new dose, the terminal tissue half live is used to calculate the
576 new WP.

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

580 The proposed algorithm within this project:

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

582 Where:

583 F_{rel} = $\quad$ Relative bioavailability new dose/old dose (a default value of 1 is used, but may be
584 $\quad$ adjusted if needed);

585 $T_{1/2}(\text{final phase})$ = $\quad$ Mean half live (days; rounded up) in WP determining tissue(s) after distribution is complete

586 WP = $\quad$ Withdrawal period (days)

587 D = $\quad$ Dose (mg/kg); it is assumed that the dosing frequency and duration will not change.
588 $\quad$ However, if the dosing interval and/or duration would change, use could be made of FARAD
589 $\quad$ subroutines, to calculate the new dose (D_{new}).

590

591
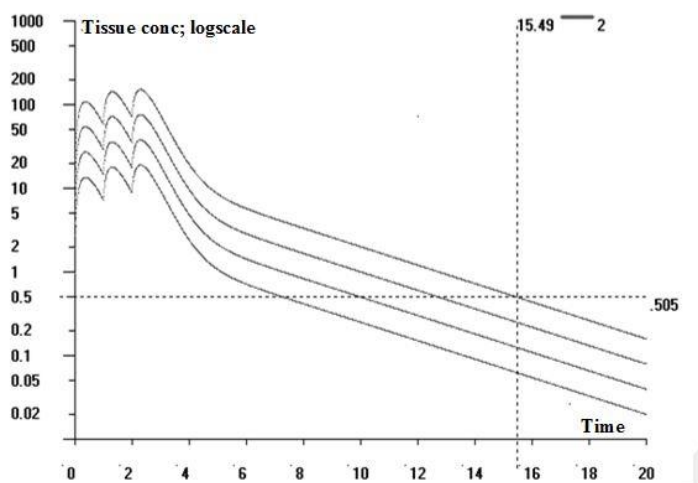
Dose	WP	Difference in WP
D	7.4	-
2D	10.1	2.7
4D	12.8	2.7
8D	15.5	2.7

592 **Figure 3.** Theoretical simulations. Under the conditions: Linear kinetics and complete distribution.
593 Proportional increase of WP at various doses

594 Because within this project only dose variations are considered and no extra label use (e.g. other
595 routes of administration, other target animal species), the conditions to be fulfilled are:

596 • Linear kinetics (for all ADME-processes) apply within the dose extrapolation range

597   o (see Figure 4 for simulations in case of non-linearity)

598 • At MRL-level, tissue distribution is complete

599   o (see Figure 5 for simulations in case of non-complete distribution)

600 Figure 3 shows the proportional increase (delta) of the WP under the conditions mentioned above.
601 Doubling the dose leads to the addition of one half-life (in this example 2.7 days).

602
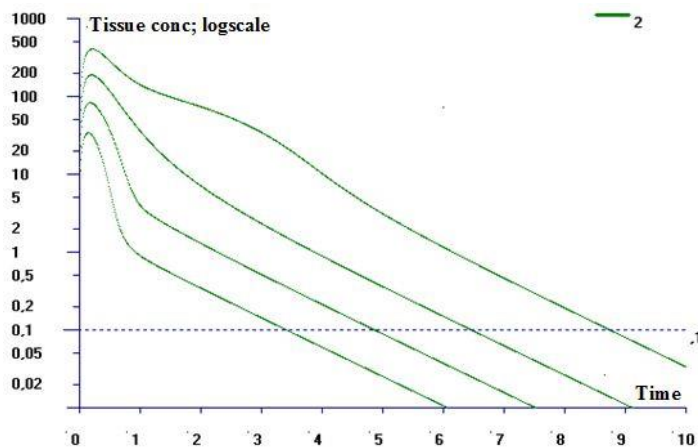
Dose	WP	Difference in WP
D	3.4	-
2D	4.9	1.5
4D	6.5	1.6
8D	8.7	2.2

603
604 **Figure 4.** Theoretical simlations Under the conditions: Non-linear kinetics, resulting in a
605 disproportional increase of WP at higher doses

606
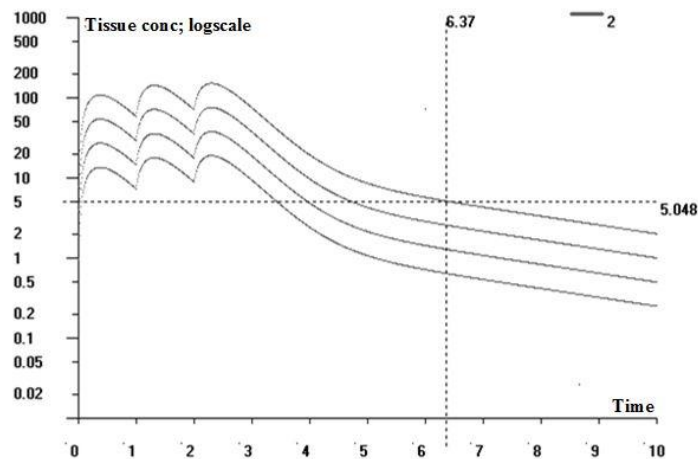
Dose	WP	Difference In WP
D	3.5	-
2D	4	0.5
4D	4.9	0.9
8D	6.4	1.5

607
608 **Figure 5.** Theoretical simulations under the conditions Linear kinetics,
609 tissue distribution not complete at MRL-level, resulting in disproportional increases of the WP at higher
610 doses

611 It is acknowledged that the current guideline on the calculation of WPs provides a statistical approach
612 that takes into account a 95% confidence limit on the 95th percentile. Due to the convex nature of the
613 95/95 interval curve, there is a probability of a slight increase of the WP (when using the statistical

method), on top the WP calculated with Equation 2, even when dose-linearity is assumed. Theoretical calculations suggest that this additional increase is around 5%. Whereas the current statistical method and the proposed algorithm (Equation 2) can not be fully compared, the addition of a safety factor of 10% to the selected worst-case half-life in tissues may be considered.

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 apply for the intended dose range (yes/no)

    b. Relative bioavailability new dose (default $F_{rel}=1$)

    c. General ADME particulars (e.g. active transport)

2. Establish the terminal half-life in tissues/milk/eggs

    a. Data sources:

        i.   Dossier data

        ii. FARAD database

        iii. Public Assessment Reports (if available)

        iv. International Journals (peer reviewed)

        v.   Publications by public committees (e.g. EMA/JECFA/EFSA)

3. If conditions (linear kinetics and complete distribution) are fulfilled, calculate the WP (extrapolated):

    a. Apply algorithm (Equation 2) to each VMP separately, calculating a new WP. There should 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.

4. If conditions are not fulfilled, perform further kinetic modelling:

    a. Apply adjusted and validated model to each VMP separately, calculating a new WP.

4.5. Injection sites

If the injection site would be the WP determining tissue, 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 determining tissue (see Figure 6). This would continue to be the case until, due to the increase of the dose, residues in one of the other tissues would become WP determining.

If the injection site would not be the WP determining tissue (anymore), then the algorithm (Equation 2) can be used. Also in this case the same injection volume at another location should be used to for instance double the dose, because altering the injection volume could lead to a different absorption rate, hence to different residue kinetics.
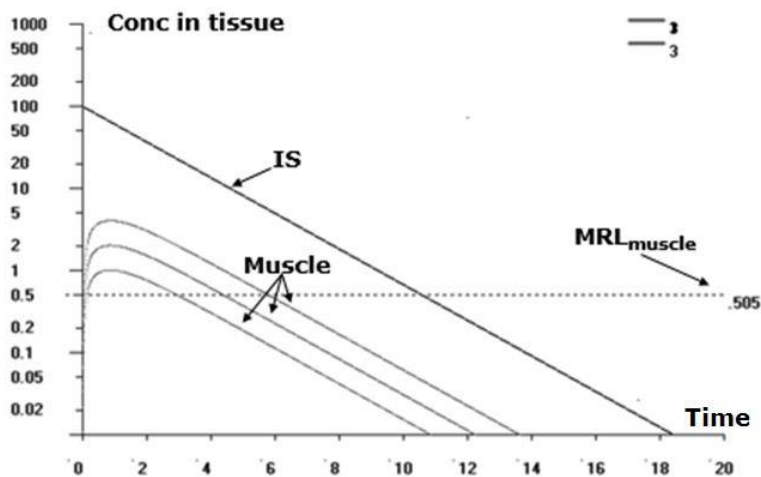
Figure 6. Theoretical simulations where the Injection sites remain WP determining at various doses, resulting in the same WP for all doses.

4.6. Some case studies from literature in eggs and milk

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.

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.

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 statistical method for tissues (WT1.4) from the CVMP guideline (EMA/CVMP, 1995) 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, 1998). But nevertheless, this example shows the validity of the algorithm used in this project, where the new WP for the 50 mg/kg bw dose is calculated using the $T_{1/2}$ of the 25 mg/kg bw dose (1.5 days), resulting in the same withdrawal period as when the WP is calculated based on the actual measured residue concentrations in tissues for the 50 mg/kg bw dose.

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

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673

Figure 7. 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
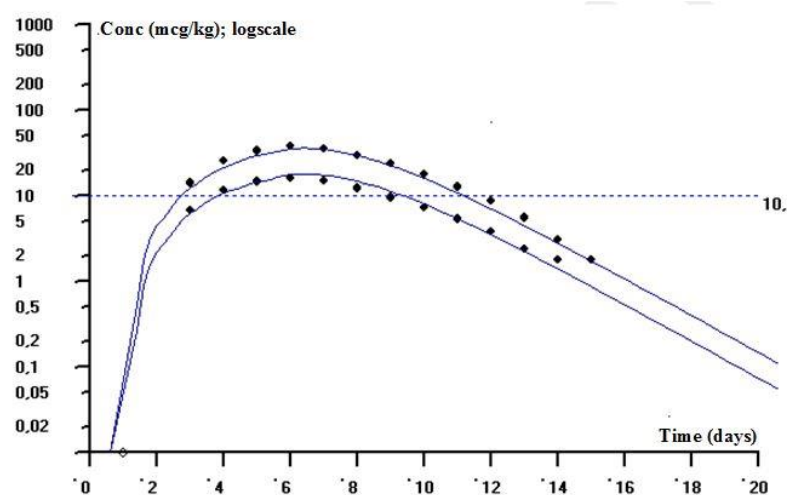
680 **Figure 8.** Fits of the time dependent course of amoxicillin residues in whole egg, Dose: 25 and 50 mg/kg bw during the first 5 days via the drinking water. Parameters for egg formation, kinetics (1 compartment) and transport rates of amoxicillin in to albumen (Kw) and yolk (Ky) were kept constant: 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 7 and Figure 8) clearly show, that the final phase of the residue depletion curve is log-linear. This justifies the use of Equation 2 for calculating the WP when using the higher dose. Further from the analysis dose linearity could be concluded, meaning at the dose range 25-50 mg/kg bw the kinetics of amoxicillin are linear.

691 <u>Example on residues in milk:</u>

692 The example for milk was taken from Malreddy *et al.* (2013). This example relates to residues of
693 gabapentin in milk following oral administration to lactating cattle at a dose of 10 and 20 mg/kg
694 bodyweight, using an 8 hour milking scheme and a fictive MRL of 0.1 μg/ml.
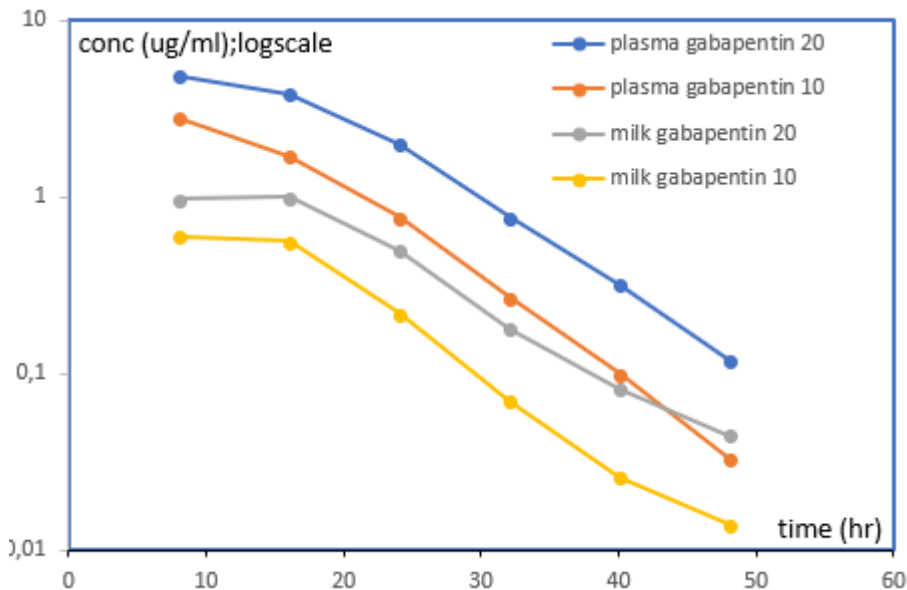
695

696 **Figure 9.** Mean plasma and milk concentrations of gabapentin following 10 and 20 mg/kg bodyweight
697 PO administration; based on Malreddy *et al*., 2013

698
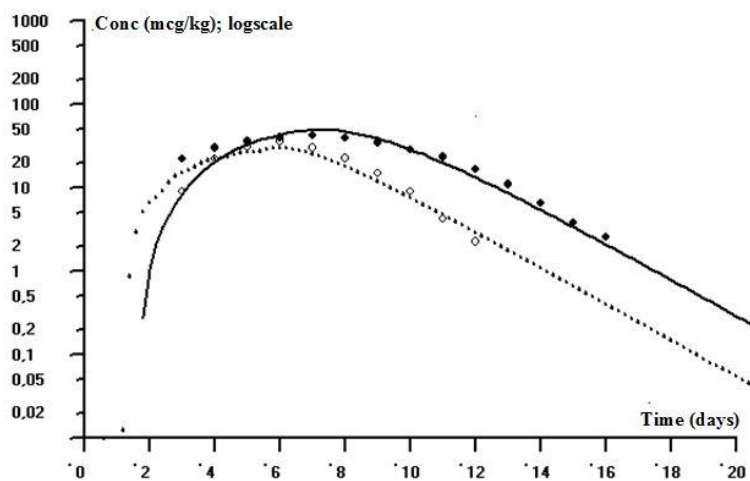
699 **Table 3.** Comparison of the predicted WP and the experimentally derived WP using data from Malreddy
700 *et al*., 2013

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

701 From Figure 9 it can be observed that the final phase of the residue depletion curve is log-linear. This
702 example also shows the validity of the algorithm used in this example, where the new WP for the 20
703 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
704 same withdrawal period as when the WP is calculated based on the actual measured residue
705 concentrations in tissues for the 20 mg/kg bw dose.
706

707 These examples in eggs and milk demonstrate the usability of the algorithm for residue depletion in
708 these food commodities.

709 # 5. Approach for addressing risks for the environment

710 ## 5.1. Introduction

711 In the EU, the Environmental Risk Assessment (ERA) is conducted for all veterinary medicinal products
712 in accordance with VICH and CVMP Guidelines. Typically, the ERA is conducted in two phases. In Phase

713 I, products with a low environmental exposure are filtered out; these products do not need further
714 assessment and substance related environmental fate and effect data are not strictly required,
715 although data showing extensive metabolism or complete degradation in manure may be provided
716 optionally. Examples of products with a low environmental exposure are products for companion
717 animals only and products that result in a Predicted Environmental Concentration in soil (PEC_{soil}) of less
718 than 100 µg/kg, based on a worst-case estimation. In Phase II, starting with Tier A, a basic set of
719 environmental effect data in representative species is produced, to estimate Predicted No Effect
720 Concentrations (PNECs) for up to three environmental compartments: soil, surface water, and if
721 needed groundwater. PECs for these compartments are also calculated, taking into account data on
722 metabolism, excretion and the environmental fate of the substance. It should be noted that a PEC in
723 groundwater (PEC_{gw}) ≥0.1 µg/l triggers further risk assessment. As a general rule, when the PECs for
724 all environmental compartments are below the relevant PNECs, no further assessment is needed.
725 However, if any of these PECs is above the PNEC for that compartment, then further data on fate and
726 effects are required for the relevant environmental compartment(s) in Tier B. In Tier B, also the risk
727 for sediment-dwelling organisms will be calculated if needed. This tiered approach progresses from a
728 crude worst-case risk estimation to a refined, more realistic risk estimation. In the situation where
729 following a full ERA a risk for the environment cannot be ruled out, i.e. the PEC is higher than the
730 PNEC, this should be considered in the overall benefit/risk balance for the product, and risk mitigation
731 measures (RMMs) may need to be recommended in the product literature.

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

5.2. The impact of dose optimisation on the ERA

5.2.1. The relation between the dose and the PEC

739 The total dose (in mg/animal for the entire treatment) is one of the inputs into the models used to
740 calculate the PEC_{soil}. The PECs for the other environmental compartments are directly linked to the
741 PEC_{soil}. The relation between the dose and the calculated PEC_{soil} is linear, meaning that a certain
742 increase in the total dose will result in the same relative increase of the PEC_{soil}. This will be the case for
743 the initial PEC_{soil} (as calculated in Phase I) as well as for the refined PEC_{soil} (as calculated in Phase II).
744 Likewise, the PECs for the other environmental compartments that are calculated in Phase II Tier A
745 have a linear relationship with the dose. Only in Phase II Tier B the relation between the dose and the
746 PECs for groundwater, surface water and sediment may become non-linear due to the use of the K_{OC} in
747 the Tier B models. Therefore, in Phase II Tier B these PECs will need to be recalculated.

5.2.2. The importance of triggers

749 As explained above, the ERA follows a tiered approach using triggers; when one of the triggers is
750 exceeded, a further targeted assessment in the next Tier is required. The main trigger in phase I is
751 based on environmental exposure (the PEC_{soil}) and the main trigger in Phase II Tier A is based on
752 environmental risk (the Risk Quotient (RQ), i.e. the PEC/PNEC; when the RQ ≥ 1, further assessment
753 is required in Tier B). Another trigger in Tier A is exposure of groundwater at concentrations of ≥ 0.1
754 µg/L. When this trigger is exceeded, an RQ for groundwater will be calculated using the available Tier A
755 data for aquatic species, and the risk for humans via consumption of drinking water will be assesed (it

756 should be noted that a new CVMP guideline on groundwater, coming into effect in November 2018,
757 specifies additional situations for which a risk assessment for groundwater will be required). When the
758 RQ for groundwater is ≥ 1, even after refinement of the PEC_{gw}, further Tier B studies are required. The
759 tiered approach implies that the final conclusion on the risk for the environment for a product with an
760 optimised (higher) dose will remain unchanged when no triggers are exceeded that were not exceeded
761 for the previous (authorised) dose.

5.2.3. Possible data gaps as a result of trigger crossing

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

5.3. Proposed approach to address the ERA

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

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

781 The environmental risks for products with an optimised dose can be addressed in a stepwise approach.
782 As explained above, the need for additional assessment of environmental risk(s) depends on the
783 individual situation, for example on whether or not triggers are exceeded. The stepwise approach is
784 explained below and is schematically illustrated in the decision tree (Figure 10).

5.3.1. Step 1: Determine the assessment situation

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

790 If the product with the optimised dose still has a lower dose than the product with the highest
791 authorised dose, no further ERA action is required. If the optimised dose is higher, but the outcome of
792 the initial assessment with the optimised dose is that the ERA can stop in Phase I (e.g. PEC_{soil} <100
793 µg/kg, or complete mineralisation of the active ingredient(s) in either the animals or in their excreta
794 occurs), then it can be concluded that no further assessment is necessary. The risks for the
795 environment have been sufficiently addressed for the optimised dose, and no further action is required.
796 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 end-points 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.

B. If the data retrieved under A are still insufficient to conduct the Tier A risk assessment, then the required information may be estimated, for example by the use of (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 these apporaches can be accepted for this specific purpose.

C. If the data are still insufficient, 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 a new CVMP guideline on groundwater, coming into effect in November 2018, specifies additional situations for which a risk assessment for groundwater will be 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 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=1 or above 1 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. This fact has to be taken into account in an overall B/R assessment for the product and the RMMs should be considered.

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Step 1

Authorised dose
PEC$_{soil}$ <100?

Optimised dose
PEC$_{soil}$ <100?

YES

YES

NO

NO

Step 2 — Identify data gaps

Step 3 — Fill data gaps

Step 4

Authorised dose
Tier A RQ<1 &
PEC$_{gw}$<0.1?

Optimised dose
Tier A RQ<1 &
PEC$_{gw}$<0.1 or
RQ$_{gw}$<1?

YES

YES

No issue

NO

NO

PEC refinement
Tier A RQ<1 &
PEC$_{gw}$<0.1 or
RQ$_{gw}$<1?

YES

NO

Step 5 — Identify data gaps

Step 6 — Fill data gaps

Step 7

Authorised dose
Tier B RQ<1 &
PEC$_{gw}$<0.1?

Optimised dose
Tier B RQ<1 &
PEC$_{gw}$<0.1 or
RQ$_{gw}$<1?

YES

YES

NO

NO

Step 8

Consider
B/R – RMMs

Note: PEC$_{gw}$ < 0.1 refers to the concentration in µg/L

853

854 **Figure 10.** Decision tree for addressing the environmental risk assessment for increased doses

855
856

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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 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 project, 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 in a given period, as opposed to an increase in the duration of dosing. An increase in total dose over a given period of time will result in a reduction in the MOS for a product, with some exceptions possible (e.g. gentamicin, where frequency of administration may also impact safety). It would be necessary to assess if an acceptable MOS for each product can be retained with the new dose. 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 in this exercise, the outcomes of studies from similar products could be pooled (see chapter 2.). In this respect, pooled studies will be useful for establishing the toxicity syndrome 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

899 formulation of each product. It is anticipated that problems with toxicity of excipients would be less
900 likely as most commonly used excipients have a wide margin of safety; nevertheless, this should still
901 be considered.

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

6.3. Proposed approach to address target animal safety

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

907 • Active substance

908 • Target animal species/category

909 • Disease indication

910 • Route of administration

911 • Pharmaceutical form

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

915 Annex 4 provides an overview of the data considered useful for reviewing target animal safety. The
916 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

920 Review the TAS studies for all products with the same active substance and pharmaceutical form that
921 are administered by the same route of administration. The aim is to:

922 • Confirm the target organs and toxicity profile of the active substance.

923 • The new MOS should be estimated based on the improved dose relative to the dose for which
924   no/an acceptable level of AEs was observed in the TAS.

925 When pooling studies within different product groups as outlined above, some attention may need to
926 be given to the relative bioavailability and differences in the PK profile for the active substance from
927 different product formulations (for example, long-acting compared to immediate release injections).
928 When calculating the MOS, studies from different products should only be pooled if the PK profiles are
929 similar (also considering that TAS studies are not anyway able to determine a precise MOS due to the
930 dose multiples used). Relevant information may be found in the pharmacokinetics studies for the
931 individual products.

932 In accordance with convention, the TAS are likely to have been conducted at 0x (negative control), 1x,
933 3x and 5x the highest original recommended treatment dose (ORTD); therefore if signs of toxicity were
934 already seen in either the 1x or 3x groups, it may be difficult to conclude that an acceptable MOS
935 remains for the increased dose. Pooling studies from different products may increase the data available
936 as different doses/dose multiples may have been used. An acceptable MOS is dependent on the
937 benefit-risk for the product.

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

941 Reproductive toxicity (where applicable): VICH GL 43 requires studies only to be conducted at 0x and
942 3x ORTD. It is assumed that if the product is approved for use in breeding animals, there would have
943 been no signs of reproductive toxicity at 3x ORTD. The new MOS should be determined based on the
944 increased dose. If this dose is lower than 3x ORTD and no adverse reactions were observed at 3x
945 ORTD, then it is probable that reproductive safety could be accepted for the improved dose. Further
946 information to support a decision may also be available from laboratory animal reproductive toxicity
947 studies and pharmacovigilance post-marketing. Additional risk management measures, if needed,
948 could include strengthening of warnings in SPC 4.7 (NtA, Volume 6C) including restrictions on use in
949 breeding animals.

950 Local tolerance: Consideration should be given to injection-site safety, which may have been
951 investigated at 1x ORTD, only. Additional risk management measures, if needed, could include
952 restrictions on the maximum volume of injection at individual sites, and/or bodyweight of animal to be
953 treated.

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

956 **Step 1a**: If needed as supplementary data, dose determination (and occasionally dose confirmation)
957 studies may have investigated doses higher than the ORTD. Useful safety information (from target and
958 non-target species) may also be available from studies presented in other sections of the dossier (see
959 Annex 4).

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

6.3.2. Step 2: Safety in the target population

966 Review the safety data from the clinical field trials for all products with the same active substance and
967 pharmaceutical form that are administered preferably by the same route of administration. The
968 following points can be considered:

969 • Is there a relationship to dose, dosing frequency or treatment duration for the observed adverse
970 events?

971 • Is there evidence of a decreased MOS in sensitive sub-populations (e.g. age groups)?

972 Additional risk management measures, if needed, could include strengthening of SPC contraindications
973 or warnings relating to sensitive sub-populations.

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

975 Review the Eudravigilance database for all products with the same active substance and
976 pharmaceutical form that are administered by the same route of administration and in the same
977 species with focus on reports where the product has been administered at overdose (subject to
978 availability). The main purpose is to gain a general impression of the safety of the products when used

| 979 | under field conditions; some specific information regarding the safety of increased doses may be |
| 980 | available in reports of overdose. |

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

If needed, studies from peer-reviewed journals 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.

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 publically 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 4, and 5 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.

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. 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 will 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-finding)

- Clinical field trials in the target population

- Eudravigilance

- Detailed information on the product composition and formulation

1015 • Laboratory animal and human safety studies – reproductive toxicity and special studies

1016 • Literature searches

1017 • Information on authorisations of similar products in other e.g. VICH participating countries

1018 An overview of the TAS-related data considered useful is presented in Annex 4.

7. Case study amoxicillin

7.1. Introduction

1021 Ampicillin and amoxicillin are two very commonly used beta-lactam antibiotics in veterinary medicine.
1022 In the EU amoxicillin is licensed as various formulations (powder, granules, tablets and suspensions for
1023 injection) for a variety of animals (food-producing and non-food producing).

1024 This case study shall be limited to the oral administration of amoxicillin to pigs, by medicated drinking
1025 water.

1026 Amoxicillin is a broad-spectrum, semisynthetic aminopenicillin antibiotic with bactericidal activity.
1027 Amoxicillin binds to and inactivates penicillin-binding proteins (PBPs) located on the inner membrane of
1028 the bacterial cell wall. Inactivation of PBPs interferes with the cross-linkage of peptidoglycan chains
1029 necessary for bacterial cell wall strength and rigidity. This interrupts bacterial cell wall synthesis and
1030 results in the weakening of the bacterial cell wall and cell lysis.

1031 Amoxicillin is usually available as amoxicillin trihydrate.

1032 The approved doses vary widely between 10 – 20 mg/kg bw, to be given once or twice daily for 3-7
1033 consecutive days. Most commonly a daily dose of 10 – 20 mg/kg bw is recommended for 3-5 days. It
1034 should be noted that the dose can be expressed in amoxicillin or amoxicillin trihydrate. The conversion
1035 factor to the trihydrate is 1.15 and to amoxicillin 0.87.

1036 Licensed products are indicated for a wide variety of infections of the respiratory, gastro-intestinal and
1037 uro-genital tract as well as skin and joint diseases. This case study will focus on the indication for
1038 respiratory disease which is most commonly caused by *Actinobacillus pleuropneumoniae*, *Haemophilus*
1039 *parasuis*, *Pasteurella multocida*, *Streptococcus suis* and *Bordetella bronchiseptica*.[1]

7.2. Dose optimisation

7.2.1. Determination of the PK parameters

1042 PK parameters can be derived from published papers and available information in marketing
1043 authorisation dossier (Annex 1). For the purpose of the pilot study, a review of published papers was
1044 performed (Table 4).

1045

---

[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.

1046

1047 **Table 4.** Overview of published scientific papers for amoxicillin

Reference	Intravenous administration dose (mg/Kg)	Oral administration dose (mg/Kg)
Agersø & Friis (1998a)	9	10
Agersø & Friis (1998b)	9	
Martínez-Larrañaga et al. (2004)	20	20
Hernandez *et al*. (2005)	15	15
Reyns *et al*. (2008)	20	20
Godoy *et al*. (2011)	15	5/9/10/15/18
Krasucka & Kowalski (2010)		28

1048 The pharmacokinetic parameters extracted from the papers are the mean value and standard deviation
1049 of the clearance, the bioavailability and the apparent clearance. An overall mean and standard
1050 deviation for each parameter were calculated from the pool.

1051 **Equation 3.** $mean_{all} = \frac{\sum mean_i \times N_i}{\sum N_i}$

1052 **Equation 4.** $SD_{all} = \sqrt{Var_{all}} = \sqrt{\frac{\sum(Var_i \times (N_i - 1))}{\sum(N_i - 1)}}$

1053 Where $mean_{all}$ is the mean of the pool, $mean_i$ the mean reported for the ith study, Var_{all} the variance of
1054 the pool, var_i the variance for the ith study.

1055 - For amoxicillin in pigs, clearance is 0.5 ± 0.18 L.h^{-1}.kg^{-1} and oral bioavailability is 0.33 ± 0.12.

1056 - The free fraction of amoxicillin in plasma was set at a mean value of 0.7 ranged 0.6 to 0.8.

1057 **Population pharmacokinetics**

1058 The availability of PK raw data or in this case study, the summary of PK parameters allows performing
1059 a meta-analysis for a given product using a non-linear mixed effect model (Figure 11 and Table 5).
1060 This approach allows integrating variability of biological origin (e.g. breed, sex, age, health status) and
1061 non-biological origin (e.g. study design, tested dose).

1062 In a peer reviewed paper (Rey *et al.*, 2014), amoxicillin concentrations in function of time were
1063 obtained from 4 different sources (3 pharmaceutical companies, 1 academic laboratory). Five
1064 formulations administered by oral routes were analysed and a common pharmacokinetic model was
1065 established. It is a two-compartment model with a zero order input rate (K0) between lag time (Tlag)
1066 and end time (Tend).

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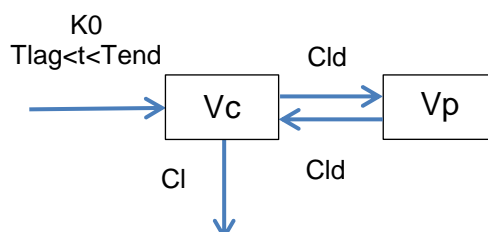
1073 **Figure 11.** Diagram of pharmacokinetic model for amoxicillin administered orally to pigs. Cl=
1074 clearance of elimination, Vc= Volume of central compartment, Vp=Volume of peripheral compartment,
1075 Cld=Clearance of distribution.

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1076 The data were analysed using software for non-linear mixed effect model. A covariate analysis was
1077 performed taking into account the formulation as the main covariate able to account for the individual
1078 intervariability. A diagonal Ω matrix was assumed.

1079 **Table 5.** Pharmacokinetic parameters obtained for a population pharmacokinetic model for 5
1080 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	

1081
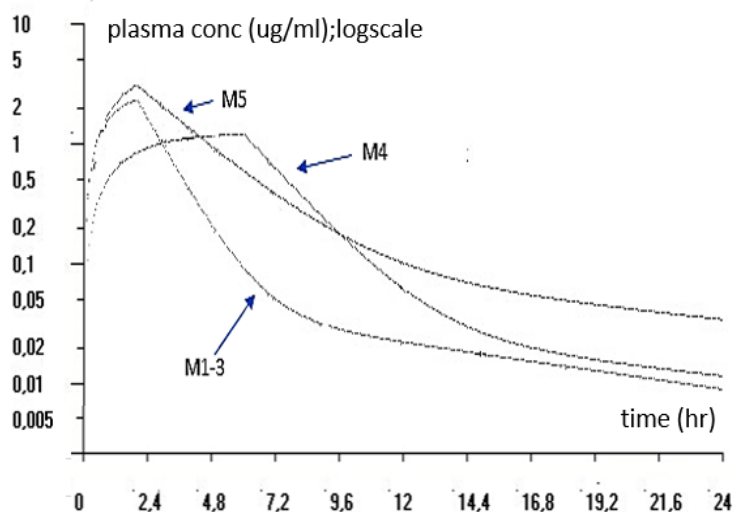
1082

1083 **Figure 12.** Simulation of a dose of 20 mg/kg based on mean parameters for the 5 formulations
1084 presented in table 5 (based on Rey *et al*., 2014).

1085 In the original publication, the target for the T>MIC was set at 40% of a period of 24h. Figure 12
1086 shows the simulation obtained with the PK model for the mean value parameter of each formulation.
1087 The parameters of formulation 2 were chosen for the pilot study because they represent the worst case
1088 scenario in terms of exposure (AUC and T>MIC).

7.2.2. Define the target bacteria

1090 The therapeutic indication targeted is swine respiratory disease with the following list of targeted
1091 pathogens.

- *Actinobacillus pleuropneumoniae,*
- *Bordetella bronchiseptica,*
- *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 2016. 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.

7.2.3. 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.

7.2.3.1. 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.

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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). The target values for a target attainment 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

7.2.3.2. 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.

7.2.4. 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.

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

7.2.5.1. 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.
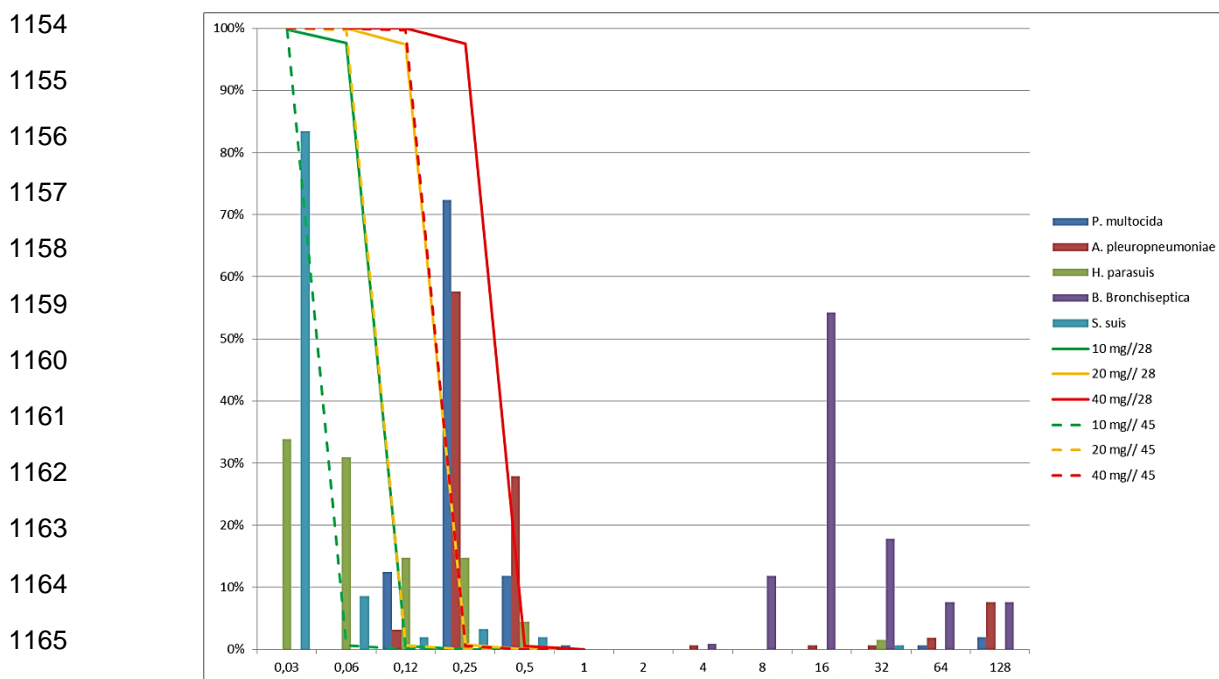
Figure 13. Graphic representation of probability of target attainment for different daily dose (10, 20, 40 mg/kg bw) according to target value of the PDI (AUC/MIC) according MIC levels and the MIC distribution for the targeted bacteria

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,* this target pathogen never reaches the PK/PD objectives. *B. bronchiseptica* should be deleted from the therapeutic indication of amoxicillin administered by the oral route to pigs when one is optimising 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 99.9[th] percentile of ECOFF (Turnidge *et al.*, 2006). In the context of this pilot project, all the requested criteria may not be fulfilled to use this 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 report, 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.

7.2.5.2. 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 optimal daily dose should be set at 40 mg/kg bw to allow an acceptable exposure of the different target pathogens.

1238 • ***Main conclusions on the amoxicillin case study in relation to dose optimisation:***

1239 As a reminder to summarise this first case study, by following the different steps, the PK/PD
1240 relationship allows to define a dosage regimen taking into account PK and PD variability but also by
1241 considering the probability to reach the target value of the selected PK/PD index for a defined drug-bug
1242 combination.

1243 For the amoxicillin case study, different conclusions can be drawn:

1244 - **Concerning the dose computed :**

1245 o Different doses can be computed in function of the therapeutic objective (e.g.
1246   Bacteriostatic, Bactericidal 2-log, Bactericidal 4-log);

1247 o Different doses can be computed in function of the target pathogens MIC distribution.
1248   Higher dose should for example, be applied to cover adequately the least susceptible
1249   bacterial species.

1250 - **Concerning the modelling using AUC/MIC or T>MIC as PDI:**

1251 o When modelling the Probability of Target Attainment (PTA; 90%) according to the selected
1252   PDI and MIC, it can be concluded that for T>MIC, the computation of the PDI requires
1253   simulation of time-concentrations curves which requires pharmacometric tools. The
1254   interest of this approach is to further refine the dosage regime in relation to the way of
1255   administration of the treatment. Indeed, the results in Table 12 revealed that fractionation
1256   of the dose increases the probability to attain the target value of the PDI. This is mainly
1257   due to the short half-life of the active substances.

1258 o T>MIC provides a better option for defining a precise daily dose for time-dependent
1259   antibiotics but it need then the definition of a frequency of administration by day to
1260   guarantee an acceptable exposure.

1261 o AUC is less precise but allows to define a daily dose allowing a good exposure and thus
1262   without taking into account the frequency of administration. The determination of a daily
1263   dose reaching the PTA of 90% using T>MIC as a PDI will not be feasible as the computed
1264   dose will be too high. The PK/PD analysis using T>MIC as PDI could be used to further
1265   refine the interval frequency after the determination of a daily dose using AUC/MIC.

1266 o The outcome of this pilot exercise, using AUC/MIC, indicates that the optimised dose to
1267   treat respiratory disease in pigs with amoxicillin in drinking water is 40 mg/kg bw to cover
1268   the major pathogens *P. multocida*, *A. pleuropneumoniae*, *S. suis* and *H. parasuis.*

1269 A recent paper (Burch & Sperling, 2018) reviewed the use of amoxicillin in swine looking at the various
1270 formulations and routes of administrations in regards to clinical efficacy. They considered
1271 epidemiological cut-off values in their PK/PD correlation and concluded that an oral dose of 20 mg/kg
1272 bw might not be suitable and should be increased.

7.2.6. Set a PK/PD breakpoint

1274 The last step of the proposed approach to address doses is the definition of clinical breakpoint, or
1275 PK/PD breakpoints when lacking clinical data (cf. chapter 3.3 – step 7). According to the data available
1276 for amoxicillin, ECOFFs vary between the targeted bacterial species. In our example, the PK/PD
1277 breakpoint could be set at 0.5 µg/mL as the PTA of 90% for strains with MIC above 1 µg/mL is never
1278 reached (Figure 13). This value seems compatible with ECOFFs of studied species but with the

1279 limitations that our dataset is too small to determine them correctly for all bacterial species (n<300).
1280 The highest daily dose tested of 40 mg/kg bw allows to reach a PTA close to 90 % when AUC/MIC is
1281 used but not with T>MIC for which the PTA value depends on the rate of administration.

7.2.7. Define an optimal daily dose

1283 According to the PK/PD modelling done in for this case study, the approved oral daily dose of 20 mg/kg
1284 bw is insufficient to sufficiently expose the target pathogens for 24 hours. A recent paper reviewed the
1285 use of amoxicillin in swine looking at the various formulations and routes of administrations in regards
1286 to clinical efficacy. They considered epidemiological cut-off values in their PK/PD correlation and also
1287 concluded that an oral dose of 20 mg/kg bw might not be suitable and should be increased (Burch and
1288 Sperling, 2018). Indeed, using AUC/MIC as a PDI, the dose of 20 mg/kg bw is not able to reach a PTA
1289 of 90% for the different target pathogens. To achieve this goal, the outcome of this pilot exercise,
1290 indicates that the optimised dose to treat respiratory disease in pigs with amoxicillin in drinking water
1291 is 40 mg/kg bw to cover the major pathogens *P. multocida*, *A. pleuropneumoniae*, *S. suis* and *H.*
1292 *parasuis*. However, as amoxicillin is a time dependent antimicrobials where T>MIC is considered as
1293 best predictors of clinical efficacy, a second step was applied to refine the daily dose firstly set using
1294 AUC/MIC. Using T>MIC, the results show that the PTA increase with dose and dose fractionation (Table
1295 12). Thus, the medication by drinking water represents a good route administration for amoxicillin
1296 allowing fractionating the dose of 40 mg/kg bw newly defined, during the day in function of the
1297 drinking rhythm and behaviour of the treated animals. Furthermore, when a medicinal product is
1298 presented in a solution prior to administration through drinking water, the product's formulation will
1299 usually not influence the bioavailability of the active substance (See Guideline
1300 EMA/CVMP/EWP/016/00-Rev.3; EMA/CVMP, 2017).

7.3. Withdrawal period

1302 The Withdrawal Periods (WP) of the various products authorised in the EU Member States vary greatly
1303 and range from 2 – 28 days (an overview is provided in Annex 3). This overview was generated around
1304 2010 and might not be completely up to date anymore. However, it is unlikely that major changes
1305 have occurred in the meantime. There is no obvious pattern why for some products the WP is rather
1306 long or short. In this context it should be noted that the most of the products are generics for which no
1307 product specific residue depletion studies were usually required[2].

1308 **Table 13.** Selection of amoxicillin products (powder for oral administration) for the treatment of
1309 respiratory disease in pigs licensed in the EU via the Mutual Recognition procedure

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

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[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.

7.3.1. 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 & 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 & Friis, 1998a). Oral bio-availability was only 31% in fasted animals and 28% in fed animals. The reported differences in bio-availability, 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 i.v. and i.m. administration is presented in Table 14 (Schwarz *et al*., 2008).

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)**	5.8±2.3	0.76±0.05	n.d.	n.d.		n.d.	0.11±0.05

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)
dose: 15 mg/kg							
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

1335 *Oral administration not defined

1336 ** in feed

1337 |*** in drinking water

1338 The most recent studies available since 2008 are briefly summarised below. In summary, the
1339 pharmacokinetic parameters assessed and evaluated where broadly in line with what has been
1340 published before.

1341 Godoy et al. (2011) made a comparative pharmacokinetic assessment of amoxicillin given to healthy
1342 pigs and pigs suffering from respiratory disease. After single intravenous bolus administration of
1343 amoxicillin to healthy pigs, the VDSS was 0.61 l/kg, total plasma clearance was 0.83 l/h/kg and MRT
1344 0.81 h. After oral bolus administration, the mean absorption time was 1.6 h and the peak plasma
1345 concentration of 3.09 µg/ml was reached after 1.2 h. The oral bioavailability was 34%.
1346 Pharmacokinetic parameters calculated (C_{maxss}, C_{minss}, C_{avss} and AUC_{24ss}) were significantly lower in
1347 healthy pigs in comparison to diseased pigs. This was due to higher bioavailability and longer
1348 absorption period observed in diseased pigs. Dose linearity was demonstrated in diseased pigs over a
1349 dose range of 4-18 mg/kg bw.

1350 Menotta et al. (2012) compared the bioavailability of a coated amoxicillin to an uncoated formulation.
1351 Oral bioavailability of the formulation with coated amoxicillin was higher than with uncoated
1352 amoxicillin, AUC was significant higher and there were statistically significant differences in C_{max}, Time
1353 to C_{max} (T_{max}) and MRT. That confirms that the galenics of the formulation may have a significant effect
1354 on the pharmacokinetic profile. However, for conventional oral formulations (powder and granules) a
1355 difference in oral bioavailability is not expected, because of the good solubility of amoxicillin trihydrate
1356 in water.[3]

1357 Dai et al. (2017) conducted a relative bioavailability study of an oral amoxicillin-apramycin combination
1358 in pigs. The study was done in a three way cross-over design comparing the pharmacokinetics of
1359 amoxicillin and apramycin either as single components, or as combination product. The test articles
1360 were given intra-gastrically to fastened pigs at a dose of 16 mg/kg bw amoxicillin. There was no
1361 difference in the pharmacokinetic profile of amoxicillin whether administered alone or in combination
1362 with apramycin. Of interest are the basic pharmacokinetics parameters for amoxicillin obtained in this
1363 study. The peak plasma concentration was reached after 1.92 h with a C_{max} of 3,25 µg/ml and $AUC_{0-\infty}$
1364 of 8.43 mg/h/l. The MRT was 3.43 and $T_{1/2}$ was 6.33 h. The data are overall consistent with previously
1365 reported data (see Table 14).

1366 In addition, several pharmacokinetic studies were made available from industry. Following, only the
1367 key findings are briefly reported.

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[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).

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1368 A pilot study was set up to investigate the plasma pharmacokinetics of amoxicillin-trihydrate in eight
1369 14-week old pigs after single (pulse) oral administration of a soluble powder, first through feed and
1370 two weeks later through drinking water. Two dosages, i.e., 11.6 and 23.2 mg amoxicillin/kg bw were
1371 tested. When administered in combination with pelleted feed, absorption of amoxicillin was somewhat
1372 delayed as indicated by the T_{max} of about 2.25 h and the terminal half-life of about 1.1 h for the 14.5
1373 mg/kg bw dose and 1.7 h for the 29 mg/kg bw dose. These values are higher than the corresponding
1374 values observed after administration in water. This indicates that absorption is the rate limiting step for
1375 elimination. The maximum plasma levels obtained do not linearly increase with the dose, i.e., 1.0 and
1376 1.25 mg amoxicillin per animal. This is also indicated by the observed area under the curve (AUC) for
1377 the two dosages, which tend to be somewhat lower for the higher dosage. The plasma-concentration
1378 profiles show that amoxicillin is rapidly absorbed as indicated by the observed T_{max} of about 0.75 h and
1379 the terminal half-life of 0.5 to 1.0 h, suggesting that rate of absorption is not limiting for elimination.
1380 This is also indicated by the observed AUCs for the two dosages, which are proportional and represent
1381 more than 99% of the total extrapolated AUC at 7.25 h after consumption of the dose. The maximum
1382 plasma levels obtained show a roughly linear increase with the dose, with C_{max} values of 1.5 and 2.7
1383 mg amoxicillin per animal for the 14.5 mg/kg bw and 29 mg/kg bw dose, respectively.

1384 In a second pilot study the pharmacokinetics of amoxicillin was assessed after repeated administration.
1385 Eight 14-weeks old pigs were divided into two medicated groups of four animals. Group I received a
1386 continuously administered daily dose of 8.0 mg amoxicillin/kg bw, mixed through the daily ration of
1387 drinking water for three consecutive days. Group II similarly received an oral dose of 16.0 mg
1388 amoxicillin/kg bw mixed through the daily ration of drinking water. Two weeks after the continuous
1389 medication, the animals received a single pulse dosage of 10. 0 or 20.0 mg/kg bw per day
1390 respectively. The average plateau plasma levels were ranging between 0.2 and 0.4 µg/ml after dosing
1391 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
1392 daily single pulse dosing peak plasma levels ranging from 0.7 to 1 µg/ml for the 10 mg/kg bw dose,
1393 and from 1.1 to 2.1 µg/ml for the 20 mg/kg bw dose were obtained.

1394 Further data were provided by Company B (1) which are summarised in the two figures below.
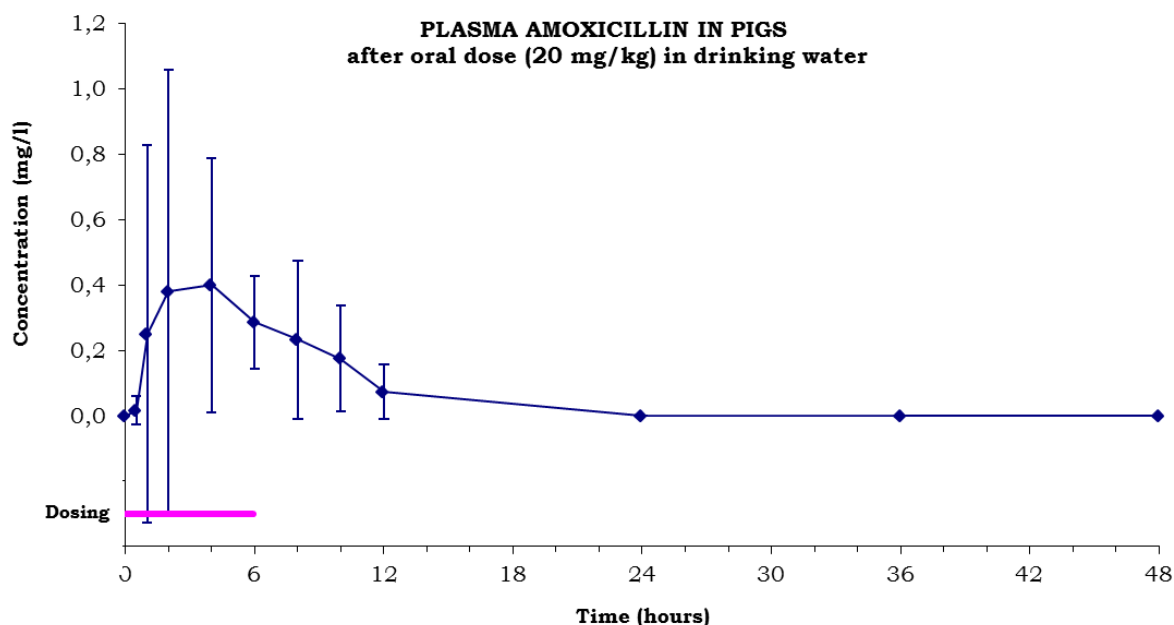
1395

1396 **Figure 14.** Amoxicillin plasma concentrations in pigs after a single oral dose. Mean values and
1397 standard deviation (+/-) are shown

PLASMA AMOXICILLIN IN PIGS
after five oral repeated doses (20 mg/kg/day) in drinking water
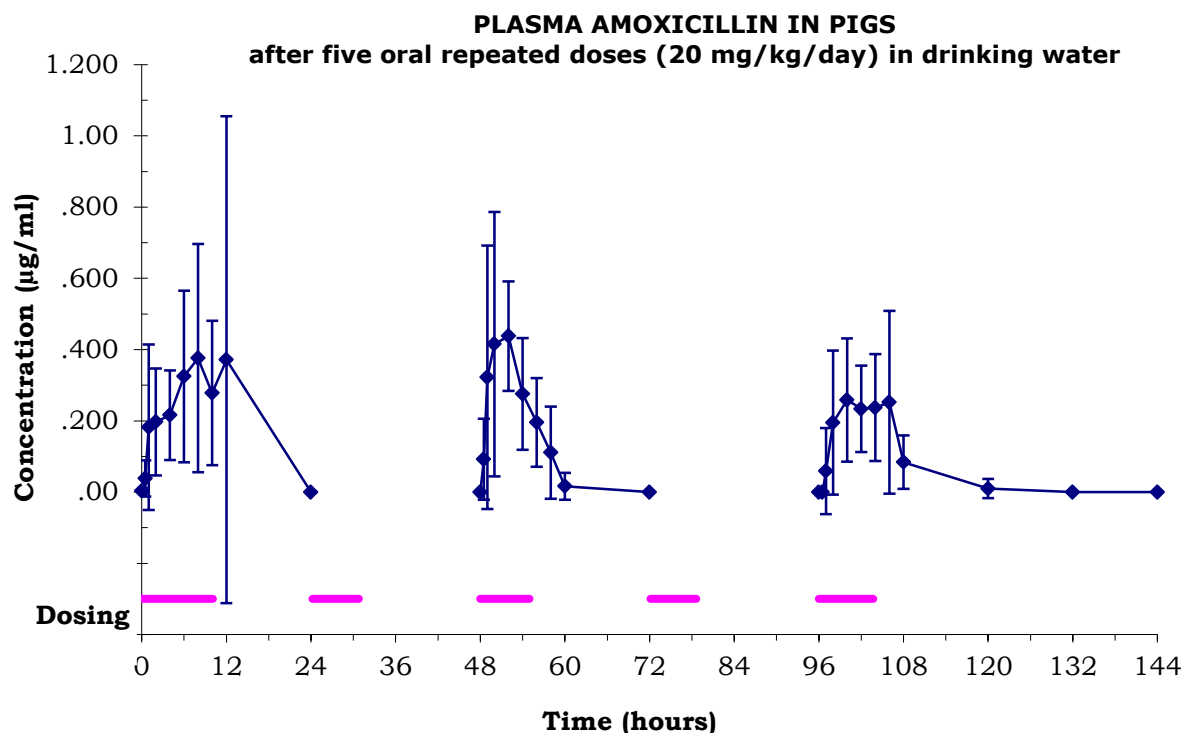
Figure 15. Amoxicillin plasma concentrations in pigs after repeated dosing. Mean values and standard deviation (+/-) are shown

7.3.1.1. Dose linearity

One of the limiting conditions for using the proposed extrapolation method to calculate a withdrawal period is that linear kinetics must apply. From studies in pigs and human, dose linearity was not always seen and it appears that it is limited by a saturated absorption[4]

The various studies assessing dose linearity are briefly described below.

Godoy *et al*. (2010) established a dose linearity for amoxicillin in <u>diseased</u> 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.

Rey *et al*. (2014) referred in his paper to the study of Godoy *et al*. and 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 was shown across the three dosages. The data are depicted below in Figure 16, Figure 17, and Figure 18.

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[4] (https://academic.oup.com/jac/article/71/10/2909/2388123/Non-linear-absorption-pharmacokinetics-of).
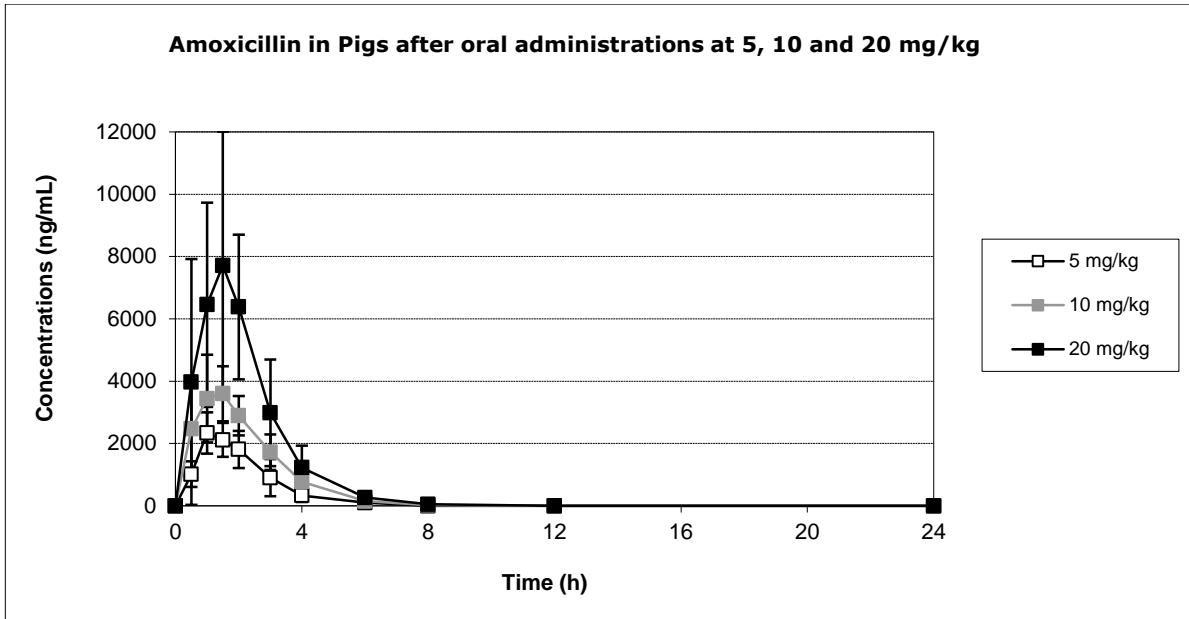
Figure 16. Amoxicillin plasma concentrations at three different dose levels. Mean values and standard deviation (+/-) are shown
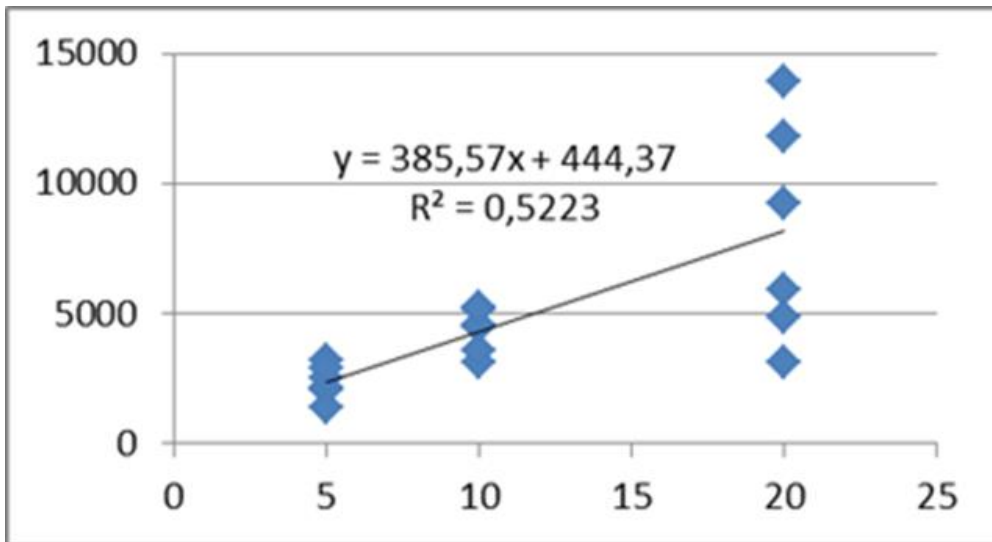
Figure 17. Dose linearity of individual amoxicillin plasma concentrations at three different dose levels (5 mg /kg bw, 10 mg /kg bw and 20 mg/kg bw). X-axis: dose (mg/kg bw); Y-axis : plasma amoxicillin concentrations (ng/ml)

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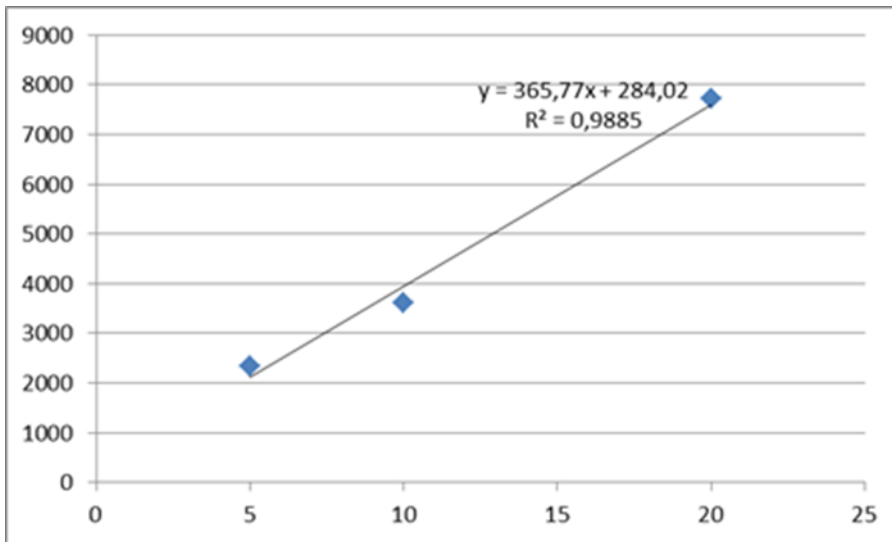
Figure 18. Dose linearity of mean amoxicillin plasma concentrations at three different dose levels (5 mg/kg bw, 10 mg/kg bw and 20 mg/kg bw). X-axis: dose (mg/kg bw); Y-axis : plasma amoxicillin concentrations (ng/ml)

An acceptable and good dose-linearity relationship ($R^2 > 0.98$) is observed for C_{max} within the dose range of 5 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[5].

7.3.1.2. 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.

Regarding C_{max}, studies have demonstrated a dose-linearity relationship between 5 and 20 mg/kg bw.

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

7.3.2. PK/PD Considerations

Using PK/PD modelling methods, within this pilot project, an optimal 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.

7.3.3. 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. Indeed a microbiological Acceptable Daily Intake (ADI) has been

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

1446 established by JECFA for amoxicillin, and this ADI covers the allergic risk associated with these two
1447 metabolites displaying almost nil antibacterial activity.

7.3.4. Radiolabelled residue depletion studies

1449 There were no amoxicillin radiolabel residue depletion studies in pigs available for evaluation. The only
1450 microbiological active residue is the parent molecule.

7.3.5. Maximum Residue Limits

1452 The CVMP (1996) did not establish an ADI for penicillins. In order to adequately protect the consumer
1453 and secure dairy production, the CVMP recommended the following maximum residue levels for six
1454 penicillins:

1455 **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

1456

1457 JECFA (2011, 2017) assessed amoxicillin at their 75th meeting in 2011 and their 85th meeting in 2017
1458 and came to the following conclusions:

1459 - An ADI of 0–0.002 mg/kg bw was established by the Committee based on a microbiological
1460 endpoint, equivalent to an upper bound value of 0.12 mg for a 60 kg person.

1461 - The Committee recommended MRLs for amoxicillin in cattle, sheep, pig and finfish tissues of 50
1462 µg/kg and in cattle and sheep milk of 4 µg/kg, determined as amoxicillin parent compound. The
1463 Committee determined also an Acute Reference Dose and a Global Estimated Acute and Chronic
1464 Dietary Exposure.

7.3.6. Tissue residue studies

1466 Only few residue depletion studies in pigs are available. JECFA (75th meeting, 2011) reviewed data
1467 from 1979 where amoxicillin was given orally as an oily suspension. Amoxicillin was eliminated very
1468 quickly and no residue depletion profile could be established in tissues and organs. It was concluded
1469 that for many studies in all species assessed, namely cattle, pigs and poultry, the sampling time
1470 intervals were too long to permit a detailed analysis of residue depletion in tissues and, consequently,
1471 there are a substantial number of reported findings <LOQ (limit of quantification).

1472 The same conclusions apply to the study published by Reyns *et al*. (2007). Residue depletion of
1473 amoxicillin residues occurred rapidly and residues were below the limit of detection (LOD) already 48 h
1474 after last administration of 20 mg/kg bw amoxicillin administered once by gavage (stomach tube).

1475 A non-GLP residue depletion study was conducted in Belgian Landrace stress-negative pigs. Twenty
1476 animals received an i.v. bolus of amoxicillin at a dosage of 20 mg/kg bw through a catheter in an ear
1477 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*., 2007). 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*., (2007))

Tissue	Chemical	Time and route of administration							
		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	10 3132[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

Martínez-Larrañaga *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 19.

1499 **Table 17.** Mean (sd) plasma concentrations (µg/ml) and tissue concentrations (µg/kg) of amoxicillin in
1500 four pigs given 20 mg/kg amoxycillin orally for five days (copied from Martinez- Larrañaga *et al.*,
1501 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

1502 Limit of quantification= 0.01µg/g, limit of detection= 0.003µg/g ND Not detectable
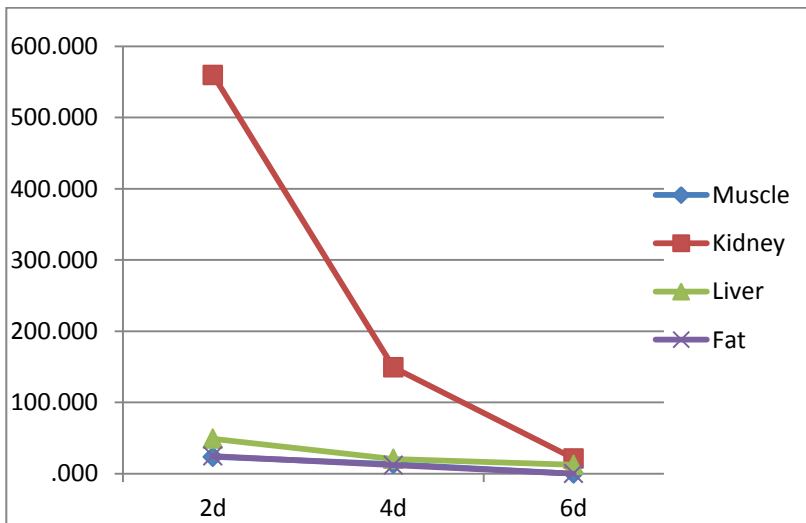
1503

1504 **Figure 19.** Amoxicillin tissue residues (µg/kg) in muscle, liver, kidney and fat from pigs given
1505 amoxicillin at a dose of 20 mg/kg bw orally for 5 consecutive days (Martínez-Larrañaga *et al.*, 2004)

1506 The elimination half-lives shown below have been calculated from the tissue residue depletion data
1507 (mean values, data from Table 17).

1508 **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	low fitting of curve with data
Fat	2 days	good fitting of curve with data

1509 In another residue depletion study, amoxicillin was administered twice daily via drinking water at a
1510 dose of 10 mg/kg bw or once daily at a dose of 20 mg/kg bw for 5 consecutive days (Company B (2)).
1511 Mean residue data shown below in Figure 20 and Figure 21. Amoxicillin residues were detectable in
1512 tissues and organs over a rather long period of time.
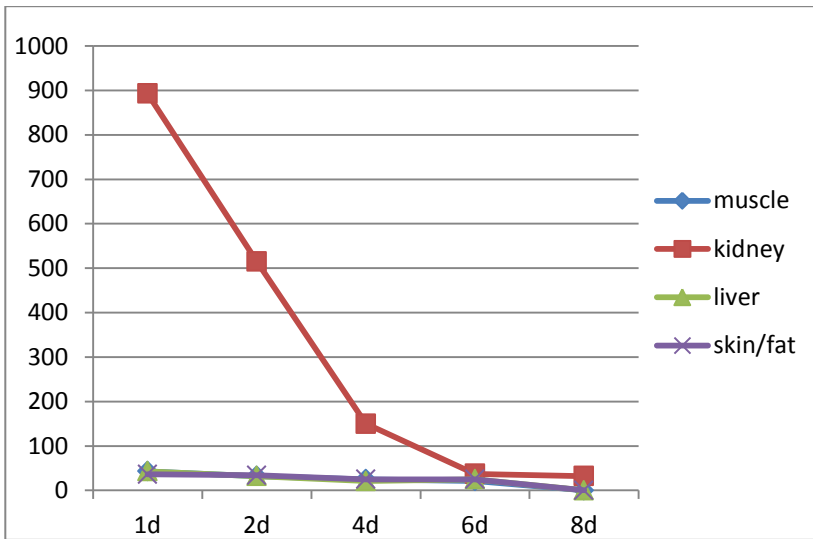
1513

1514 **Figure 20.** Amoxicillin residues (µg/kg) in pigs after oral administration twice daily via drinking water
1515 at a dose of 10 mg/kg bw amoxicillin in 4 animals per group; HPLC method, LOQ: 20 µg/kg
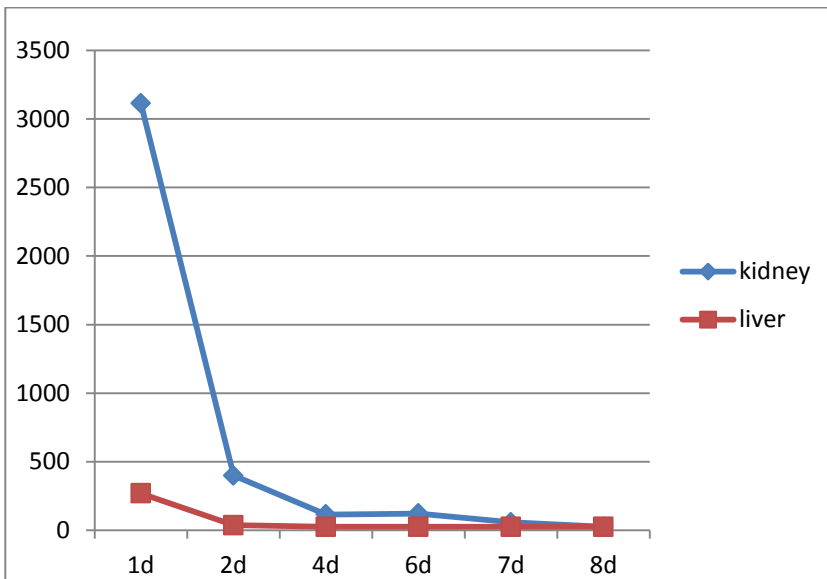
1516

1517 **Figure 21.** Amoxicillin residues (µg/kg) in pigs after oral administration of 20 mg/kg bw amoxicillin,
1518 once a day in liquid meal for 5 days, 4 animals per group, HPLC method, LOQ: 20 µg/kg

1519 The elimination half-lives shown below have been calculated from the two tissue residue depletion
1520 studies (10 mg/kg bw given twice daily for 5 consecutive days and 20 mg/kg bw given once daily for 5
1521 consecutive days (data from Company B(2)).

1522

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
was 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.

7.3.7. 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.

7.3.8. 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.

Tissue residues are also 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 should be thus not considered at this time.

1548 For the extrapolation of a new withdrawal period considering a higher dose, tissue residue elimination /
1549 half-life is to be considered which is rather short and below 48 hours. As a worst case approach a half-
1550 life of 48 h was used in the extrapolation of the WPs.

1551 ### 7.3.9. Withdrawal time calculation

1552 The new withdrawal periods were calculated using Equation 2.

1553 It has been noted that the current withdrawal periods for the amoxicillin products vary considerably
1554 between products. There is no obvious reason for this. One explanation could be that the products do
1555 differ in their oral bioavailability. However, this may not explain the great differences in all the cases.
1556 However in this pilot project it was agreed to extrapolate from the **current** WPs of the products (see
1557 2.2.).

1558 **Table 21.** Current WPs and the WPs calculated for a dose of 40 mg amoxicillin/kg bw for the products
1559 listed in Table 13

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

1560 ## 7.4. Environmental risk assessment

1561 Because there may be different authorised doses for the same or similar products, as a general rule,
1562 the situation for the product with the highest authorised (total) dose for the same target animals is
1563 used for the comparison, provided that an ERA exists for that product at that dose for the relevant
1564 target species. In the case of amoxicillin products for use in drinking water for pigs, ERAs were
1565 available addressing the risks at a dose of 20 mg/kg bw per day for 5 days.

1566 ### 7.4.1. Step 1: Determine the assessment situation for amoxicillin

1567 For the products containing amoxicillin for use in drinking water for pigs at doses of 20 mg/kg bw per
1568 day for up to 5 days, the existing ERAs went into Phase II because the PEC_{soil}-trigger of Phase I was
1569 exceeded. Considering that the optimised dose of 40 mg/kg bw per day for up to 7 days is higher than
1570 the currently authorised dose, it was concluded that the ERA for the optimised dose would also enter
1571 Phase II.

1572 In the available Phase IIA assessments, fate and effect studies were considered, and the RQs were
1573 determined for the various test species representing the terrestrial and aquatic environments. The RQs
1574 for terrestrial species were in the range of 0.005-0.084, and the RQs for aquatic species were in the
1575 range of 0.012-0.43.

1576 When doubling the dose from 20 to 40 mg/kg bw per day for 5 days (the maximum duration for most
1577 of the products), the RQs will be increased by a factor of 2, resulting in a maximum RQ of 0.86. This
1578 RQ remains below 1. In addition, the dose increase will not result in a (Phase II Tier A) $PEC_{groundwater}$
1579 higher than 0.1 μg/L. However, when the duration is extended to 7 days (as for some authorised
1580 products), the highest RQ (for aquatic species) would increase to 1.2. While this is only a slight
1581 exceedance of the RQ of 1, it would indicate the need for a Tier B assessment. Within the limited

1582	sample of products available for this pilot project, no Tier B data were available. Beyond this pilot
1583	project, it should first be investigated if Tier B data are available from any of the MAHs. However
1584	within the context of this pilot project and in lieu of Tier B data, it was considered that most products
1585	have a treatment duration of 3-5 days, and all products have roughly the same PK when given via the
1586	drinking water at the same dose. Therefore, it was concluded that 3-5 days could be sufficient for all
1587	products concerned and having the same indication,. A limitation to 5 days as the maximum treatment
1588	duration was considered as a possible Risk Mitigation Measure (RMM), which could be applied to all
1589	such products concerned. Overall, it was concluded that the optimised dose does not give rise to
1590	concerns in relation to environmental risks. Further consideration of steps 2-8 of the proposed
1591	approach was not necessary.

7.4.2. Conclusion on the ERA

| 1593 | It was concluded that doubling the dose of amoxicillin from 20 mg/kg bw per day to 40 mg/kg bw per |
| 1594 | day for a maximum duration of 5 days will not present a risk for the environment. |

7.5. Target animal safety

1596	As noted in the introduction, the approved doses of amoxicillin for administration in drinking water to
1597	pigs vary widely between 10 – 20 mg/kg bw, to be given once or twice daily, for 3-7 consecutive days.
1598	According to the outcomes of the PKPD modelling, it is proposed that the dose should be doubled to 40
1599	mg/kg bw for the given swine respiratory disease indication.

7.5.1. 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.

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 toxicity studies were not available to the pilot project.

Conclusions: 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.

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

Data not available to the pilot project.

7.5.2. Step 2: Safety in the target population

Data not available to the pilot project.

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

Data not available to the pilot project.

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

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

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*.

1658 <u>Toxicological evaluation of certain veterinary drug residues in food (JECFA 75th meeting, 2011)</u> In
1659 laboratory animal toxicological studies, NOAELs were largely based on the highest doses tested and
1660 were from 250 to 2000 mg/kg bw per day. Dogs receiving doses of 500 mg/kg bw showed
1661 **gastrointestinal effects** due to disturbance of the GI flora.

1662 Human toxicity: Gastro-intestinal, allergic effects and hepatotoxicity are reported. In humans the
1663 incidence of hepatotoxicity is identified at <0.02 to 3 per 100,000 prescriptions. It was concluded that
1664 amoxicillin is unlikely to cause reproductive or developmental toxicity in humans.

1665 <u>Textbooks</u>

1666 Prescott, J.F., & Dowling, P.M. (Eds.). (2013). *Antimicrobial therapy in veterinary medicine. John Wiley*
1667 *& Sons.: 'Penicilllins and beta-lactam antibiotics are generally **remarkably free of toxic effects even***
1668 ***at doses grossly in excess of those recommended.** The major adverse effects are **acute***
1669 ***anaphylaxis and collapse;** milder hypersensitivity reactions…are more common…. Anaphylactic*
1670 *reactions are less common after oral rather than parenteral administration…Less common adverse*
1671 *reactions include **haemolytic anaemia and thrombocytopenia**.' 'One hazard with broad-spectrum*
1672 *penicillins is the potential to **disturb the normal intestinal flora**.'*

1673 *<u>Conclusions</u>: Published studies on the toxicity/safety of amoxicillin in pigs were hard to locate on a*
1674 *basic internet search (PubMed, Google scholar). According to grey literature and standard texts,*
1675 *amoxicillin has a wide margin of safety. Hepatotoxicity and renal toxicity may occur rarely.*
1676 *Gastrointestinal disturbances may occur due to disruption of the microbiota. Amoxicillin is unlikely to*
1677 *cause reproductive or developmental toxicity. The adverse event of most concern in humans is*
1678 *anaphylaxis, which is generally regarded as idiosyncratic. Although it takes a higher oral dose to*
1679 *induce an allergic reaction than if the drug is administered parenterally, it is not clear if increasing the*
1680 *dose within the therapeutic range would increase the risk of hypersensitivity developing.*

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

1684 No specific studies are available that would demonstrate a MOS above the approved dose (20 mg/kg
1685 bw per day) consistent with current VICH requirements. However, based on two GLP TAS studies,
1686 despite some limitations in the studies, it has been demonstrated in 10 healthy pigs that doses of 100
1687 mg/kg or higher administered for at least 5 days were well tolerated.

1688 Published literature indicates that amoxicillin is safe in laboratory species at doses well in excess of
1689 those used therapeutically. Hepatotoxicity and renal toxicity may occur rarely. Gastrointestinal
1690 disturbances may occur due to disruption of the microbiota. Amoxicillin is unlikely to cause
1691 reproductive or developmental toxicity. The most common and concerning adverse events are
1692 hypersensitivity reactions – it cannot be concluded if these idiosyncratic reactions would increase in
1693 frequency following an increase to the dose regimen.

1694 **Overall it is concluded that the proposed dose of 40 mg amoxicillin/kg bw per day for 5 days**
1695 **in drinking water is likely to be adequately tolerated in pigs.**

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

1698 Excipients used in different formulations include:

1699	•	Pentasodium triphosphate
1700	•	Silica Colloidal anhydrous
1701	•	Trisodium phosphate anhydrous
1702	•	Na carbonate
1703	•	Na citrate
1704	•	Lactose monohydrate – lactose intolerance may be dose-dependent.
1705	•	Na Glycine carbonate – mildly toxic by ingestion.
1706	•	Na hexametaphosphate
1707	•	Mannitol – potential for laxative effect, depending on level of intake.

1708 The above excipients are all commonly used in veterinary medicinal products. It seems unlikely that a
1709 doubling of intake would have implications for target animal safety, but this would be considered on a
1710 product-by-product basis according to the individual composition since some precautions are identified
1711 above.

1712 7.5.7. Step 7: The conclusions above are incorporated into the final
1713 benefit-risk for the dose increase for each individual product

1714 Overall it is concluded that VMPs administered at the proposed dose of 40 mg amoxicillin/kg bw per
1715 day for 5 days in drinking water are likely to be adequately tolerated in pigs for the treatment of the
1716 indication for respiratory disease.

1717 *7.6. Overall conclusion and recommendations on amoxicillin*

1718 The approaches on dose optimisation, WP, ERA and TAS as described in chapters 3, 4, 5, and 6,
1719 respectively, were tested in the case study on amoxicillin products, orally administered via the drinking
1720 water, for the treatment of respiratory infections in pigs. The most common dose currently authorised
1721 for this indication is 20 mg/kg bw per day for 5 days.

1722 In order to optimise the dose, the following pathogens were considered to be relevant: *Actinobacillus*
1723 *pleuropneumoniae, Bordetella bronchiseptica, Haemophilus parasuis, Pasteurella multocida,* and
1724 *Streptococcus suis*. The optimised dose was determined as 40 mg/kg bw per day. It was noted that,
1725 due to the low susceptibility, it was not possible to establish a dose for *B. bronchiseptica*, and therefore
1726 pigs infected by this pathogen should not be treated with amoxicillin via the drinking water.

1727 For the establishment of the WP, only a limited number of studies were available for this pilot project.
1728 Since the depletion of residues of amoxicillin after oral administration to pigs is very rapid, most of the
1729 older residue studies confirmed that residues are already below LOD after a few days. However, this
1730 challenge could be overcome, by the use of the hourglass approach. Data and insights from multiple
1731 sources (e.g. FARAD, literature, published thesis's, registration dossiers) were combined to find the
1732 relevant PK parameters and eventually the terminal half-life of the depletion of residues could be
1733 determined. A "worst-case" and thus rather conservative half-life of 2 days was used for the
1734 extrapolation of WPs, resulting in relatively low increases of the WPs.

1735 For addressing the environmental risks, adequate Phase I and Phase II ERA data were available for the
1736 authorised dose of 20 mg/kg bw per day for 5 days. For the optimised dose, the RQs remained below 1
1737 when the duration is maximally 5 days, and above 1 when the duration is 7 days. It was considered

1738 that the duration of 3-5 days may be sufficient for products with the same indication, which would
1739 justify the limitation of the duration to maximally 5 days, in order to limit the exposure to the
1740 environment. Overall, the optimised dose for amoxicillin does not give rise to concerns for the
1741 environment.

1742 In relation to TAS, no specific safety issues were identified after consideration of all provided data from
1743 the registration dossiers and other relevant sources. It was concluded that amoxicillin administered at
1744 the optimised dose is likely to be adequately tolerated in pigs.

1745 8. Case study oxytetracycline

1746 8.1. Introduction

1747 Oxytetracycline (OTC) is a commonly used broad spectrum tetracycline antibiotic in veterinary
1748 medicine. In the EU oxytetracycline is licensed in various formulations (powders, solution for injection,
1749 suspension for spray, premix and tablets), for a variety of animals (food producing and non-food
1750 producing).

1751 This case study will be limited to the solution for injection formulation to be used for respiratory
1752 infections in cattle.

1753 Oxytetracycline is a broad spectrum antibiotic effective against both Gram positive and Gram negative
1754 bacteria with a bacteriostatic effect. OTC binds to 70S and 80S ribosomes blocking the attachment of
1755 aminoacyl-transfer RNA to the ribosomal messenger RNA thereby blocking the ability of bacteria to
1756 produce proteins. This prevents the bacteria from growing and multiplying.

1757 Oxytetracycline is normally available as the dihydrate or hydrochloride salt.

1758 The solution for injection is available in 10% ("short acting") and 20% ("long acting") formulations.
1759 The approved doses are:

1760 • 20% formulations: 20 or 30 mg/kg bw, single injection; in some approved labels: repeated after
1761 48 or 72 hours in severe cases.

1762 • 10% formulations: between 4 – 20 mg/kg bw per day, daily injection for between 1 and 5 days

1763 Licensed products are indicated for a wide variety of infections primarily septicaemia, respiratory and
1764 gastro-intestinal infections, as well as foot rot, soft tissue infections and furunculosis and enteric
1765 redmouth disease in aquaculture.

1766 This case study will focus on the indication for respiratory disease caused by *Pasteurella multocida*,
1767 *Mannheima haemolytica* and *Haemophilus somni*.

1768 8.2. Dose optimisation

1769 8.2.1. Pharmacokinetics

1770 One of the challenges of the case study for oxytetracycline injectable products is the possibility that
1771 the pharmacokinetics differ between the various formulations. Depending on how much products differ
1772 in their pharmacokinetic profile, there may be a need for a product-by-product PK/PD analysis which
1773 might result in different outcomes for the optimised dose. Therefore, the possible existence of
1774 formulation-specific pharmacokinetics was investigated.

1775 First, the composition was considered for a range of products (i.e. the OTC injectables for cattle
1776 authorised in The Netherlands), including 20% ("long acting"; LA) and 10% ("short acting"; SA)
1777 formulations (an overview is given in Annex 5). As it turned out, all formulations have a comparable
1778 composition / similar composition / similar galenics, namely containing water and other solvents,
1779 chelators, complexing agents, preservatives, and substances for adjusting the pH. The organic solvents
1780 and complexing agents in particular, can have the ability to delay / influence the release of the active
1781 ingredient from the site of injection and thus influence the (absorption) pharmacokinetics of the
1782 formulation. These substances were quite similar across formulations. Therefore, it appears that no
1783 major differences in the PK would be expected from the design of the composition of the product.
1784 Indeed, Nouws *et al*. (1985) tested a range of LA (long acting) and SA (short acting) OTC formulations
1785 in dairy cows and found that the pharmacokinetics were roughly the same. In addition, OTC half-lives
1786 in tissues were similar for LA and SA formulations (see 8.3).

1787 Whereas the compositions of the formulations are similar in terms of the inactive ingredients, it has to
1788 be noted that there is a 2-fold difference in strength between the LA and SA formulations, and that
1789 these products have different patterns of use. Therefore, under field conditions, there will be
1790 differences in the volume and the number of injections, and these differences may influence the
1791 absorption from the injection sites and thus the PK profile. In an unpublished study report provided by
1792 the industry, pharmacokinetic profiles were shown to be different between an LA and SA formulation.
1793 It was considered that the difference in the number of injections given could well explain the difference
1794 in pharmacokinetics.

1795 In view of the above, it was decided to analyse two datasets separately, one representative for an LA
1796 formulation and another one representative for a SA formulation.

1797 In this case study, PK profiles from different sources (Marketing Authorisation Holders) were used for
1798 the computation of a daily dose. The pharmacokinetics for different concentrations of oxytetracycline
1799 formulations (20% and 10%) were determined using old datasets provided by different pharmaceutical
1800 companies for doses ranging from 5 to 20 mg/kg bw administered intramuscularly to calves, young
1801 cattle and cows. The OTC plasma concentrations for different sampling times were analysed using a
1802 non-linear mixed effect model using Monolix® (Lixoft) and simulations of different dosage regimen
1803 were performed in R using mlxR package. The PK model was a mono-compartmental model using an
1804 extravascular administration route. The PK parameters of the two main OTC concentrations present in
1805 the EU market are reported in the following table.

1806 **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

1807

1808 The next figure is the graph of observed data and percentiles of distribution of the Population PK model
1809 with the 90th percentiles for the two tested formulations.

1810

| Formulation 20% – 20 mg/kg bw | Formulation 10% - 20 mg/kg bw single or 2x/48h |
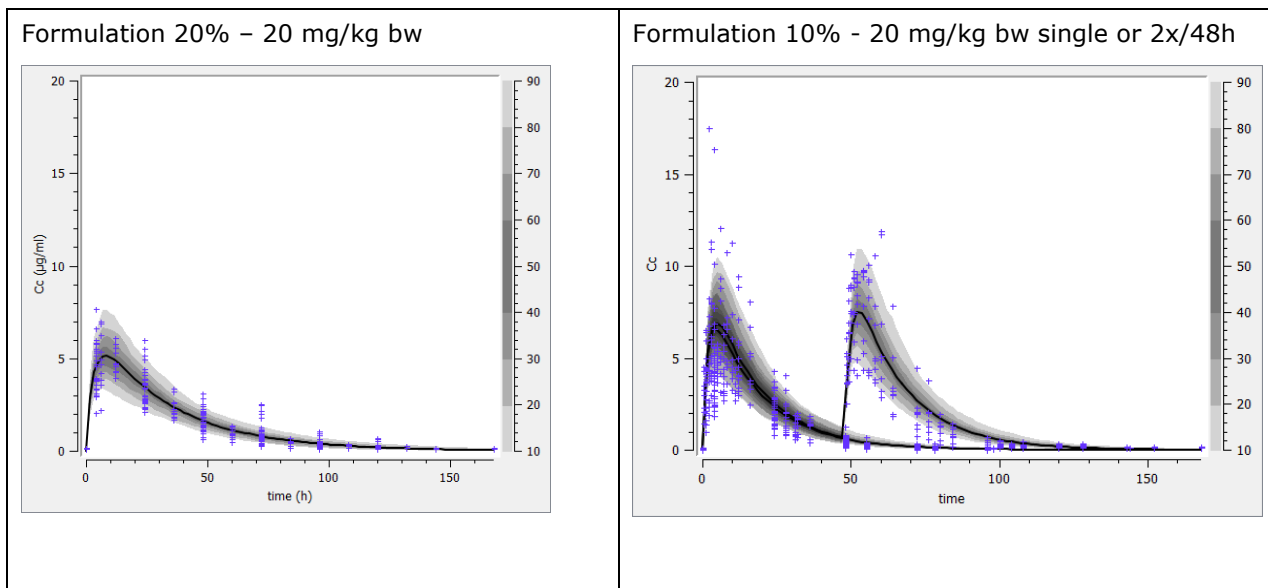
Figure 22. Representation of the distribution of plasmatic concentration in function of time obtained by population PK model for a long acting formulation dose (20 mg/kg bw) and a short acting formulation dose (11 mg/kg bw)

8.2.2. 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 99.9[th] 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 this 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.

8.2.3. 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.

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8.2.4. 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.

8.2.5. 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

- 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 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%

	Interval	P. multocida		M. haemolytica		H. somni	
	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%
	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%

1862

1863

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%
	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%

	Interval	P. multocida		M. haemolytica		H. somni	
	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.

8.2.6. 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

1906 -   After 48 h, the single administration of 20 mg/kg bw of 20% formulation leads to a PTA below
1907   90% for all the target pathogens which justifies the second administration.

1908 -   According the PK/PD modelling, PTA can be improved by increasing the administrated dose of a
1909   formulation or by repeating the administration with a shorter time interval.

1910 By defining an optimal frequency of administration (48 h versus 36 h), PTA can also be improved,
1911 especially in this case study for *M. haemolytica*. For this target pathogen, using an administration of 20
1912 mg/kg bw 36 h apart of a 20% formulation, the PTA is above 90% for 3 days.

8.2.7. Set a PK/PD breakpoint

1914 As for the amoxicillin case study, the next step of the proposed approach to address doses is the
1915 definition of clinical breakpoint, or PK/PD breakpoints when lacking clinical data (cf. chapter 3.3 – step
1916 7). According to the data available for oxytetracycline, in our example, the PK/PD breakpoint can be
1917 set at 2 µg/mL. It is compatible with values of ECOFF of bacterial species targeted. *Mannheima*
1918 *haemolytica* has the highest ECOFF and is the less susceptible species.

8.2.8. Define an optimal daily dose

1920 For the oxytetracycline case study, it was decided to analyse two datasets separately, one
1921 representative for a LA formulation (20% formulation) and another one representative for a SA
1922 formulation (10% formulation). According to the chapter 8.3 of this report, no or slight differences
1923 where identified between SA and LA formulation regarding PK profiles. However, the 2-fold difference
1924 in strength between the LA and SA formulations will have an impact on in the volume and the number
1925 of injections, and these differences may influence the absorption from the injection sites and thus the
1926 PK profile. Then this difference in the rate of absorption could influence the daily dose defined by a
1927 PK/PD approach.

1928   = For the SA – 10% formulation, according to the PK/PD modelling with the provided data, the
1929   dose of 10 mg/kg bw administered each 24h allows reaching a PTA of 90% for bacteriostatic
1930   activity for all the target pathogens, except during the first 24h for *M. haemolytica* where the
1931   PTA is close to this target value (86.1%).

1932   **It can then be concluded that, for the SA – 10% formulation, there is no need to**
1933   **increase the daily dose and that the dosage regimen 10 mg/kg bw each 24h provided**
1934   **a sufficient exposure for all the target pathogens tested.**

1935   = For the LA - 20% formulation, the modelling showed that the exposure is sufficient to reach
1936   the PTA target value only for the two periods 0-24h and 24-48h. According to the SPC of
1937   approved product, the dosage regime of the LA formulation is a single injection with repetition
1938   after 48 or 72 hours in severe cases. Thus, it can be concluded that the current dose of 20
1939   mg/kg bw reach the PTA of 90% only for the two first days. Then, to improve the PTA for the
1940   next days, a second injection should be realised 48h apart or ideally 36h apart for the least
1941   susceptible pathogens and not 72h as suggested. Based on the PK/PD modelling, to reach a
1942   PTA of 90% up to 72h with a single injection, the daily dose should be increased to 80 mg/kg
1943   bw. However, another approach to improve the PTA is to further refine the interval between

1944	the two administrations. Indeed, with the approved dose of 20 mg/kg bw, the PTA is higher
1945	than 90% for the bacteriostatic and bactericidal activity against the three bacterial species
1946	during three days with a 20% and a 10% formulation when a second injection is administered
1947	48h or 36h respectively. However, in field conditions, the 20% formulation is more adapted
1948	than the 10% formulation due to the limitation of the volume that needs to be injected.
1949	According to the PK/PD modelling and the rational principles of use of antibiotics, it is not
1950	necessary to increase the dose of the LA formulation (up to 80 mg/kg bw) to artificially
1951	increase the duration of activity and rather refine the interval frequency of administration.

1952	**It can then be concluded that, for the LA – 20% formulation, there is no need to**
1953	**increase the daily dose but further refine the interval between two injection and that**
1954	**the dosage regimen of 20 mg/kg bw with a second injection between 36 to 48h**
1955	**provided a sufficient exposure for all the target pathogens tested.**

8.3. Withdrawal period

8.3.1. Introduction

1958 After systemic absorption, oxytetracycline (OTC) distributes rapidly into the extracellular spaces of
1959 animal tissues. It also can cross the placental and the blood-brain barriers. OTC undergoes little or no
1960 metabolic degradation in cattle, and is eliminated mainly unchanged in the urine. Tubular secretion and
1961 passive reabsorption mechanisms are reported to be the mechanisms involved (Mevius *et al*., 1986).
1962 In bovine some (2-10%) epimerisation of OTC into 4-epi-OTC takes place. The marker residue used for
1963 determination of the withdrawal periods is defined as the sum of both compounds.

1964 After parenteral administration the WP determining tissue is known to be the site of injection

1965 Different OTC injectable formulations are authorised in the EU. For example, in the Netherlands there
1966 are some 25 OTC injectables authorised for use in bovine. A number of their particulars are listed in
1967 Table **26**.

1968 Table **26** shows that there is hardly a correlation present between withdrawal periods (WPs) for tissues
1969 and offal and the dose of OTC administered.

1970 Possible explanations:

1971 1. The WP for tissues is determined by the depletion rate of residues of OTC from the site of
1972 injection. The amount of OTC deposited per injection site is more or less comparable for the
1973 various products.

1974 2. Relatively large safety factors have been applied (to account for inadequacies in the (older)
1975 residue studies), masking a possible effect between dose and WP.

1976 3. Inadequate sampling of the injection site leading to unspecific spreading of the WPs

1977 4. Influence of injection site location on residual OTC concentrations on the site of injection.

1978 Since the residues on the injection site determine the WP for tissues, increasing the dose (within
1979 limits) by simply increasing the number of injections would have no effect on the WP for tissues. It
1980 should however be noted that the animal welfare situation should be considered, when applying this
1981 method. It could be argued that, in field conditions, 2-3 injections per animal/dosing would be a
1982 maximum.

1983 **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

1984 * no Respiratory Infection claim

1985 As an example Table 27 shows the max weight that could be treated, based on a maximum of 3
1986 injection sites per dosing.

1987 **Table 27.** Theoretical max weight (kg) to be treated for 10% OTC , 20% OTC (in parenthesis) and
1988 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}

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8.3.2. Plasma kinetics

In most of the studies reported in public literature (e.g. Nouws *et al*., 1985, Mevius *et al*., 1986, Toutain & 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.
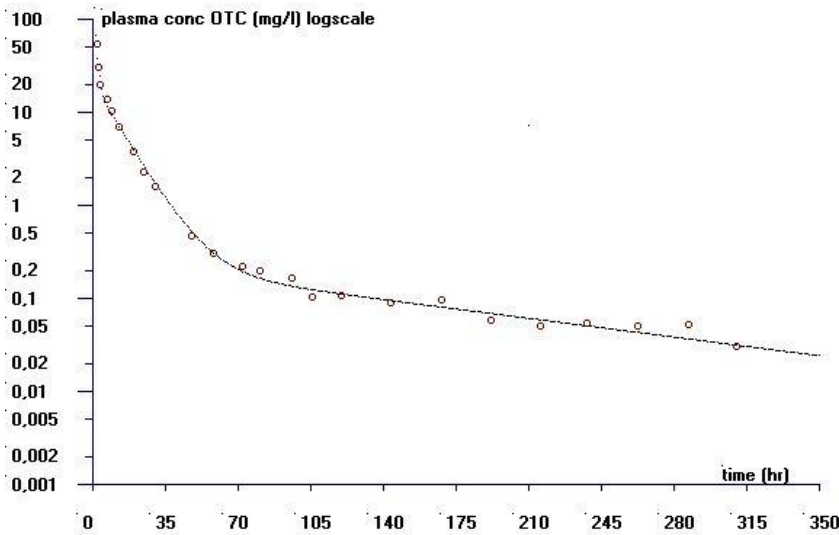
Figure 23. Measured concentration (mean ± SD) and mean fitted plasma-concentration time curve for oxytetracycline after single i.v. administration of 40 mg/kg bw to veal calves (n=5); based on Meijer *et al*., 1993
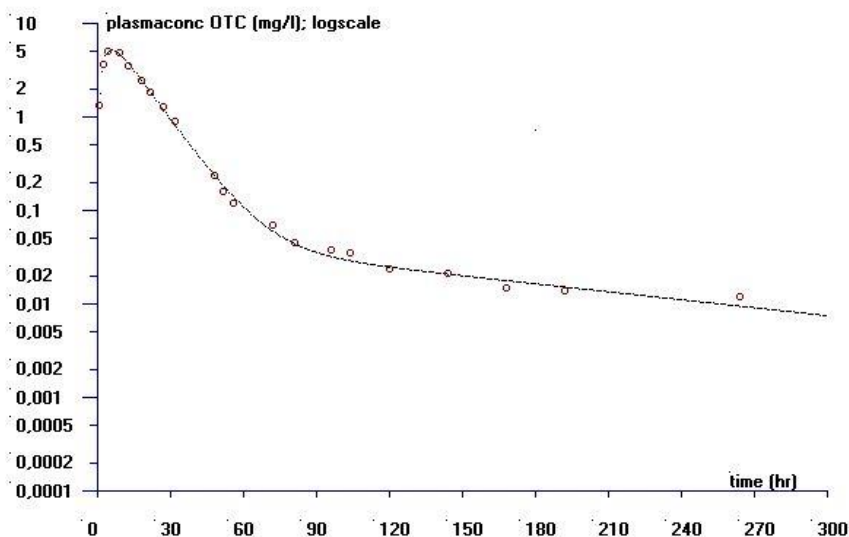
Figure 24. Measured concentration (mean ± SD) and mean plasma-concentration time curve for oxytetracycline after single i.m. administration of 20 mg/kg bw to veal calves (n=5); based on Meijer *et al*., 1993

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)

	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.v. administration of 20 mg/kg bw to veal calves (n=5, SD = Standard Deviation)

	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

2011   **Table 29** shows that an absolute bioavailability (F%) of approximately 100% for OTC could be
2012   calculated from the data after i.m. administration of 20 mg/kg bw to calves.

2013   Studies covering only the first 120 h after administration all show a bi-phasic elimination. This pattern
2014   is roughly the same for the 10% and 20% products (see figures below).
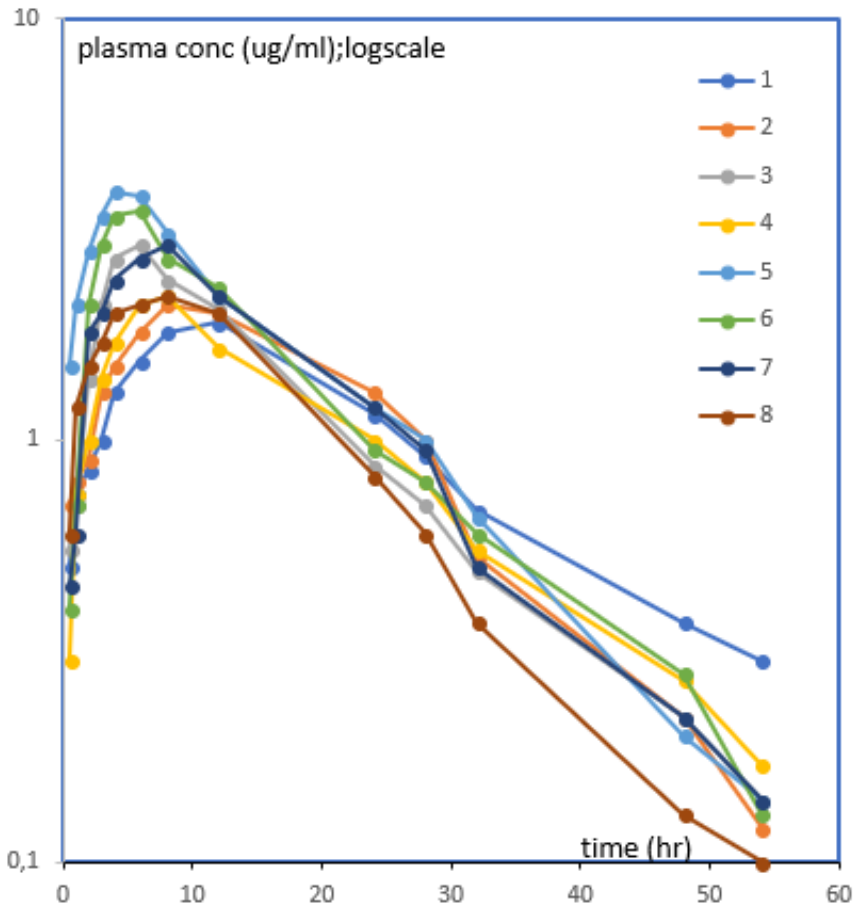
2015

2016   **Figure 25.** Mean plasma OTC concentration following intramuscular administration of Oxytetracycline-
2017   10% formulations to dairy cows at a dose level of 5 mg/kg; based on Nouws *et al*., 1985
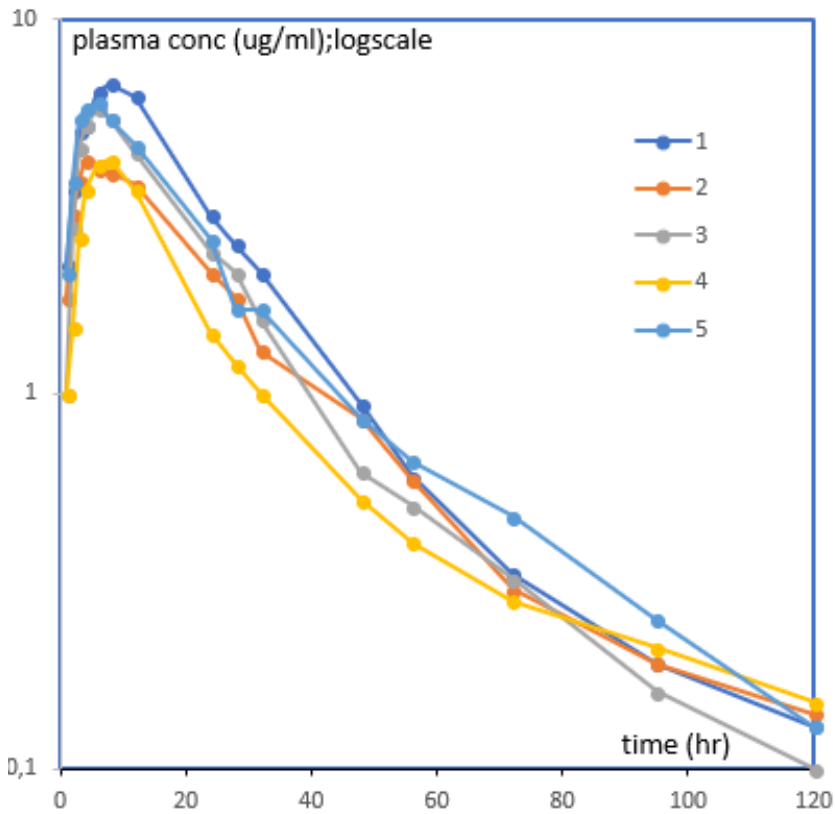
2018

Figure 26. 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 Nouws *et al.*, 1985

For the eight 10% formulations (i.m.) in Figure 25 the $T_{1/2}$ of first the elimination phase was 9-14 h during the first 60 h period (Nouws *et al.*, 1985).

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For the five 20% formulations (i.m.) in
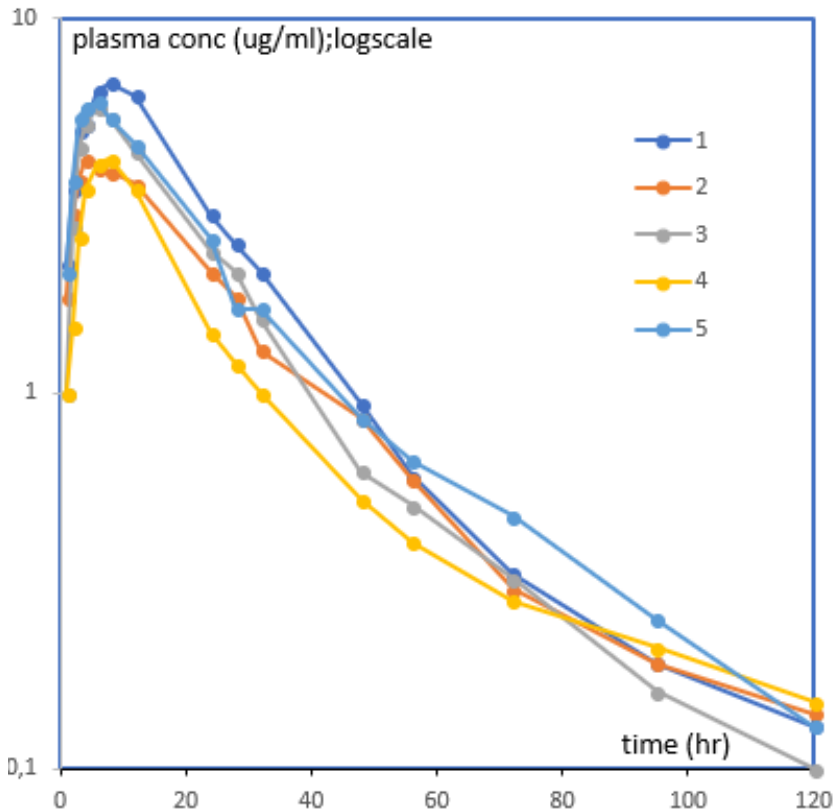
Figure 26 the $T_{1/2}$ of first elimination phase was 9–12 h when using data points <48 h. When the plasma concentrations were followed over a longer period of time (up to 120 h), a second phase could be detected ($T_{1/2}$= 25-44 h). It was noted that this phase probably was the result of the change-over situation from the first elimination phase to the final phase of 5-6 days (see Figure 24).

8.3.3. Intramuscular vs Subcutaneous administration

Studies (Clarke *et al*., 1999; study with product 20) comparing i.m. versus s.c. administration (see Figure 27 and Figure 28) show that the plasma kinetics for both routes of administration are highly comparable.
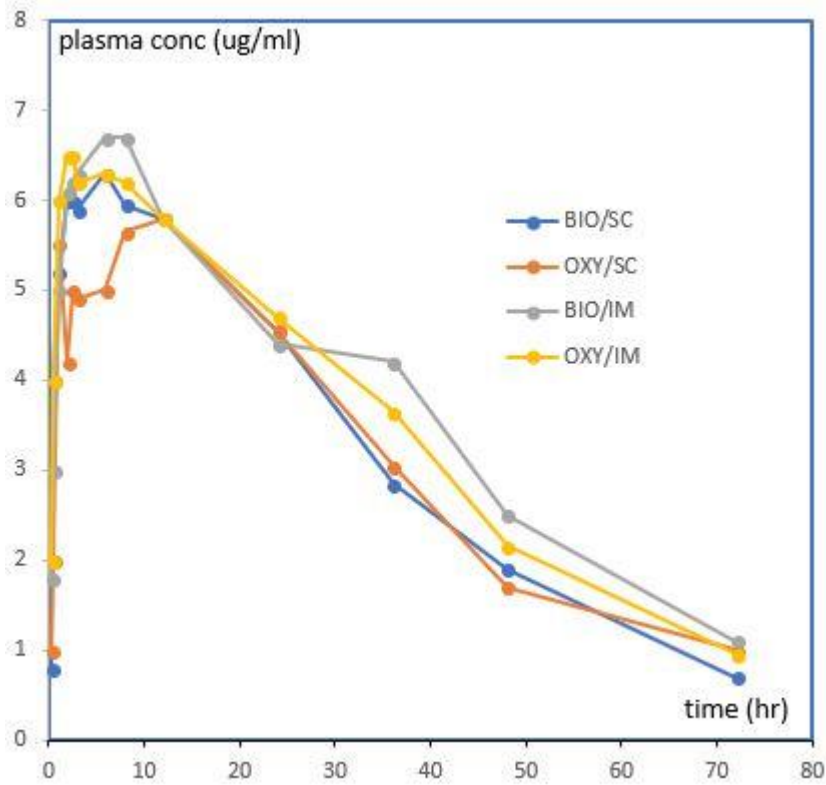
2034
2035
2036   **Figure 27.** Serum concentrations of oxytetracycline after subcutaneous (s.c.) or intramuscular (i.m.)
2037   administration (20 mg/kg bw) of BioMycin 200 (BIO) or OXY shot LA (OXY) formulations to cattle. Data
2038   represent mean concentrations ± SD; based on Clarcke *et al*., 1990.

2039
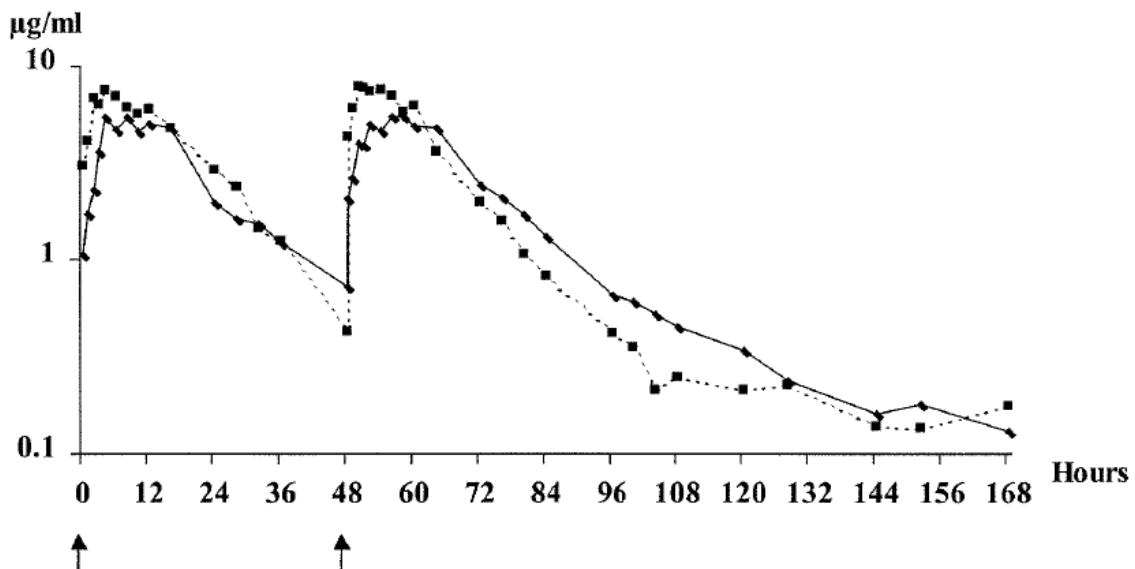
2040

2041   **Figure 28.** Plasma kinetics after s.c. (solid line) and i.m. (dashed line) administration of a 10%
2042   product to calves (study product 20) at a dose of 20 mg/kg bw

8.3.4. 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*	calve	105	Meijer *et al*., 1993
20	Im	130*	calve	105	Meijer *et al*., 1993
20	Iv	66	cattle	212-275	Toutain & Raynaud, 1983
20	Im	78	calve	372-420	Achenbach, 2000
20	Im	83	calve	372-420	Achenbach, 2000
20	Sc	90	calve	372-420	Achenbach, 2000
20	Sc	86	calve	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*	calve	203-234	FARAD, 1997b
11	Sc	102*	calve	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.

It seems that the total body clearance is relatively constant and independent of dose and route of administration (mean clearance = 88 ± 23 ml/kg.hr).

It is concluded that the assumption of linear kinetic behaviour appears to be justified, under the condition that the dose would be moderately (e.g. factor 2-4) increased.

8.3.5. 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

8.3.6. 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.
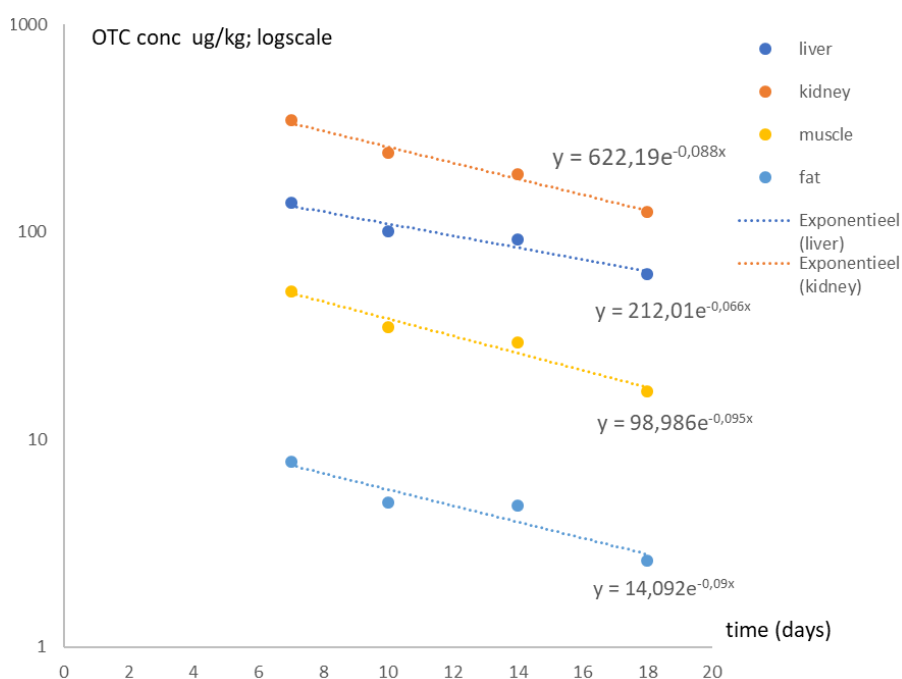
Figure 29. Residue depletion in cattle tissue following the last of 5 i.m. administrations with a 10% OTC injectable formulation at a dose of 10 mg/kg bw per day

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)	reference
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	

Product type	Adm	Total dose (mg/kg)	tissue	$T_{1/2}$ (days)	reference
			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 4
			muscle	4.6	
			fat	3.5	

2074 A mean tissue half-life of 5.9 ± 2.3 days could be calculated.

2075 So **if** the withdrawal period for tissues would be determined by the depletion of OTC from the regular
2076 tissues and not by the depletion from the injection site, then a terminal half-life of 6 days could be
2077 used in the extrapolation equation (Equation 2).

2078 ## 8.3.7. Residues in the injection site(s)

2079 Figure 30 shows the depletion of OTC from the injection site as measured in one of the studies
2080 (Achenbach, 2000), following the s.c. administration of a 20% product at a single dose of 20 mg/kg bw
2081 and with a maximum injection volume of 10 ml per injection site.
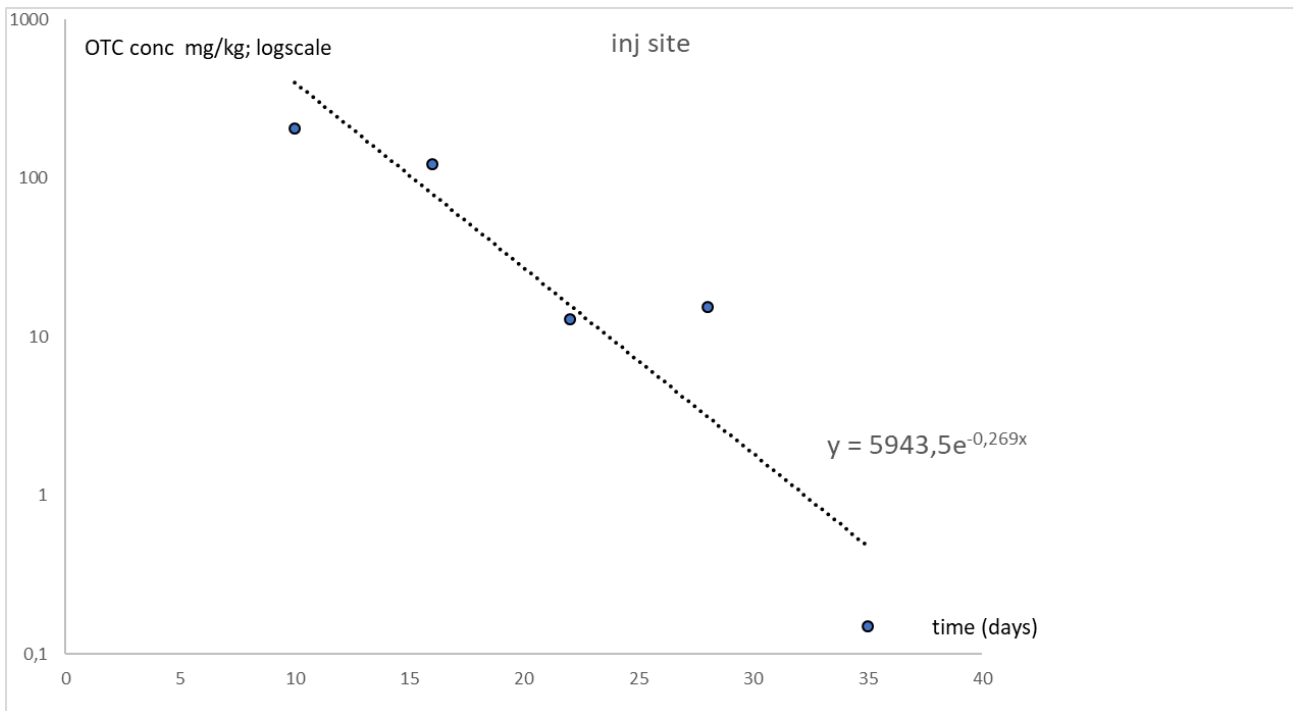
2082

2083 **Figure 30.** Mean OTC concentration (mg/kg) in injection site following the s.c. administration of a
2084 20% product at a single dose of 20 mg/kg bw; from Achenbach, 2000.

2085

2086 **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 4

2087 ** Inj sites Left and right side of the neck measured separately

2088 Table 32 shows the estimates of the $T_{1/2}$ for the final depletion of OTC from the injection site for a
2089 number of products. The $T_{1/2}$ was found to be significantly smaller than the 6 days, calculated from the
2090 tissue depletion curves.

2091 In calves 10 days after injection (10-20 ml) some 0-0.72% of the amount injected was left at the site
2092 of injection (Nouws *et al*., 1990).

2093 Three theoretical scenarios could be considered as far as increasing the dose of OTC is concerned:

2094 1. When dose increase can be performed by increasing the number of injection sites, no change in
2095   WP for tissues would be necessary, but animal welfare could be at stake.

2096 2. When increasing the dose would be performed by increasing the injection volume then an
2097   alternative approach would be necessary (see below). In this situation animal welfare (too large
2098   injection volumes, irritation) could also be at stake.

2099 3. Dose increase could also be achieved by limiting the maximal weight of the animal to be treated.
2100   In that case (if the max volume remains unaltered) no change in WP would be needed.

2101 ***8.3.7.1. Proposed approach of WP extrapolation in case of an increase of injection***
2102 ***volume/injection site***

2103 Figure 31 shows the relation between max dosing volume and withdrawal period for tissues for the
2104 originator products listed in Table **26** (the generics were not taken into account).

2105 The influence of the injection volume on the WP seems to be marginal. This would seem to be a rather
2106 controversial conclusion. For example, injecting twice the amount on the site of injection, theoretically
2107 would lead to a higher WP, adding another 2-3 days. The explanation for the WP-data not showing this
2108 probably lies in the fact that in many cases the WP was established using a large safety factor to
2109 account for deficiencies in the studies. This would obviously mask the effect of an increase in injection
2110 volume.

2111 Although the influence of the injection volume on the WP seems to be marginal in the present dataset,
2112 as a worst case approach, it is proposed that in case of a increased injection volume, in the
2113 extrapolation equation (Equation 2) the half life of 6 days from the tissue depletion data is to be used.

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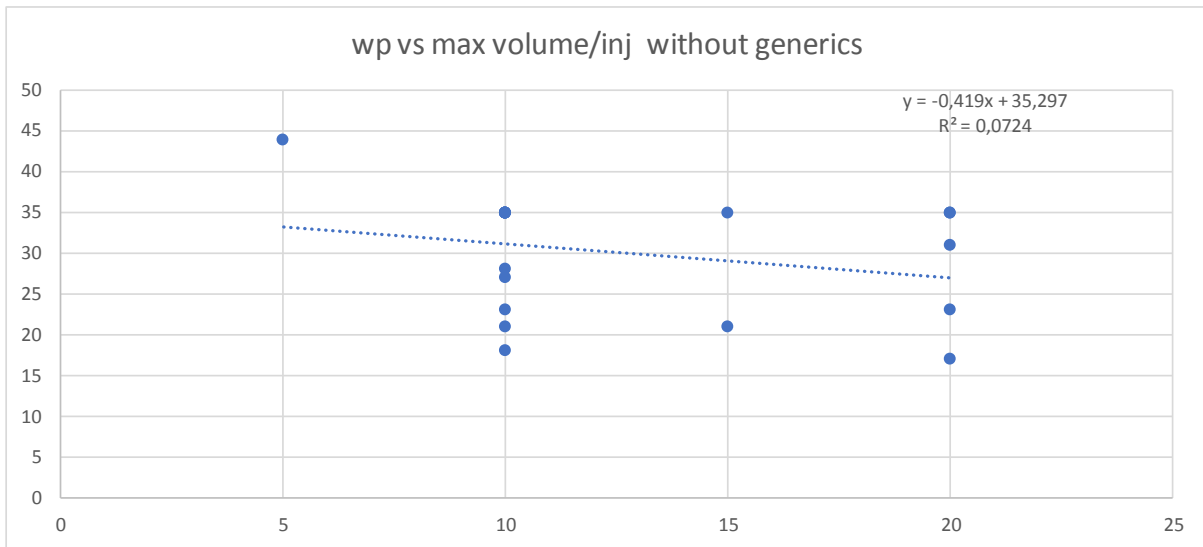
Figure 31. The withdrawal period (y-axis, in days) for cattle of various oxytetracycline injectable VMPs as a function of the injection volume per injection site (x-axis, in ml)

8.3.8. Residues in milk
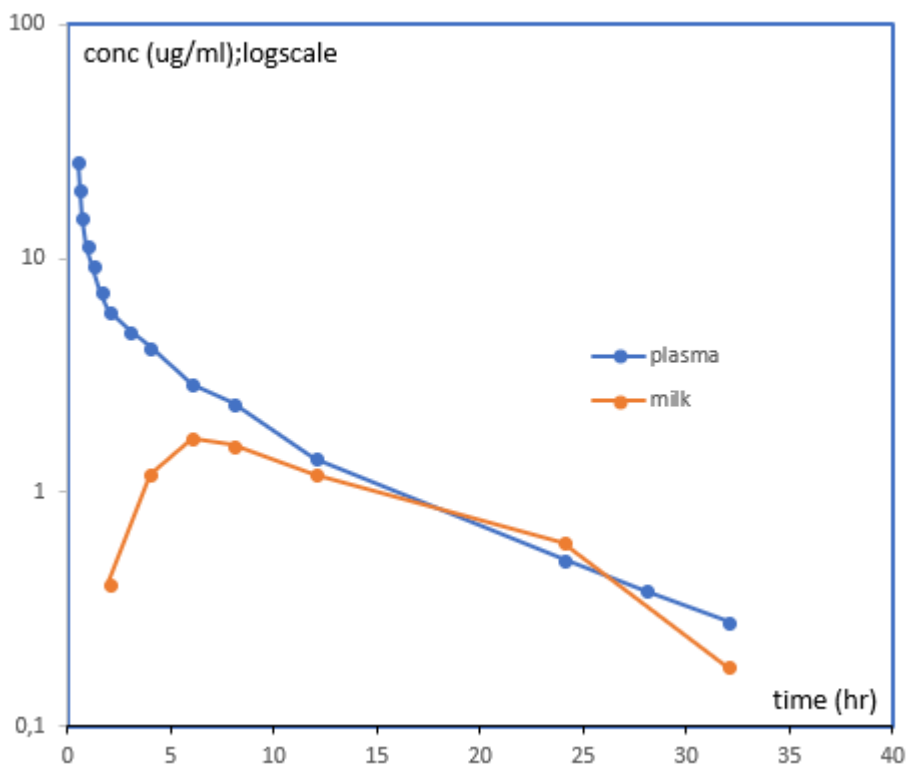
Figure 32. Oxytetracycline concentrations in plasma and milk (mean and s.d.) following intravenous administration of Engemycine-10% at a dose of 5 mg/kg bw; based on Nouws *et al.*, 1985

In Figure 32, after an initial rise, the time dependent course of the concentration of OTC in milk generally seems to mimic the pattern in plasma. This pattern was confirmed by other data from Nouws (see Figure 33 and Figure 34). The ratio milk/plasma was reported to be in the range of 1 to 2 (Nouws *et al.*, 1985).

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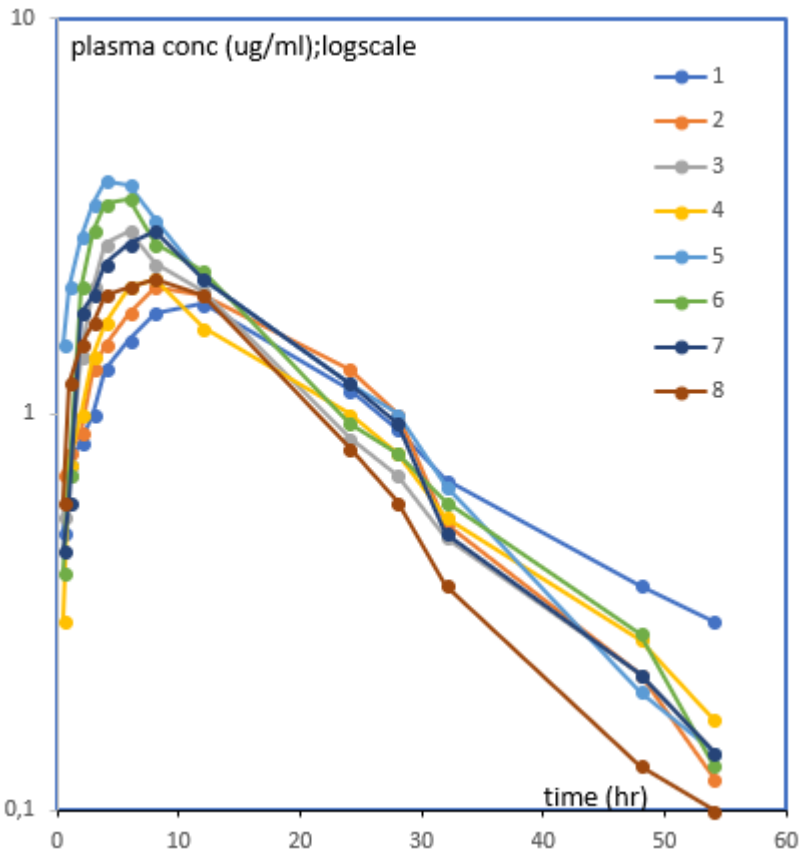
Figure 33. Mean plasma OTC concentrations following muscular administrations of Oxytetracycline-10% formulations to dairy cows at a dose level of 5 mg/kg bw; based on Nouws *et al.*, 1985
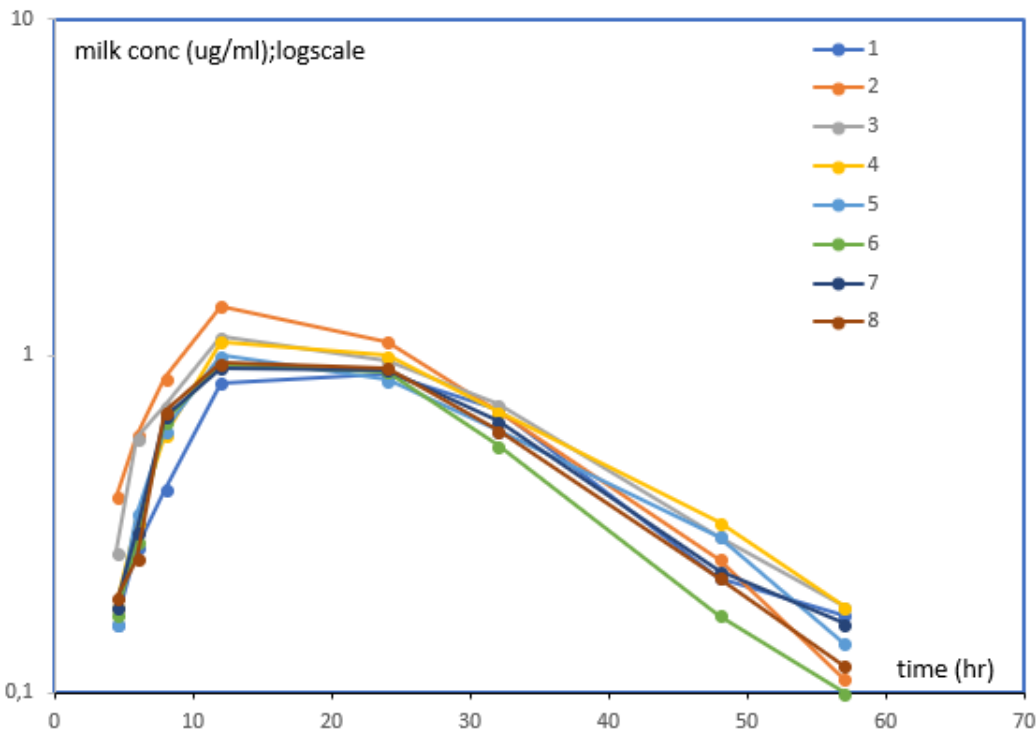
Figure 34. Mean milk OTC concentrations following muscular administrations of Oxytetracycline-10% formulations to dairy cows at a dose level of 5 mg/kg bw; based on Nouws *et al.*, 1985

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2131   In the figure below it is shown that when the milk concentration curve is monitored for a longer period
2132   of time, again (as expected) a long (approx. 6 days) terminal depletion phase can be observed (study
2133   6), comparable to the one seen in plasma.
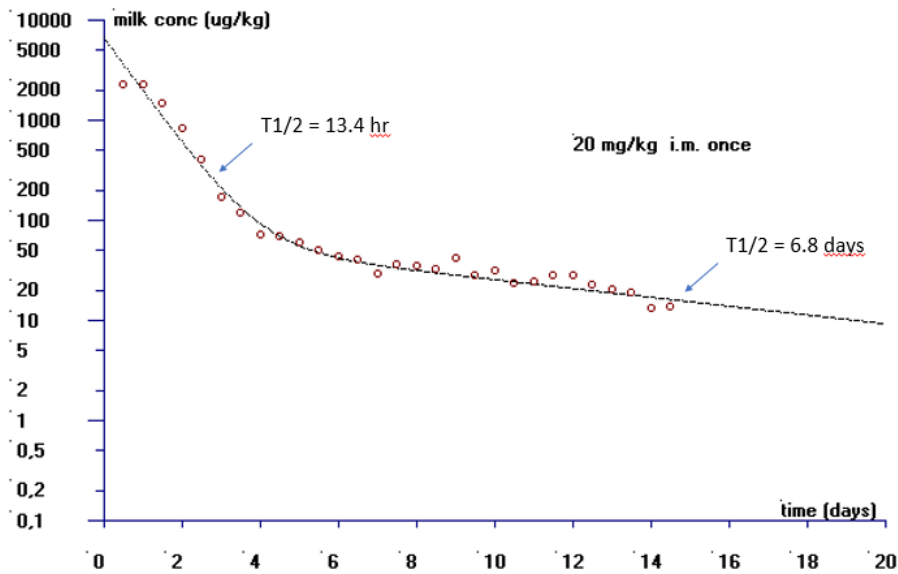
2134

2135   **Figure 35.** Depletion of OTC concentrations in a cow's milk over time after a single intramuscular
2136   injection of OTC at a dose of 20 mg/kg bw; data from animal no.6 in Study 6
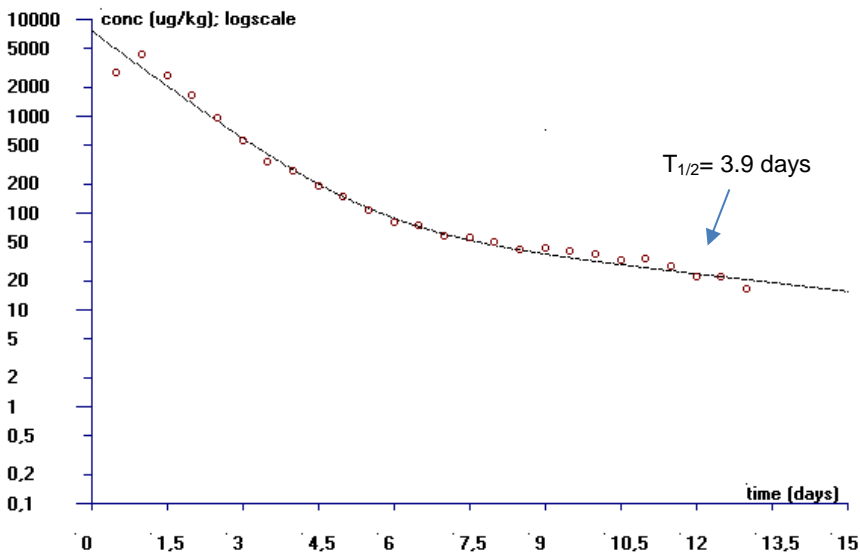
2137

2138   **Figure 36.** Depletion of OTC mean concentrations in cow's milk over time after a single intramuscular
2139   injection of OTC at a dose of 20 mg/kg bw; data from all 10 animals in Study 6

2140   Since the depletion curve of OTC residues in milk, runs parallel with the plasma and tissue
2141   concentrations, as a worst case, the terminal half-life of 6 days (calculated from the tissue depletion
2142   data) should be used in the extrapolation equation (Equation 2).

2143

8.3.9. Withdrawal time calculation

The new withdrawal periods were calculated using Equation 2.

Using PK/PD methods for the 10% formulations an optimised 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 optimised 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. For milk a terminal $T_{1/2}$ of 6 days would be used in Equation 2, leading to an addition of 6 days for each doubling of the withdrawal period, adding up to an additional 8 days.

The two other possible scenarios for increasing the dose that could be considered are specified below. The $T_{1/2}$ final phase 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.

2164   1.   Increasing the dose could be performed by increasing the injection volume. In this situation
2165   animal welfare (too large injection volumes, irritation) could also be at stake, so the maximum
2166   injection volume was set to 20 ml per injection site. The results are listed in Table 35.

2167   2.   Dose increase could also be achieved by using a maximum number of injections of 3 and
2168   subsequently limiting the maximal weight of the animal to be treated. In that case (if the max
2169   injection volume would remain unaltered) no change in WP would be needed for tissues as the
2170   injection site will remain the WP determining tissue and residues at the IS unchanged. For milk
2171   equation 2 can be used. The results are listed in Table 36.

2172 **Table 35.** Extrapolated WPs for the 10% formulations for a dose of 10 mg/kg bw, using a maximum
2173 number 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

2174

2175 **Table 36.** Extrapolated WPs for the 10% formulations for a dose of 10 mg/kg bw, using a maximum
2176 number injections of 3 without altering the maximum injection volume, and the resulting introduction
2177 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	10%	8	18	5	7	10	300
10	10%	4	35	4	12	20	600
11,13	10%	4	35	10	18	10	300
21,9	10%	4	35	4	12	20	600
22	10%	4	35	10	18	10	300

2178

2179 For the 20-30% formulations the repeated injection would lead to no changes in withdrawal periods for
2180 tissues of these products. For milk a terminal $T_{1/2}$ of 6 days would be used in Equation 2, leading to an
2181 addition of 6 days for each doubling of the withdrawal period. Taking into account the interval of 36-48
2182 hours between the two doses, where a certain fraction if the first dose is already eliminated at the time
2183 the second dose is given, it could be calculated that as a worst case it still would lead to an increase of
2184 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

8.4. 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.

8.4.1. Step 1: Determine the assessment situation for oxytetracycline

In accordance with the PK/PD modelling (see 8.1.), the optimised 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 optimised 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 $PEC_{groundwater}$ 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 optimisation for oxytetracycline does not lead to additional environmental risks.

8.4.2. Conclusion on the ERA for oxytetracycline

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

8.5. 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 PKPD 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

8.5.1. 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)

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

Based on studies in laboratory spp, the target organs for OTC toxicity are the liver and kidneys. Injections cause local tissue reactions. Anaphylaxis has been observed in cattle.

Study reports (n=27) were provided for investigations of <u>local (injection site) tolerance</u>. 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, or Product OTC1 at the recommended dose of 20 mg/kg bw, 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) 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).

Conclusion: For Product OTC1, the maximum injection volume should be 10 ml per site.

Product OTC2 is a formulation containing 200 mg OTC per ml, administered as a single injection. A single study was provided for which one of the aims was to investigate injection site safety.

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 2/3 weeks.

Conclusion: Product OTC2 caused marked IS reactions at a maximum injection volume of 10 ml; hence there is a rationale to restrict the injection volume.

Conclusions: 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.

2253 *Multiple proprietary IS safety studies were provided for one 20% formulation (including other OTC*
2254 *formulations as controls) and a single study investigated IS safety of 2 versions of another 20%*
2255 *formulation. It is apparent that OTC injections (regardless of strength) are irritating and there is a*
2256 *rationale to restrict the IS volume. It seems plausible that oxytetracycline itself is an irritant, although*
2257 *tolerability to individual formulations may be affected by their excipient composition.*

2258 ***8.5.1.1. Step 1a: Review supplementary data from dossiers, if needed e.g. dose-finding***
2259 ***studies***

2260 Data not available to the pilot project.

8.5.2. Step 2: Safety in the target population

2262 Data not available to the pilot project.

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

2264 Data not available to the pilot project.

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

2267 Literature review – A review was conducted using PubMed and the terms <oxytetracycline> <cattle>
2268 and <toxicity> or <safety>.

2269 In a study from TerHune & Upson (1989), 30 healthy calves were administered OTC LA formulation at
2270 40 mg/kg bw IM. Reactions and toxicosis were limited to anaphylaxis (n=1) and IS swellings (n=2).

2271 Textbooks

2272 Prescott & Dowling (2013) states that tetracyclines are irritants and may cause damage at injection
2273 sites. Calcium-binding may cause acute cardiac toxicity. Anhydrotetracyclines damage plasma
2274 membranes and bind to serum albumin.

2275 Plumb's Veterinary Drug Handbook (6th Ed) (Plumb, 2008) indicates that tetracyclines are excreted in
2276 milk in a ratio of milk:plasma of 0.25 to 1.5.

2277 Grey literature

2278 Information available from SPCs of EU-authorised products

2279 SPC 4.3 – Contraindications: Several products include contraindications from use in animals suffering
2280 from renal or hepatic damage or with known hypersensitivity to oxytetracycline.

2281 SPC 4.9 – Dosing and administration: Several products include restrictions on the injections volume at
2282 any one site from between 10 to 20 ml.

2283 SPC - warnings for the target spp.

2284 Warnings relate to possible occurrence of gastrointestinal disorders, allergic reactions, photosensitivity,
2285 hepatotoxicity, nephrotoxicity, tooth discolouration and injection site reactions. The incidence of
2286 adverse events is not clear from the SPCs of these long-authorised products.

2287 Concerns also relate to use during pregnancy and effects on foetal development. For one product it is
2288 advised that although oxytetracycline is excreted in the milk, concentrations are generally low and the
2289 product 'can be safely administered to lactating animals'.

2290 OTC is reputed to have 'low general toxicity' although the MOS is not available from SPCs.

2291 <u>CVM FOIA reports</u>

2292 In the USA Liquamycin LA-200 is authorised for treatment of pneumonia in cattle at a single dose of 20
2293 mg/kg bw, or for other indications at 6.6 – 11 mg/kg bw for 4 days.

2294 <u>NADA 113-232 Liquamycin LA 200</u>

2295 Study 2532D-60-96-164 investigated the local safety of SC injection at 20 mg/kg bw as part of a
2296 residues depletion study in 26 calves with average weight 253 kg. SC injections resulted in transient
2297 swellings from as early as D1. These peaked at D7 but resolved clinically without intervention. The SC
2298 route resulted in smaller lesions than IM. Histopathological exam revealed that lesions did not
2299 completely resolve within the 28 day WP.

2300 <u>NADA 141-312 Hexasol injection</u> (OTC 300 mg/ml + flunixin meglumine 20 mg/ml)

2301 P-FLO-020 investigated the safety of Hexasol when administered at 0, 1x, 3x and 5x the RTD of 29.9
2302 mg OTC + 2 mg flunixin/kg for 3 administrations at 72 h apart to 24 M/F calves (6/group) aged 3 to 5
2303 months and weighing 100 to 147 kg. There was a dose-dependent increase in AST to 5x ULN until D7;
2304 no evidence of hepatotoxicity was found and this was considered to be related to muscle inflammation.
2305 Creatinine and urea increased in the 5x group and peaked at the high ULN at D4. 2 calves in the 5x
2306 group had much higher levels and were euthanised on D7; examination of the kidneys detected cortical
2307 tubular necrosis consistent with mild renal toxicity.

2308 *Conclusion - This study showed that a dose of 90 mg/kg bw (n=6), repeated on 3 occasions at 72 h*
2309 *apart, was a 'no effect level' for renal toxicity; pathology was present at 150 mg/kg bw. A dose of 150*
2310 *mg/kg bw was a no effect level for liver toxicity.*

2311 <u>NADA 141-143, 2003 Tetradure 300 containing oxytetracycline 300 mg/ml</u>

2312 Published data from Griffin *et al.*, 1979, Lairmore *et al.*, 1984, Riond & Riviere, 1989, TerHune &
2313 Upson, 1989, Vaala *et al.*, 1987, were considered.

2314 079/96: A GLP TAS study to investigate the safety of Oxytet 30 following IM injection to cattle. OTC
2315 was administered at 1x, 2x and 4x the RTD of 30 mg/kg bw on 3 occasions at 72 h apart to 24 cattle
2316 aged 6 to 9 months and weighing 214 to 286 kg. A maximum injection volume was 10 ml per IS.

2317 Localised IS reactions were noted in all groups and reflected the total dose administered with the
2318 highest incidence of lameness in the 4x group.

2319 Anorexia was observed in the 4x group after the 3rd injection and lasted 8 days.

2320 The most notable findings were increased urea and creatinine in the 4x group which was accompanied
2321 by histopathological changes indicating renal dysfunction detected at necropsy at D21. No post-
2322 mortem changes were noted in the 1x and 2x groups. No hepatic pathology was noted.

2323 *Conclusion - This study showed a no effect level for renal toxicity up to 60 mg/kg bw (n=8), repeated*
2324 *on 3 occasions at 72 h apart; renal pathology was seen at 120 mg/kg bw.*

2325 041/95: GLP PK study to support safety of IV and IM administration of Oxytet 30 at 30 mg/kg bw
2326 dose. The study involved 12 cattle weighing from 409 to 441 kg. No evidence of collapse, neurological

2327 effects or changes in gait were observed. Hardness and swelling were noted to varying degree at IS for
2328 both routes, but resolved by D 28.

2329 089/96: GLP IS safety study. A dose of 30 mg/kg bw and 60 mg/kg bw was administered IM at a max
2330 of 10 ml/site on 3 occasions at 72 h apart in the neck, rump and leg. IS were monitored and examined
2331 by histopath at 15 days after the final injection. No IS reactions were noted at the neck sites, although
2332 some localised tissue necrosis may still be present at 21 days.

2333 *Overall conclusions - Based on the TAS studies available, there appears to be a 'no effect level' up to*
2334 *60 mg oxytetracycline/kg bw after IM injection repeated on 3 occasions at 72h intervals, above which*
2335 *there may be impacts on renal function. However, it should be considered that this conclusion is based*
2336 *on findings in small numbers of animals. Lower doses (33 mg/kg bw) administered IV may also result*
2337 *in toxicity.*

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

2341 The data available indicate that OTC has renal toxic effects with a NOEL at 60 mg/kg bw by
2342 intramuscular administration and less than 33 mg/kg bw IV.

2343 Irritant effects limit the volume that can be administered at each IS, and this may vary with the
2344 formulation. For some 200 mg/ml formulations, the maximum IS volume is 10 ml. Where this is based
2345 on safety reasons, this should be taken into account if there is a dose increase that might lead to a
2346 need for multiple injections.

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

2349 The following excipients have been included in different EU-authorised formulations:

2350 • 2-Pyrrolidone

2351 • Benzylalcohol

2352 • Citric acid monohydrate

2353 • Dimethylacetamide

2354 • Disodium Edetate Dihydrate Ethanolamine

2355 • Glycerolformal

2356 • Hydrochloric Acid

2357 • Macrogol 1500

2358 • Magnesium Chloride Hexahydrate

2359 • Magnesium Oxide

2360 • Methyl-4-hydroxybenzoaat (E218)

2361 • Monoethanolamine

2362 • N-methyl-2-pyrrolidone

2363 • Polyethylene Glycol 200

2364 • Povidone K 17

2365 • Propyl-4-hydroxybenzoaat (E216)

2366 • Sodium formaldehyde sulphoxylate dihydrate

2367 The excipients may impact on local tolerance and this should be taken into account on a product-by-
2368 product basis.

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

2371 For oxytetracycline injections, the optimised doses suggested by the PK/PD modelling for the
2372 treatment of bovine respiratory disease fell within the range of doses already approved for different EU
2373 10% and 20% formulations, with the only modification being a reduction in the interval for repeat
2374 injections of the 20% formulations from 48 - 72 h to 36 – 48 h.

2375 The data available indicate that oxytetracycline has renal toxic effects which manifest above a dose of
2376 60 mg/kg bw (repeated on 3 occasions) – this would impact on the scope for any dose increase. The
2377 suggested dose of 20 mg/kg bw repeated once after 36 h (total 40 mg/kg bw) for 20% formulations is
2378 expected to give a C_{max} and overall exposure below this threshold for renal toxicity, and therefore is
2379 likely to be adequately tolerated in cattle for the treatment of the indication for respiratory disease.

2380 In terms of those 10% formulations for which the dose of 10 mg/kg bw represents a dose increase, it
2381 may be of more practical significance that local irritant effects can limit the volume that can be
2382 administered at each injection site. The maximum tolerated injection volume may vary with the
2383 formulation. It is suggested that the maximum dose volume at any site should not exceed that already
2384 stated in the SPC for individual products, or where not stated should be based on a review of the TAS
2385 data for the individual product. The number of injections that can practically be administered would
2386 have to be taken into account and could result in a restriction on the maximum bodyweight of animal
2387 for which a product could be used.

8.6. Overall conclusion on oxytetracycline

2389 The approaches on dose optimisation, WP, ERA and TAS as described in chapters 3, 4, 5, and 6,
2390 respectively, were tested in the case study on oxytetracycline products, administered by injection, for
2391 the treatment of respiratory infections in cattle, including lactating cattle. The solution for injection is
2392 available in 10% ("short acting") and 20% ("long acting") formulations. The approved doses are 4 – 20
2393 mg/kg bw per day, daily injection for between 1 and 5 days for the 10% formulations, and 20 or 30
2394 mg/kg bw, single injection, repeated after 48 or 72 hours in severe cases for the 20% formulations.

2395 In order to optimise the dose, the following pathogens were considered to be relevant: *Pasteurella*
2396 *multocida*, *Mannheima haemolytica* and *Haemophilus somni*.

2397 Because formulation-specific differences in PK may exist, the compositions and the PK of various
2398 products were analysed, revealing no significant differences in PK. However, the difference in strength
2399 will require different injection volumes which may impact on the absorption kinetics. Therefore, the
2400 PK/PD analysis was done for the 10% and 20% formulations separately.

2401 The optimised doses for the 10% and 20% formulations were 10 mg/kg bw and 20 mg/kg bw,
2402 respectively. These doses fell within the range of doses already approved for authorised products in

2403 the EU, with the only modification being a reduction in the interval for repeat injections of the 20%
2404 formulations from 48-72 h to 36–48 h.

2405 For the establishment of the WP, a "worst-case" and thus rather conservative half-life of 6 days was
2406 used for the extrapolation of WPs for both tissues and milk, resulting in low to moderate increases of
2407 the WPs.

2408 For addressing the environmental risks, adequate Phase I and Phase II ERA data were available for the
2409 authorised dose of 20 mg/kg bw. For the optimised doses (5x10 mg/kg bw or 2x20 mg/kg bw), the
2410 RQs remained below 1. Therefore, the optimised doses for oxytetracycline do not give rise to any
2411 additional concerns for the environment.

2412 In relation to TAS, The data available indicate that oxytetracycline has renal toxic effects which
2413 manifest above a dose of 60 mg/kg bw (repeated on 3 occasions) – this would impact on the scope for
2414 any dose increase. The suggested dose of 20 mg/kg bw repeated once after 36 h (total 40 mg/kg bw)
2415 for 20% formulations is expected to give a C_{max} and overall exposure below this threshold for renal
2416 toxicity, and therefore is likely to be adequately tolerated in cattle for the treatment of the indication
2417 for respiratory disease.

2418 In terms of those 10% formulations for which the dose of 10 mg/kg bw represents a dose increase, it
2419 may be of more practical significance that local irritant effects can limit the volume that can be
2420 administered at each injection site. The maximum tolerated injection volume may vary with the
2421 formulation. It is suggested that the maximum dose volume at any site should not exceed that already
2422 stated in the SPC for individual products, or where not stated should be based on a review of the TAS
2423 data for the individual product. The number of injections that can practically be administered would
2424 have to be taken into account and could result in a restriction on the maximum bodyweight of animal
2425 for which a product could be used.

2426 # 9. Discussion and conclusions

2427 ## 9.1. Dose optimisation by PK/PD analysis

2428 ### 9.1.1. Cases studies analysis

2429 For the purpose of the pilot study, the PK/PD index AUC_{24h}/MIC is considered for tetracyclines (Andes &
2430 Craig, 2002) and amoxicillin (Lees *et al.*, 2015). To investigate the differences between different PK/PD
2431 indices, T>MIC is also considered for amoxicillin (Rey *et al.*, 2014). This comparison of PK/PD indices in
2432 the application of the methodology will allow review of advantages (such as applicability, feasibility)
2433 and drawbacks (such as data requirements, complexity) of each PK/PD index.

2434 The calculation of AUC/MIC is simple to perform and allows back calculation to set a dose or a
2435 breakpoint. It requires a good pharmacokinetic dataset to estimate AUCs and does not require
2436 extensive pharmacometrics. The calculation of time above MIC requires robust estimates of the
2437 distribution of pharmacokinetic parameters (means and variances) from different experimental studies.
2438 An expertise in pharmacometrics using nonlinear mixed effects is needed for this step. The time to
2439 maintain MIC is not a simple parameter but a variable function of different conditions and depends not
2440 only on the dose but also from the shape of the time concentration curve. Thus, it cannot be derived
2441 from a simple formula and needs to be computed. The use of population pharmacokinetics, allows
2442 simulation of probable product exposures which can be obtained with any dosage regimen. This is
2443 important for a time-dependent antibiotic such as amoxicillin for which the input rate (absorption) is at
2444 least as important as the total administered dose and not dose-proportional. Indeed, the time to

2445   maintain MIC will be highly dependent not only on the dose administered but also on the formulation,
2446   the route of administration and the inter-individual PK variability (for example in body weight, sex,
2447   age, social rank).

2448   As an example, for pigs, for oral *ad libitum* administration, plasma concentrations are related to the
2449   feeding and water intake behaviour. This behaviour can be modified by disease state. The
2450   pharmacokinetic data set used by Rey *et al.* was obtained with healthy animals as it was submitted for
2451   marketing authorization for a veterinary medicine. Infection could modify the feeding and water intake
2452   behaviour and also product disposition. As discussed in the paper by Rey *et al*., the effect on
2453   disposition must vary according to the type of disease. Exposure of diseased animals could increase or
2454   decrease in comparison with healthy animals. Both PK/PD indexes AUC/MIC and T>MIC are dependent
2455   on animal status, product bioavailability, disposition and clearance.

2456   The use of a PK/PD approach requires a definition of the PTA to be achieved such as:

2457   -   T>MIC: 40% of 24 hours greater than the MIC of 90% of the pig population

2458   -   AUC/MIC: Ratio expected for bacteriostatic or bactericidal effect of 90% of the pig population.

2459   The relationship between T>MIC and antibacterial efficacy has been determined *in vitro* in several
2460   experimental animal studies (Craig, 1998) and retrospective analysis of clinical trials in human
2461   medicine seems to confirm those findings (Ambrose *et al.*, 2007). For AUC/MIC, the targets were
2462   derived from *in vitro* activity of amoxicillin in serum on a limited set of *P. multocida* strains (Lees *et al*.,
2463   2015). The choice of this index was justified in the paper because a concentration-dependent killing
2464   profile was observed *in vitro* in serum and confirmed in *ex vivo* studies. In addition, it was shown that
2465   for antibiotics like the β-lactams, where efficacy has been found to be correlated to T>MIC, the best
2466   PK/PD index shifts towards AUC/MIC as half-life increases (Nielsen & Friberg, 2013) while for an
2467   AUC/MIC dependent antibiotic a decrease in half-life will lead to a shift into a T>MIC relationship.
2468   When the half-life was increased to 2 h, the AUC/MIC became the most important PK/PD index
2469   (Nielsen *et al.*, 2011).

2470   Mechanisms based on PK/PD modelling based on *in vitro* studies are also proposed as a flexible and
2471   powerful tool to describe the effect of antibacterial agents. The simulations are based on a model
2472   characterizing *in vitro* time-kill curve experiments combined with a pharmacokinetic model. The
2473   approach selected the previously PK/PD indices for different classes of antibacterial product. The target
2474   level and optimal dosing regimen should be based on quantitative description of the full time course of
2475   PK as well as PD and tailored to the population to be treated (Nielsen *et al.*, 2011).

2476   9.1.2.   PK/PD and prevention of resistance

2477   The 'mutant selection window' (MSW) is a concept well described in the scientific literature (Zhao &
2478   Drlica, 2001) for certain classes of antibiotics (e.g. fluoroquinolones). It postulates that an antibiotic
2479   concentration zone exists where resistant mutants, are selectively amplified. The lower limit of the
2480   MSW is the lowest concentration that inhibits the growth of the susceptible cells and is often
2481   approximated by the MIC. The upper limit is the minimum concentration that inhibits growth of the
2482   least-susceptible single-step mutant subpopulation, the mutant prevention concentration (MPC).
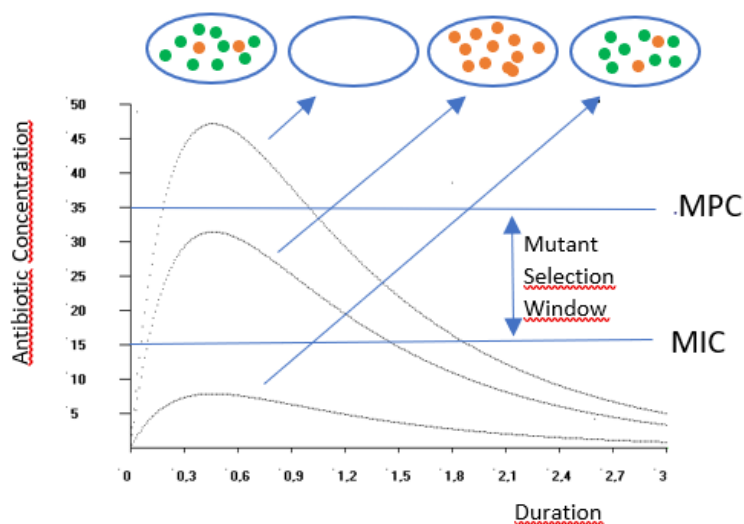
2483

Figure 37. Concept of mutant selection window (based on Canton & Morosini, 2011)

2485 This MSW also updates the classical concept of "sub-inhibitory" concentrations favouring the
2486 emergence of resistance, although the threshold to be considered is not the MIC of the majority wild
2487 pathogen population but the MIC of the least susceptible pathogenic sub-population, which in fact
2488 corresponds to the MPC.

2489 Then, to clearly take into account the notion of concentration preventing mutation in a PK/PD
2490 modelling, it is necessary first to define MPC distribution values for each molecule/bacterial species
2491 combination. It will allow obtaining three new PK/PD indices by replacing the MIC by the MPC:

2492 - AUC/MIC → AUC/MPC

2493 - T>MIC → T>MPC

2494 - C_{max}/MIC → C_{max}/MPC

2495 Currently, MIC distribution is well standardised notably for surveillance monitoring programs and the
2496 information is easily accessible. However, applying MPC principles, when available, may serve to
2497 optimise antibiotic therapy and reduce resistance selection.

2498 9.1.3. Limitations of the modelling approach

2499 *9.1.3.1. Impact on gut microbiota*

2500 One of the main challenges in relation to AMR is to reduce the exposure of intestinal microbiota in
2501 order to control the dissemination of resistance factors in the environment. Dose optimisation should
2502 aim to lower exposure of the treated animals over time. The proposed PK/PD methodology could be a
2503 useful tool for determining doses that are effective against the bacterial populations targeted by the
2504 antibiotic therapy however it is unable to integrate the potential impact on gut microbiota.

2505 *9.1.3.2. Use of the MIC as a PD indicator*

2506 The PK/PD relationship is based only on the determination of a MIC as an indicator of effectiveness.
2507 However, the MICs are determined *in vitro* in a standardized environment and are not always
2508 representative of site of infection. It should be noted that those aspects are currently under

2509 investigation notably studies comparing *in vitro* MIC obtained either in a standardized broth medium or
2510 in serum or biological fluid such as transudate/exudate. Evidence suggest that potency of certain
2511 antimicrobials measured in serum (as MIC) differs markedly from MICs determined in artificial broths
2512 and may need also to be considered for the dose optimisation (Dorey and Lees, 2017; Dorey *et al.*,
2513 2017; Lees *et al.*, 2018). In addition, in numerous situations, the MICs are not predictive of *in vivo*
2514 antibacterial activity as for example for intracellular pathogens or in a biofilm environment (Ferran *et*
2515 *al.*, 2016). Furthermore, some antibiotics present other modes of action (e.g. anti-inflammatory,
2516 immunomodulatory activities) which MIC does not take into account (Fischer *et al.*, 2011).

9.1.3.3. Host immune response

2518 The PK/PD relationship does not take into account the immune response of the host which will have an
2519 effect on growth of bacteria and its complete clearance from the body or a control of bacterial
2520 population in animal. The efficacy and memory effect of immune response are dependent of several
2521 conditions (inoculum size, immune capacity). A relationship between the bacterial population and the
2522 immune cells population can be described and added in a more complicated model. The dosage
2523 regimen (dose, frequency, duration of treatment) will be in relation with the recovery rate and the risk
2524 of relapse. At this stage of research on PK/PD modelling, the models are still under investigation (Gjini
2525 & Brito, 2016).

9.1.3.4. Duration of treatment

2527 Until now, one of the main limitations of the PK/PD methodology applied to the revision of the dosages
2528 of older antibiotics is that it helps determine a dose but does not give any information on the duration
2529 of treatment. Limiting the durations of antibiotic treatment to the minimum necessary can help reduce
2530 costs and adverse effects, but the main benefit is to reduce the duration of exposure of the commensal
2531 microbiota to antibiotics, which is an essential element in preventing the emergence, amplification and
2532 circulation of bacterial resistance. A number of studies have assessed the impact of the duration of an
2533 antibiotic treatment on the amplification of resistance within the commensal flora.

9.1.3.5. Need for a clinical confirmation

2535 The application of the PK/PD relationship for dose determination is accepted according to the revised
2536 efficacy guideline (EMA/CVMP/627/2001-Rev.1). However, when a PK/PD relationship is used, a clinical
2537 confirmation is always needed to assess the efficacy of the newly defined dose. If the proposed
2538 methodology leads to a substantial increase of the daily dose for old products, it may be necessary to
2539 define a clear regulatory process that should be applied in this context. It is unlikely that, for products
2540 that are now widely used in the field and have proven their clinical benefit, new effectiveness efficacy
2541 studies should be required under the regulations and according to current requirements. Thus, an
2542 important limitation of the approach is the lack of information on reliable PDT and corresponding PTA
2543 for certain type of infections in animals. Mode of administration

2544 The method proposed thus far considers the intake of the medicinal product to be "perfect". For
2545 injection routes, this is hardly a problem, provided that good hygiene measures are followed and
2546 needles and syringes suited to the dosage are used. In contrast, bioavailability studies by the oral
2547 route are all based on the forced drenching of animals. While pets receive their antibiotic by drenching,
2548 oral treatments of livestock food-producing animals are most often collective and based on "voluntary"
2549 intake by the animals, either by a solid medium via medicated feed, or by a liquid medium via drinking
2550 water.

2551

2552 - *Administration via feed*

2553 The main limitation is therefore the feed intake of each animal within the batch. When feeding *ad*
2554 *libitum*, the amount of feed consumed is more variable than the amount of water drunk. This leads to a
2555 greater variability of serum concentrations following administration of the same antibiotic (Soraci *et*
2556 *al*., 2014).

2557 - *Administration via drinking water*

2558 Compared to feed, administration via drinking water presented several advantages as for example
2559 treatment durations are usually shorter than via feed which lower the exposure of commensal flora, it
2560 is easier to target a smaller batch of animals and treatment can be started more quickly. However, the
2561 limitations and uncertainties are rather linked to the compliance of the dosage finally administered to
2562 the animals: accuracy of the dosage, quality of the medicated water and homogeneity.

2563 For oral *ad libitum* administration, plasma concentrations are related to the feeding and water intake
2564 behaviour (depending on e.g. the health status, the animal social rank), meaning that it induces new
2565 individual variabilities that the method presented here cannot take into account.

9.1.4. Data requirements

2567 In order to use the PK/PD analysis approach for the dose optimisation of established veterinary
2568 antibiotics, the following data are considered essential:

2569 • PK data

2570   o PK raw data from studies for individual product

2571   o Mean values for each PK parameters (CL, F, f …)

2572 • PD data

2573   o MIC distribution for each target bacteria

2574 Furthermore, the following data would be desirable:

2575 • Time-kill curves

2576 • PK/PD modelling

2577 • Literature search

2578 • *In vivo* experiment - correlation between prediction and clinical outcome

9.1.5. Conclusions on the PK/PD analysis

9.1.5.1. The importance of the dose optimisation of established veterinary antibiotics

2581 The importance of revising the dosages is based on a need to optimise the doses of older antibiotics
2582 because repeated exposure to inappropriate concentrations represents a major risk in terms of
2583 antimicrobial resistance in target pathogens. An optimal dosage must be determined to ensure the
2584 efficacy of the treatment, but also to prevent the emergence, selection and/or dissemination of
2585 resistant micro-organisms in a bacterial population. Inter-individual variability, in terms of exposure to
2586 the antibiotic, is certainly one of the risk factors with the greatest influence on the emergence of
2587 antibiotic-resistant organisms. Accordingly, a dosage should be based on a PK/PD approach and should

2588 take the inter-individual variability into account, regarding both pharmacokinetics and
2589 pharmacodynamics.

2590 The methodology for revising the dosages of older antibiotics is based on a PK/PD approach that can
2591 integrate both pharmacokinetic (clearance, bioavailability) and pharmacodynamic variability (in terms
2592 of MIC) in the search for the optimal dose. The use of a PK/PD approach in the dose determination
2593 phase prior to a clinical validation phase will therefore make it possible to select a dosage leading to a
2594 sufficient exposure of the target bacterial population to an effective concentration of the antibiotic, in
2595 the majority of animals treated.

2596 The current doses of established antibiotics generally provide a clinical benefit without this being
2597 optimised with regard to the risk of antimicrobial resistance, whether it concerns the pathogenic
2598 bacteria targeted or the commensal microbiota.

2599 ### 9.1.5.2. The feasibility of the PK/PD approach

2600 The PK/PD approach requires consolidated data to be available both on the pharmacokinetics of the
2601 antibiotics in the species considered, and the pathogens' susceptibility to antibiotics, in the form of MIC
2602 distributions. The effectiveness indices (PK/PD indices) are central to the PK/PD methodology applied
2603 to antibiotics, whether in the area of human or animal antibiotic therapy, because they are required to
2604 be predictive of a high probability of therapeutic success, in potentially varying clinical situations.
2605 Currently, there were few available data however, especially for the issue of older antibiotics. Ideally
2606 these PK/PD indices (and their threshold values) would be confirmed by clinical trials performed in the
2607 target species. For old antibiotics, the PK/PD integration approach is eligible to dose optimisation in the
2608 treatment of acute diseases in animals when the substance belongs to an antimicrobial class with
2609 scientific evidences from experimental and clinical trials supporting the setting of PDI and PDT.

2610 ## 9.2. Withdrawal Period adjustment by PK analysis

2611 ## 9.2.1. Case studies analysis

2612 For the purpose of testing the approach of adjustment of the WP using an algorithm based on PK
2613 modelling (see chapter 4.), two case studies were performed. The idea was to test a simple case
2614 (amoxicillin products for oral use in pigs) together with a more difficult one (injectable oxytetracycline
2615 products for use in cattle; including dairy cattle). However, as it turned out, both cases each had their
2616 own specific difficulties.

2617 Whilst the problem how to deal with the injection site and the i.m versus s.c. administration had to be
2618 addressed in the oxytetracycline case, the amoxicillin case turned out to be unexpectedly difficult, due
2619 to lack of usable residue data.

2620 Since the depletion of residues of amoxicillin after oral administration to pigs is very rapid, most of the
2621 old residue studies that could be found in registration files, only confirm that the residues are already
2622 below LOD after a few days.
2623 For oxytetracycline, the erratic sampling of the injection site caused some fitting problems as well as
2624 the fact, that increasing the dose could pose a challenge regarding the maximum amount of injections
2625 that would be practical versus the maximum weight of an animal to be treated.
2626 In both cases however, the particular challenges could be overcome, by the use of the 'hourglass'
2627 approach.

2628 Using data and insights from multiple sources (FARAD, literature, published thesis's, registration files,
2629 *etcetera*) and combining them in order to find the relevant PK parameters and eventually the terminal

2630 half-life, in both cases reliable solutions for the extrapolation of the WP could be found. Therefore the
2631 two cases show that it is possible to use the proposed algorithm for extrapolating the Withdrawal
2632 Period.

2633 ### 9.2.2. Concluding remarks on Withdrawal Period extrapolation

2634 Although the WPs in the EU are usually based on residue depletion studies (as the "golden standard"),
2635 it is acknowledged that this approach has some limitations in relation to the predictive value for the
2636 true WP under field conditions, where the products are used in different breeds and different weight
2637 classes of the (diseased) target animals (see 4.1.).

2638 Therefore, the standard approach may not be scientifically superior per se to the PK modelling
2639 approach using data from literature and all products. In the proposed extrapolation approach, the
2640 pharmacokinetic parameters for the substance are extracted from all available sources, in order to get
2641 the best estimates as the basis for extrapolation. The uses of multiple information sources and
2642 established pharmacokinetic principles ensure the scientific basis of the proposed extrapolation
2643 approach.

2644 So this approach for the adjustment of existing WPs is most probably not inferior to the approach of
2645 the conduct of new residue depletion studies.

2646 As already pointed out, it should be noted that the third step in the proposed extrapolation-process is
2647 to apply the algorithm to each VMP separately. This would mean that the relative differences in the
2648 existing withdrawal periods will remain, not only to ensure minimal disturbance of the market whilst
2649 maintaining consumer protection, but also to take into account the potential effect of the formulation
2650 on the parameters influencing the (absorption) kinetics of the products.

2651 ### 9.3. Addressing environmental risks by a data review approach

2652 ### 9.3.1. Case studies analysis

2653 The environmental risk assessment for the case studies on amoxicillin in pigs and oxytetracycline in
2654 cattle turned out to be fairly easy. For amoxicillin, the doubling of the dose from 20 to 40 mg/kg bw
2655 per day for 5 days did obviously increase the PECs with a factor of 2. The Risk Quotients remained
2656 below 1 when the duration is maximally 5 days, and above 1 when the duration is 7 days. It was
2657 considered that the duration of 3-5 days may be sufficient for products with the same indication, which
2658 would justify the limitation of the duration to maximally 5 days, in order to limit the exposure to the
2659 environment. For oxytetracycline, there was already an ERA for a single dose of 20 mg/kg bw. Dose
2660 optimisation resulted in two regimens: 2 x 20 mg/kg bw for the LA formulations, or 5 x 10 mg/kg bw
2661 for the SA formulations, both of which would increase the environmental exposure as compared to the
2662 existing ERA. However, even with these posologies the Risk Quotients remained below 1, and therefore
2663 there was no trigger crossing and consequently no need to enter another Phase or Tier of the ERA.

2664 ### 9.3.2. Conclusions on the ERA data review

2665 A data review approach was set up and tested in two case studies. The case studies showed that the
2666 data review approach was feasible, and that there were no additional concerns for the environment
2667 with the new optimised doses. This conclusion was reached without the need for additional
2668 experimental studies.

2669 It has to be recognised that the case studies were easy in the sense that there was no trigger crossing
2670 when going from the current dose to the optimised dose. Therefore, the data review approach as
2671 outlined in chapter 5. , was not tested to the full extent. There may be other cases where the approach
2672 can be more challenging. Nevertheless, within the limitations of this pilot, the approach was successful.

9.4. Addressing target animal safety by a data review approach

9.4.1. Case study analysis

2675 The data review methodology proposed to address target animal safety was not followed
2676 comprehensively in the two case studies due to the lack of availability of pivotal study data for these
2677 old products from either pharmaceutical companies or regulatory agencies, and the time needed to
2678 perform searches to fill data gaps from publicly available material. Although the methodology could be
2679 time consuming, the expectation is that it would be followed until sufficient evidence is available to
2680 give confidence in the conclusions.

2681 In regards to the amoxicillin case study, only two proprietary TAS studies were available that, although
2682 not to current VICH requirements and performed in only a small number of animals, gave a reasonable
2683 level of evidence to support a margin of safety for the proposed revised dose in the target species. No
2684 specific studies could be found on a basic literature search to support field safety in pigs; however
2685 standard texts and reports representing use of the substance over decades in laboratory species and
2686 humans give reassurance of a wide margin of tolerance. It was possible to fully identify the target
2687 organs and toxic profile of the substance based on the totality of the data available.

2688 For the oxytetracycline case study, the CVM Freedom of Information summary reports provided the
2689 most informative data on systemic tolerance; although it has to be considered that this is only
2690 available in high level summary format. For oxytetracycline, the optimised dose regimens suggested by
2691 PK/PD modelling fell within the range of doses approved in the EU; however, the margin of safety for
2692 renal effects would have to be taken into account for any further dose increase. The proprietary studies
2693 provided to the project by industry related to injection site safety with the focus being on local
2694 tolerance and injection volume, rather than dose. These studies clearly highlighted that local tolerance
2695 is likely in practice to be the key dose-limiting factor for oxytetracycline injectable formulations, with
2696 some variability between different formulations according to excipient composition.

2697 For both the amoxicillin and oxytetracycline studies, no proprietary data were available from either
2698 field safety studies or post-marketing pharmacovigilance. Outside the pilot project scenario, these data
2699 should be sought to give greater confidence in the final conclusions.

9.4.2. Conclusions on the TAS data review

2701 For the amoxicillin formulations, the data review approach can give reasonable confidence that the
2702 proposed dose increase to 40 mg amoxicillin /kg x 5 days in drinking water would be adequately
2703 tolerated in pigs for the treatment of respiratory disease. Amoxicillin is a well-established molecule
2704 with a wide margin of safety in many species and with further probing of dossiers sufficient data are
2705 likely to be available to draw conclusions on the safety of the dose increase in pigs. The oral
2706 formulations are administered as solutions and have relatively simple excipient formulations, and
2707 therefore safety can be extrapolated between them with a degree of confidence.

2708 Although no increase in dose outside of the EU-approved ranges was suggested for oxytetracycline
2709 injections, the possibility of a hypothetical dose increase was explored. As the margin of safety for the
2710 active substance is not large, further supporting data would have been needed beyond what could be

2711 provided within the constraints of this pilot project. This may have been available from a wider review
2712 of product dossiers. For this case, the oxytetracycline injectable formulations are more complex than
2713 the oral amoxicillin solutions. The data review methodology identified that local injection site reactions
2714 may be dose-limiting in practice. Local tolerance can vary according to individual product composition
2715 and would have to be considered on a product-by-product basis; therefore proprietary studies would
2716 be required to establish the maximum injection volume where not already stated in a product's SPC.
2717 Where data are not available, a default value could be established according to the worst case
2718 scenario. If restriction of injection volume would lead to an impractical number of injection sites, a
2719 simple risk management solution would be to limit the maximum bodyweight of animal to be treated.
2720 In conclusion, use of the data review approach would be possible for this case, but the need for
2721 individual product review could be burdensome.

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

2724 The main purpose of the pilot project was to develop and test a novel approach for dose optimisation,
2725 WPs, ERA and TAS, without the need for conducting further experimental studies. This approach may
2726 be useful to review and improve the situation of established veterinary antibiotics where the
2727 authorised dose may not be effective anymore. At the same time, application of this approach will lead
2728 to a certain level of harmonisation between authorised products across the EU. In this respect, this
2729 approach can also be used as part of other regulatory harmonisation exercises (e.g. possibly initiated
2730 by future EU legislation on veterinary medicines).

2731 A number of general principles for the regulatory implementation of this approach and the related
2732 harmonisation of VMPs (discussed below) were defined, but the appropriate regulatory procedures, the
2733 appropriate legal basis, and other related legal issues were not defined or discussed. The latter points
2734 need further discussion.

9.5.1. Selection of candidates

2736 Chapter 2.1. offers a method to select and prioritise (groups of) established veterinary antibiotics for
2737 which dose optimisation may be required. Application of this method allows putting resources where
2738 they are most needed, and provides clarity on the order at which the products will be reviewed, which
2739 would facilitate short and long term planning of related work at the sides of regulators and industry.

9.5.2. Extent of harmonisation

2741 As explained above, the dose optimisation of products or groups of products will lead to a certain
2742 degree of harmonisation. The minimum desired level of harmonisation would be a harmonisation of
2743 individual products with authorisations in different Member States (i.e. at product level). This has been
2744 explained in chapter 2.2. (the hour glass method). However, because of the group-wise analysis,
2745 some aspects such as the optimised dose, may be applied to different products within the same group,
2746 as was done for the case studies with amoxicillin and oxytetracycline. This may in particular apply to
2747 similar products which have been licenses nationally some time ago, resulting in different summaries
2748 of product characteristics, but which are essentially similar.

9.5.2.1. Same-product harmonisation

2750 The same product with authorisations in different Member States can have differences in the
2751 indications (i.e. inclusion of certain diseases), the causal organisms (i.e. inclusion of certain

2752 pathogens), the dose, the withdrawal periods, and the special warnings and precautions for use. There
2753 are several possibilities for within-product harmonisation, and the selected level of harmonisation has
2754 consequences for the approaches to address dose, WP, ERA, and TAS, and for the final outcome. For
2755 example, one could calculate an optimised dose for each disease, or even per causal pathogen for
2756 these diseases, resulting in differentiated optimised doses that can be applied to the authorisations
2757 depending on which diseases/pathogens has been already licensed in the various Member States.
2758 However, such an approach would require many calculations for the doses and withdrawal periods, and
2759 may also have different outcomes for ERA and TAS, depending on the highest label dose. Moreover in
2760 practical terms this may not offer advantage since for first line antimicrobials treatment is often started
2761 before the causative pathogen has been identified and many infections (including respiratory disease)
2762 are syndromes with mixed bacterial etiology. In addition, differences of the SPC of the product
2763 between Member States would remain. Another possibility would be to aim for the largest possible
2764 denominator and thus l a full harmonisation per product. That would include the sum of all authorised
2765 indications/pathogens for which a dose optimisation was possible applied to all authorisations of this
2766 product across the EU, irrespective of the current indications authorised in the individual MSs. This
2767 approach is not only easier to apply but would maximise the availability of efficacious veterinary
2768 antibiotics for various diseases at the same time. A full harmonisation per product is preferred,
2769 resulting in identical SPCs in all MSs where the product is authorised. A full harmonisation also implies
2770 a single WP for meat and offal, and a single WPs for milk or eggs, where applicable. It should be noted
2771 that current WPs for the same product can be very different between MSs. Therefore, the
2772 establishment of a single WP will require the selection of a "Reference WP" that can be used as a
2773 starting point for the extrapolation. Is proposed that this Reference WP will be scientifically established
2774 on the basis of available residue data, and not on the shortest or the longest WP by default.

9.5.2.2. Between-product harmonisation

2776 As explained in chapter 2.2. , there are scientific and practical reasons to harmonise at the level of
2777 individual products. Nevertheless, the analysis conducted according to the hour glass method may
2778 reveal that certain products in a group are so similar that for the same indication and the same
2779 species, the same optimised dose could apply. However, as indications can differ between products is
2780 proposed not to harmonise indications across these products. For example, if product A has only
2781 respiratory tract infections on the label, and similar product B has both respiratory tract infections and
2782 urinary tract infections on the label, then the respiratory tract infections could be harmonised between
2783 products when possible (i.e. they will have the same optimised dose), but product A will not get the
2784 urinary tract infections indication. In addition, is proposed that WPs are not harmonised across
2785 (similar) products. Where differences in excipient formulation could have an impact on local tolerance,
2786 this aspect needs to be considered on a product-specific basis.

9.5.3.   Level of assessment

2788 Established veterinary antibiotics have been authorised through national, decentralised, or mutual
2789 recognition procedures, and therefore have national marketing authorisations. Therefore, in principle,
2790 any changes to the marketing authorisations fall within the remit of the National Competent Authorities
2791 (NCAs). However, it should be noted that:

2792 • the process of dose optimisation, WP, ERA and TAS requires input from National Competent
2793   Authorities through the authorisation dossiers from all MSs;

- the process of dose optimisation, 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 optimisation, 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 optimisation 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 optimisation 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.

9.6. Need for further research

One of the objectives of this project 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 optimising dosing with respect to minimising risks from AMR where progress is not possible without generation of additional data. Non-experimental approaches for dose optimisation, WP, ERA and TAS were developped. 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. Indeed, sufficient data was available to conduct the case studies for amoxicillin and oxytetracycline. The therefore it was concluded that considerable progress was made without the need for generation of additional data, and did not further investigate possibilities for funding.

As explained in 9.1.3.5. , 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 a very data-rich basis for the modelling and review approaches.

Whereas the PK/PD methodology allows for optimising 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.

10. 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.

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2835   2. It is recommended that the implementation of the recommendations of this report will take place
2836   at the central level, i.e. that CVMP will conduct the scientific assessment. It was noted that the
2837   outcome could result in an e.g. Commission Decision.

2838   3. It is recommended to develop a clear procedure to establish a list of the candidate products for
2839   dose optimisation, with a prioritisation of these candidates, in line with the principles discussed in
2840   chapter 2 of this report. In establishing the actual list, it is recommended that relevant
2841   stakeholders are consulted. For example, the FVE can be consulted to obtain information of
2842   dosages used in the field, and VetCAST can be consulted to obtain information products for which,
2843   according to their knowledge, the current dosing regimens is not in line with PK/PD principles.

2844   4. It is recommended that selected candidate products for dose optimisation are grouped at the
2845   *animal-species-disease-route of administration-pharmaceutical form* level.

2846   5. It is recommended to follow the hour glass approach (see chapter 2) for collection and integration
2847   of data and for the application of model outputs.

2848   6. It is recommended that procedures for dose optimisation, withdrawal periods, ERA, and TAS, result
2849   in harmonisation at product level and where applicable also between similar products as outlined in
2850   paragraph 9.5.2.2 .

2851   7. It is recommended that the dose optimisation and the consideration of withdrawal period, ERA, and
2852   TAS, are conducted in accordance with the principles presented in chapters 3, 4, 5, and 6 of this
2853   report.

11. Glossary

2855   ADME   Absorption, Distribution, Metabolism, Excretion

2856   AE   Adverse Event: any observation in animals, whether or not considered to be
2857   product-related, that is unfavourable and unintended and that occurs after any use
2858   of VMP (off-label and on-label uses). Included are events related to a suspected
2859   lack of expected efficacy according to approved labelling or noxious reactions in
2860   humans after being exposed to VMP(s).

2861   AMEG   Antimicrobial Advice Ad Hoc Expert Group

2862   AUC   Area Under the Curve: the total concentration integrated over a given time interval

2863   AMR   Antimicrobial resistance

2864   B/R assessment   Benefit-risk assessment: A process of assessing benefits and risks in accordance to
2865   the benefit-risk assessment policy. This assessment includes the mitigation of risks
2866   from a proposal of benefit-risk management options. The benefit-risk balance is
2867   the outcome of the benefit-risk assessment.

2868   CBP   Clinical breakpoint: A selected MIC value to distinguish between treatable and
2869   non-treatable organisms

2870   CLSI   Clinical and Laboratory Standards Institute

2871 C_{max} The maximum (or peak) serum concentration that a product achieves in a
2872   specified compartment or test area of the body after the product has been
2873   administrated

2874	CVMP	Committee for Medicinal Products for Veterinary Use
2875 2876	DDDvet	Defined Daily Doses for Animals; The DDDvet is the assumed average dose per kg animal per species per day
2877 2878 2879 2880	Dose optimisation	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).
2881 2882 2883	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.
2884	EGGVP	European Group for Generic Veterinary Products
2885	EMA	European Medicines Agency
2886	ERA	Environmental Risk Assessment
2887	EUCAST	The European Committee on Antimicrobial Susceptibility Testing
2888 2889 2890 2891	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).
2892	f	free or unbound fraction
2893	GLP	Good Laboratory Practice
2894 2895 2896	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/
2897 2898	Horizon 2020	Horizon 2020 is a EU Research and Innovation programme with nearly €80 billion of funding available over 7 years (2014 to 2020)
2899	LA	long acting
2900 2901	MAH	Marketing Authorisation Holder: A person or entity who/which holds the authorisation of a VMP.
2902	MBC	Minimum Bactericidal Concentration
2903 2904	MIC	Minimum Inhibitory Concentration: the lowest concentration of a chemical which prevents visible growth of a bacterium.
2905 2906 2907	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.
2908 2909 2910 2911	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.
2912	MS	Member State of the European Union

2913	NCA	National Competent Authority
2914	OECD	Organisation for Economic Co-operation and Development
2915	OIE	World Organization for Animal Health
2916	ORTD	Original Recommended Treatment Dose
2917	PBT	Persistent, Bioaccumulative and Toxic
2918	PEC	Predicted Environmental Concentration
2919	PD	Pharmacodynamics
2920 2921	PDI	PK/PD-index: The quantitative relationship between a pharmacokinetic parameter (such as AUC, peak level) and a microbiological parameter (such as MIC)
2922	PDT	target value of the PK/PD index
2923	PK	Pharmacokinetics
2924 2925 2926 2927 2928	PK/PD modelling	A technique that combines the two classical pharmacologic disciplines of pharmacokinetics and pharmacodynamics. 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 product dose.
2929	PNEC	Predicted No Effect Concentration
2930 2931 2932	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.
2933	PTA	Probability of Target Attainment
2934	QSAR	Quantitative Structural Activity Relationship
2935 2936	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).
2937	RMM	Risk Mitigation Measure
2938	RQ	Risk Quotient, i.e. PEC/PNEC ratio
2939	SA	short acting
2940 2941 2942	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.
2943	SPC	Summary of Product Characteristics
2944	TAS	Target Animal Safety
2945	vPvB	very Persistent and very Bioaccumulative
2946	VCIA	Veterinary Critically Important Antimicrobial Agents
2947	VHIA	Veterinary Highly Important Antimicrobial Agents
2948	VIA	Veterinary Important Antimicrobial Agents

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2949	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.
2953	VMP	Veterinary Medicinal Product
2954	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.
2957	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.
2961	WT	wild type

2962

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13. Annexes

Annex 1

Data available

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/CO2)	
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	

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From MAA applications, extensions, variations			
		Mode of administration Sampling scheme Analytical method PK/PD analysis	

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3179 **Annex 2**

3180 **Definition of important PK, PD and PK/PD indices (from 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 [34]
MBC	MBC is the lowest concentration at which 99.9% reduction in bacterial count is achieved	mg/L or μg/mL	Tayler et al., 1983 [44]
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 [45]
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 [34]
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 [34]
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 [34]
PK/PD integration			
$T > \text{MIC}$	The cumulative percentage of 24 h period in which the drug concentration exceeds the MIC at steady state pharmacokinetic condition	%	Mouton et al., 2005 [34]
AUC/MIC	The area under the concentration time curve divided by MIC	No unit	Mouton et al., 2005 [34]
CMax/MIC	The peak concentration of drug divided by MIC	No unit	Mouton et al., 2005 [34]

3181

3182 **Annex 3**

3183 **Withdrawal Periods of various 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

3184

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

3185

Trade name	Country	Posology for pigs	WP for pigs (days)
Aciphen	DE	2-10 mg/kg KGW über 2-5 Tage	3
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 2,5 almapharm	DE	2-10 mg 2x tgl.	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
Amoxicillin-Trihydrat 10%	DE	10 mg/kg KGW 2x tgl. über 3-5 Tage	3
Amoxicillin C20 GKS	DE	Masthähnchen: 1 g/Tier u. Tag m. d. Trinkwasser; Schwein: 100* mg 2x tgl. m. d. Trinkwasser über 3-5 Tage	4

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Trade name	Country	Posology for pigs	WP for pigs (days)
		*To be checked whether this is a typo!	

3186

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) for 5 following days orally in wetfeed.	14

3187

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

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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
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

3189

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)
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

3190

Trade name	Country	Posology for pigs	WP for pigs (days)
Amoxindox 50	IT	40 mg product/kg b.w./day (corresponding to 20 mg amoxycillin trihydrate/kg b.w./day) for 5 days.	1
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

3191

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

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3195   **Annex 4**

3196   **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	Characterise toxicity syndrome, target organs Identify the margin of safety (MOS)	Healthy animals Final formulation (or close) 0, 1x, 3x, 5x ORTD, for 3x dose duration Clinical observations, clinical pathology, necropsy, histopathology Local tolerance: injection site safety (1xORTD)	Local tolerance Formulation-specific AEs Palatability issues at higher dose
Reproductive TAS studies preferably according to principles of VICH GL 43	Identify safety 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 pre-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) Variable dose range, e.g. 0x, 0.5x, 1x, 2x ORTD Efficacy endpoints Dose confirmation studies: usually 1x ORTD, possibly natural disease outbreak, larger animal numbers	May also report safety outcomes
Clinical field 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

From MA applications, extensions, variations			
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 Information on excipients – e.g. MRL summary reports, Codex reports, GRAS list	According to study design. Toxicity data		
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.	

Overview of compositions of OTC formulations authorised in The Netherlands

Product		Alamycin LA	Alamycin LA 300	Cydosol LA	Oxy LA inj	Tridox Pro Inj	Vetroxy LA	Alamycin 10	Cydosol 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

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