anses
French agency for food, environmental and occupational health & safety

Investigate, evaluate, protect

Methodology for revising the dosages of older antibiotics

ANSES Opinion
Collective Expert Report

April 2017 Scientific Edition

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Maisons-Alfort, 4 April 2017

OPINION
of the French Agency for Food, Environmental and Occupational Health & Safety

on the internal request "2014-SA-0080 - MA Methodology for revising dosages"

ANSES undertakes independent and pluralistic scientific expert assessments.
ANSES's public health mission involves ensuring environmental, occupational and food safety as well as assessing the potential health risks they may entail.
It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.

It provides the competent authorities with the necessary information concerning these risks as well as the requisite expertise and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).

Its opinions are published on its website.
This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 4 April 2017 shall prevail.

On 28 March 2014, ANSES issued an internal request to conduct the following expert appraisal: determination of a methodology for revising the dosages of older antibiotics in veterinary medicine.

1. BACKGROUND AND PURPOSE OF THE REQUEST

In its Measure 17 (Theme 2), the EcoAntibio 2017 Plan provides for maintaining the MAs of older antibiotics regarded as non-critical for human medicine, and in particular focusing on revalidation of the therapeutic regimens.
Feedback from use in the field indicates dosages that are ill suited to certain bacterial populations encountered in the targeted diseases.
In addition, the development of knowledge of pharmacokinetics (PK) and pharmacodynamics (PD), and of the PK/PD relationships of antibiotics, has made it possible in some cases to assess the relevance of the dosages, while taking into account the risk of selection of antibiotic resistance in the targeted bacteria. In the framework of this internal request, a Working Group (WG) was set up to define a **methodology** for revising the dosages of older antibiotics, encompassing both animal and public health objectives.
The actual revision of the dosages of older antibiotics will need to be conducted at the European level, where this methodology will be proposed. The conclusions of the work following this internal request are therefore not designed to recommend doses for older antibiotics, but rather to define a scientifically robust methodology for their revision.

2. ORGANISATION OF THE EXPERT APPRAISAL

The expert appraisal was carried out in accordance with French Standard NF X 50-110 "Quality in Expert Appraisals – General Requirements of Competence for Expert Appraisals (May 2003)".

The expert appraisal falls within the sphere of competence of the Expert Committees (CES) on "Veterinary Medicinal Products" and "Animal Health and Welfare" (SABA). ANSES entrusted the expert appraisal to the WG on the "Methodology for revising the dosages of older antibiotics", which met seven times between November 2014 and October 2016, including a hearing with the Veterinary Medicinal Product and Reagent Industry (SIMV), as the leader of Measure 17 of the National EcoAntibio Plan.

The methodological and scientific aspects of the work were presented to the CES on "Veterinary medicinal products" between November 2014 and March 2016, and then to the CES on "Animal Health and Welfare" between October 2016 and February 2017. The work was adopted by the CES on "Animal Health and Welfare" on 7 February 2017.

ANSES analyses the links of interest declared by the experts prior to their appointment and throughout the work, in order to avoid potential conflicts of interest with regard to the matters dealt with as part of the expert appraisal. The experts' declarations of interests are made public via the ANSES website (www.anses.fr).

The scientific and regulatory references, and the guidelines used in carrying out this expert appraisal are listed in Section 9 of the report.

3. ANALYSIS AND CONCLUSIONS OF THE CES SABA AND THE WG

The report begins with some preliminary considerations on dose determination, and then defines the scope and the choice of compounds of interest, on which a review of the research and literature on the pharmacokinetic profiles was conducted.

A proposed methodology for revising the dosages of older antibiotics, and its application to an intentionally limited scope, is then presented. Lastly, the limitations of the method are described, along with the consequences of revising the dosages on animal health (efficacy, animal tolerance, resistance of target bacteria) and on public health (consumer safety, safety of the environment, but also the impact on commensal flora).

The report concludes with the various points highlighted as a result of this work, and makes some recommendations.

1) Preliminary considerations when determining a dose

The methodology for determining effective doses has evolved considerably over the past forty years, and has been accompanied by a refinement of the regulatory framework. Different periods in the history of the dosages in veterinary antibiotic therapy were thus identified through an analysis of the successive guidelines. Then, the establishment of effective doses based on pre-clinical and clinical elements followed dose determination based primarily on clinical grounds.

The contribution of PK/PD relationships to dose determination consists in introducing information relating to the PK of the antibiotic and to its PD in the dosage selection process. The different PK/PD indices used in antibiotic therapy are reviewed in the report.

Their value as criteria for predicting efficacy in antibiotic therapy and preventing resistance is highlighted because they in fact correspond to a model of plasma exposure to the antibiotic, which

is compared to the minimum inhibitory concentration (MIC), used as an indicator of the pathogen's susceptibility to the tested antibiotic.

The correlations between the PK/PD indices and the clinical efficacy of different classes of antibiotics have been determined from experimental infection models developed in rodents (rats, mice), for human antibiotic therapy. Clinical studies in humans, whether prospective or retrospective, have helped quantify their levels of correlation with efficacy (clinical, microbiological) and propose threshold values (or critical values) for these indices associated with high cure probabilities (> 80-90%).

More recently, mathematical physiological models explored *in silico* the correlations between the PK/PD indices and antibacterial activity for the beta-lactams.

Within a bacterial population susceptible to an antibiotic, resistant clones can appear continuously following a spontaneous mutation on the bacterial genome. In the absence of selection pressure related to an antibiotic treatment, these resistant mutants generally remain largely in the minority within the inoculum. Selection is exercised when the antibiotic concentrations reduce the wild majority population while at the same time not affecting the mutant sub-population.

Thus, prevention of resistance in the pathogenic bacteria targeted by the antibiotic therapy goes hand in hand with the therapeutic objective because it relates to the same bacterial species located in the same biophase. This is why the PK/PD indices described are used to predict both the efficacy and the prevention of resistance. Studies on animal models, confirmed by clinical trials in humans, have shown that the values of the PK/PD indices must be higher when the objective is to maximise prevention of resistance.

2) Scope and choice of species, indications and compounds of interest

The WG decided to apply the PK/PD approach to dose determination within an investigative scope that is intentionally limited to a few antibiotics, selected to treat a single type of bacterial disease, and arbitrarily choosing a single causal agent in order to facilitate comparisons.

The animal species selected were cattle (calves) and pigs, as they are major food-producing species. Respiratory disorders were selected because they constitute one of the main reasons for the use of antibiotic therapy.

Pasteurella multocida was selected from among the aetiological agents responsible for these disorders because, although it is not the major pathogen in pigs, it is common to both animal species, and also because of the availability in the literature of MIC distributions of the selected antibiotics with regard to this bacterial species.

The five selected antibiotics were tetracycline, oxytetracycline, doxycycline and amoxicillin due to their widespread use, along with a more recent antibiotic, florfenicol. These choices were consolidated by an analysis of the antibiotics sales and exposure data.

3) Review of the literature search and analysis

Pharmacokinetic data relevant for calculating the dosages were collected by the Working Group in advance: clearance (Cl) and bioavailability (F) as pharmacokinetic parameters controlling the blood concentrations of the antibiotic, as well as the free fraction of the antibiotic in plasma (fu).

The quantity and quality of the data collected for tetracycline in calves proved insufficient for enabling the dosage revision methodology to be applied to them.

It should be noted that in pigs, the oral bioavailability of oxytetracycline and that of tetracycline are very low, and that of doxycycline and of amoxicillin are intermediate.

Only florfenicol has very good bioavailability in both species.

When the literature data are sufficient for applying the methodology developed, the inter-animal variability represented by the distribution of the values of the PK parameters in the population can also be taken into account.

4) Methodology for revising the dosages of antibiotics

a) Construction of the methodology

The PK/PD approach makes it possible to calculate a dose taking into account in combination the pharmacokinetic and pharmacodynamic properties of a medicinal product. The relationship between the dose and the PK/PD parameters is given by the following equation:

$$\text{Dose}_{\text{per unit of time}} = \frac{\text{Plasma clearance}}{\text{Bioavailability}} \times \text{Target concentration}$$

where $Dose_{per\ unit\ of\ time}$ is the dose of the antibiotic expressed per unit of time, and *Target concentration* is the mean plasma concentration associated with the desired therapeutic effects.

In the case of antibiotics, the target concentration must make it possible to reach the threshold value (or critical value) of the PK/PD index correlated with their efficacy.

It has been shown that the **AUC_{24h}/MIC index** can be used for all the antibiotics studied in this report.

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

$$\text{Target concentration} = \frac{\left(AUC/MIC\right)_{\text{critical value}}}{24h} \times \frac{\text{MIC}}{\text{fu}}$$

where $\left(\text{AUC}/\text{MIC}\right)_{\text{critical value}}$ is the critical value of the PK/PD index expressed in hours, *fu* is the free fraction of the antibiotic in plasma, and MIC is the minimum inhibitory concentration of the antibiotic for the bacterium in question.

By incorporating the determination of the target concentration in the equation cited above, an equation is ultimately obtained that enables calculation of the daily dose (over 24h) needed to obtain the level of plasma exposure targeted by the PK/PD index:

$$\text{Dose}_{\text{daily}} = \frac{\text{Plasma clearance}}{\text{Bioavailability}} \times \frac{\text{MIC}}{\text{fu}} \times \left(AUC/MIC\right)_{\text{critical value}}$$

Determining a dose therefore requires the values of parameters derived from three distinct components to be documented:

- **The value of the MIC** of the pathogen,
- **The value of the pharmacokinetic parameters**: clearance, free fraction of the antibiotic in plasma (fu) and bioavailability,
- **The threshold value of the PK/PD index (here AUC_{24h}/MIC)**, which sets a goal of plasma exposure to the antibiotic, relative to the susceptibility of the pathogen.

For each antibiotic, threshold values for the PK/PD index were extracted from the literature for three different cases: a bacteriostatic effect with no alteration of the immune defences, a bacteriostatic effect with alteration of the immune defences, and a bactericidal effect (see Table 8 in the report).
These three cases represent three levels of increasing requirement with regard to the expected efficacy.

The MIC values used are:
- the critical concentration ("breakpoint") provided by the Veterinary Committee of the CA-SFM-Vet, which delineates the *Susceptible* category of the antibiotic susceptibility tests (antibiograms),
- the epidemiological cut-off, or ECOFF, provided by EUCAST.

The Working Group explored the extent of the PK and PD variabilities when establishing the methodology. Thus, the methodology integrated either a **MIC point value (ECOFF or critical concentration)** or **the distribution of the MICs**, which accounts for the variability of the susceptibility of the strains. Similarly, a **mean value of the pharmacokinetic parameter** or **the distribution of the values** of the pharmacokinetic parameter, can be integrated in the equation. Table 10 of the report summarises all the situations and the report describes all the applications of the methodology, by varying the PK factor, then the PD, and then the two together.

b) Implementation of the methodology for the selected antibiotics

The methodology can be applied in two ways: either by searching for doses incorporating all the above criteria according to the indications sought (curative and/or metaphylactic treatment, germs targeted), or on the basis of the MA dose, and searching for the indications that may be claimed (according to the MICs of the targeted pathogen). For florfenicol in calves and in pigs, the doses calculated according to the PK/PD methodology were of the same order of magnitude as the doses from the MA, which was not the case for the other, older, antibiotics tested.

In general, most of the doses calculated according to the PK/PD methodology for tetracycline, oxytetracycline, doxycycline and amoxicillin in both species were higher than the doses from the MA, regardless of the targeted effect (bacteriostatic or bactericidal) or the targeted MIC.

The tetracycline doses were systematically higher in pigs due to lower bioavailabilities than in calves.

Taking the interindividual variability of the pharmacokinetic parameters into account logically led to calculated doses that were even higher than those obtained with the mean values of the pharmacokinetic parameters.

The dose calculations produced with the distribution of the apparent clearance values (Cl/F) derived from a pharmacokinetic population analysis gave very similar results to those obtained with the distribution established from the literature data; the report therefore concluded that the population analysis made only a limited contribution in the case of this exercise relating to older antibiotics.

For the antibiotics tested and the bacterium considered (*Pasteurella multocida*), the critical concentrations from the CA-SFM-Vet were systematically higher than the ECOFFs derived from the MIC distributions from EUCAST. As a result, given the relationship of proportionality between the dose and the MIC, the antibiotic doses are systematically higher for the critical concentrations from the CA-SFM-Vet than for the ECOFFs.
In addition, taking into account the MIC distributions with regard to *Pasteurella multocida* led to calculated doses that were generally lower than those obtained with the MIC point values (critical concentration from the CA-SFM-Vet or the ECOFF).

To conclude, the methodology integrates both the variability of pharmacokinetic origin and the MIC distribution of the pathogens.

5) Limitations of the method

The methodology developed above cannot be used when the antibiotic's efficacy cannot be linked to its level of plasma exposure, or when the MICs are not predictive of antibacterial activity, for example for intracellular pathogens or in a biofilm environment.

In addition, the MIC does not take into account other modes of action of certain antibiotics: anti-inflammatory and immunomodulatory activities.

Indeed, in spite of current doses sometimes being far lower than the calculated doses, reports of therapeutic failures remain infrequent in practice. This apparent contradiction could be related to field uses such as metaphylaxis that are more favourable to the antibiotic, and/or a high proportion of spontaneous cures (efficacy studies compared with a placebo are non-existent for older antibiotics), and/or the use in the field of doses that are already higher, but also the existence of effects other than antibacterial ones (immunomodulation, for example) that underlie the therapeutic efficacy of the antibiotics and cannot be taken into account by the PK/PD approach.

The PK/PD methodology used in this report is suited to determining doses that are effective against the bacterial populations targeted by the antibiotic therapy, but in the current state of knowledge it is unable to incorporate the control of dissemination of resistance factors in the environment, essentially *via* the intestinal microbiota. Dosage optimisation should therefore aim to limit exposure of the treated animals over time. The PK/PD approach provides no insight on the duration of the treatment. However an increase in the daily dose could warrant a reduction in the duration of treatment in a certain number of cases.

The clinical efficacy of the new dosages and, if applicable, the reduction in treatment duration, should nevertheless be confirmed by field data.

The variability in the intake of feed and drinking water, over time and according to the individuals, is also a factor that can influence the therapeutic dose when implementing a collective treatment.

The limitations and questions on administration via drinking water and/or milk are a major issue for the sectors concerned. In the context of an increase in doses, at the very least, studies should be conducted, or reviewed if they already exist, on the solubility and stability in stock solution while complying with the new dosage adopted.

6) Consequences of a revision of the dosages on animal health and public health

- **On animal tolerance**: if the revision of the dosage implies an increase in the dose, a re-assessment of the tolerance for the animal becomes necessary. It does not necessarily require new studies, however, if data on an overdose situation are available for the medicinal product concerned. Strengthening of the monitoring of animals treated with the new dosages should in any case be advocated.
- **On the environment**: an environmental risk assessment should be proposed for many older medicinal products by refining stage I (calculation of exposure) or even by going to stage II by providing experimental data to define the PNECs (predictable no effect concentrations) and probably also data for calculating a refined PEC (predictable environmental concentration). The PECs originally proposed by the standard scenarios can be high depending on the antibiotic and the dosage selected, leading to calculations of the RQ (Risk Quotient = PEC/PNEC) >1, indicating a potentially high risk to the environment. It would then be necessary to provide additional studies, firstly to refine the PEC, taking into account data on the degradation of antibiotics in livestock manure, and secondly to refine the PNEC through chronic toxicity studies.
- **On the consumer of foods of animal origin**: estimating the withdrawal period for new dosages (oral route) is possible from tissue or plasma data provided that they were supplied in the original dossier. In addition, the quality of these data must be sufficient to carry out modelling and simulations, and the linearity hypothesis must be verified. According to the calculation procedure used in the original dossier, a safety factor may be added to the time point chosen as the withdrawal period for the new dosage. However, if the quality of the data is insufficient, the value of the withdrawal period should be confirmed through an *in vivo* study.
- **On antibiotic resistance**: the methodology for revising the dosages of older antibiotics is based on a PK/PD approach that can integrate both pharmacokinetic (clearance, bioavailability) and pharmacodynamic variability (in terms of MIC) in the search for the optimal dose. This methodology can be used to select a dosage that guarantees, in the majority of animals treated, exposure of the target bacterial population to an effective antibiotic concentration, which is a positive element for limiting the selection of resistance. The current doses of "older" antibiotics generally provide a clinical benefit without this being optimised with regard to the risk of antibiotic resistance, whether it concerns the pathogenic bacteria targeted or the commensal microbiota.

7) Conclusions and recommendations

The efficacy indices (PK/PD indices) are central to the PK/PD methodology applied to antibiotics, whether in the area of human or animal antibiotic therapy, because they are required to be predictive of a high probability of therapeutic success, in potentially varying clinical situations. The WG worked with the PK/PD index values available in the literature, and obtained from *in vitro* or *in*

vivo models that were as relevant as possible with respect to the animal species (pigs, cattle), bacteria (*Pasteurella multocida*) and antibiotics (tetracyclines, amoxicillin, florfenicol) studied.

There were few available data however, and besides the issue of older antibiotics, major progress will be made in animal antibiotic therapy when these PK/PD indices (and their threshold values) are determined from controlled clinical trials performed in the target species.

The major advantage of the PK/PD approach is that it makes it possible, when determining the doses, to take into account the variability of the susceptibility of the pathogenic bacterial strains and the inter-animal variability of the pharmacokinetics of the antibiotics. The different options tested made it possible to reach a conclusion on the optimal options:

- for the pharmacokinetic component, **the interindividual variability of the processes of absorption and elimination can be taken into account** through a classic literature analysis, when available, without having to turn to analyses of population pharmacokinetics,
- for the pharmacodynamic component, the most rational approach involves including in the dose calculation the **MIC distributions** relating to the antibiotic/pathogenic bacterium combination.

Given the greater dispersion of the MIC values of the bacterial strains in comparison with the individual values of the pharmacokinetic parameters, it is **the susceptibility of the pathogens that has the greatest impact on the dispersion of the individual doses calculated.**

It is therefore fundamental to have databases (MIC distributions) that are as large and unbiased as possible.

Implementing the methodology therefore involves **collecting MICs that are representative** of the bacteria potentially targeted by the antibiotic in the different geographical areas, farming systems, etc. Assuming that the disparity of the MICs obtained in the different conditions could lead to very large differences in doses, proposed doses adapted to specific epidemiological situations could then be considered. Lastly, the identification of any change over time in the MICs of the pathogens should also lead to periodic dose re-assessments. It is also possible that the years of use of the older antibiotics have contributed to a gradual increase in the MICs to their current values, which is responsible for the large increase in doses calculated for these antibiotics.

The report explored the limitations of the PK/PD approach. Among these, the methodology developed cannot currently be used to propose **an optimal duration** for an antibiotic therapy. The increase in certain limits for daily doses could, however, be offset at least partially by **a decrease in the treatment durations**, if current durations allow, and if the clinical data confirm the efficacy of a reduced treatment duration.
In addition, the increase in individual doses could be offset by a **decrease in the number of animals treated**, through the generalisation of targeted intervention strategies, based on the stalls or pens occupied by the sick animals and the animals immediately around them, instead of treating a whole room or building.

The dosage calculation methodology does not directly take into account the component on exposure of the commensal microbiota, in particular digestive, known to be one of the main gateways for transmission to humans of risks of antibiotic resistance. The upward re-assessment of the daily doses of antibiotics could lead to an increase in the quantities of antibiotics consumed, which is unacceptable in the current context. For this reason, this re-assessment should be accompanied by measures to offset, or even reverse, its effect on consumption.

In the area of antibiotic therapy, these measures should propose:

- A decrease in the treatment durations, if current durations allow,

- A drastic change in the methods of therapeutic intervention in farming, by seeking to reduce the numbers of animals treated when infectious episodes occur (increasingly early detection and diagnosis, "targeting" of treated animals, etc.).

However, the previous proposals are part of a broader context of optimised animal health management, with the establishment of actions seeking to optimise farming conditions and the robustness/resilience of the animals when faced with disease, and to develop alternatives in the areas of prevention or therapy.

In addition, these steps to revise the dosages of older antibiotics should be accompanied by regulatory measures. The objective of the exercise should be to maintain the availability of the drugs and avoid these MAs falling out of use, which could ultimately have the effect of encouraging the use of antibiotics considered critical to human health. An improvement in data protection for the MA holders undertaking work on their older MAs is necessary.

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety endorses the conclusions and recommendations of the CES SABA and the Working Group on the "Methodology for revising the dosages of older antibiotics".

The French Agency for Food, Environmental and Occupational Health & Safety also makes the following recommendations:

- The methodology should be publicised and promoted among European medicinal product stakeholders (authorities and manufacturers),
- The establishment of multiple doses adapted to different indications (germs) and objectives (curative and/or metaphylactic) should be encouraged, rather than searching for the indications that can be treated with the current dose. Indeed, this second solution does not support a wider scope in terms of indications and objectives for the drug, but should be reserved for MA holders that do not carry out a revision of their older MAs,
- The highest dose should be regulated by establishing an appropriate withdrawal period,
- Controlled studies in the target species should be encouraged, to help define the PK/PD indices and their threshold values,
- Databases providing information on MIC distributions should be available,
- Support should be offered for regulatory developments to provide a period for protection of data on older veterinary medicinal products for the MA holders that revise their dosages.

Dr Roger GENET

KEYWORDS

Antibiotics, dosages, antibiotic resistance

Methodology for revising the dosages of older antibiotics

Internal request "2014-SA-0080 – MA: Revision of dosages"

Collective Expert Appraisal
REPORT

"Expert Committee on Animal Health and Welfare"

"Working Group on the Methodology for revising the dosages of older antibiotics"

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PREAMBLE: Outside experts, Expert Committee and WG members, or designated rapporteurs are all appointed in their personal capacity, *intuitu personae*, and do not represent their parent organisation.

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Mr Éric BOUSQUET – SIMV Member (VIRBAC pharmaceutical company)

Mr Bertrand RIDREMONT – SIMV Member (MSD Santé Animale pharmaceutical company)

CONTENTS

Acronyms and abbreviations

ACDkg: Animal Course Dose - dose required to treat one kg of body weight over the entire duration of treatment

MA: Marketing authorisation

ANMV: French Agency for Veterinary Medicinal Products

AUC_{24h}: Area under the curve of plasma levels measured over 24h

CES: ANSES Expert Committee

CA-SFM-Vet: Veterinary Committee of the French Microbiology Society's Antibiogram Committee

Cl: Clearance

CLSI: Clinical and Laboratory Standards Institute

Cmax: Maximum concentration

C_{mean}: Mean concentration

MIC: Minimum inhibitory concentration

CNRS: National Centre for Scientific Research

MPC: Mutant prevention concentration

CRD: Research and development agreement

CV: Coefficient of variation

CVMP: Committee for Veterinary Medicinal Products of the EMA

CD: Claimed dose

EC: European Community

EC_{50}: Efficacy concentration – modelled concentration for which effects are expected to be observed on 50% of the population of a species

ECOFF: Epidemiological cut-off – MIC threshold value beyond which persistent strains are considered to be resistant

EEC: European Economic Community

SD: Standard deviation

EMA: European Medicines Agency

EMEA: Europe Middle-East Africa

ENV: National Veterinary School

ERA: Environmental Risk Assessment

EUCAST: European Committee on Antimicrobial Susceptibility Testing

F: Bioavailability

MSW: Mutant selection window

fu: Free fraction of the antibiotic in plasma

GL: Guideline

WG: Working Group

HAS: French National Authority for Health

HPLC: High-performance liquid chromatography

IFREMER: French Research Institute for Exploitation of the Sea

IM: Intramuscular

INRA: National Institute for Agricultural Research

IV: Intravenous

Kg bw: Kilogram of body weight

MRL: Maximum residue limits

LOQ: Limit of quantification

ONIRIS: Nantes-Atlantique National Veterinary, Food and Nutrition School

PD: Pharmacodynamics

PEC: Predictable environmental concentration

PK: Pharmacokinetics

PNEC: Predictable no effect concentration

RESAPATH: French Surveillance Network for Antimicrobial Resistance in Pathogenic Bacteria of Animal Origin

SPC: Summary of Product Characteristics

RQ: Risk Quotient = PEC/PNEC

SABA: Animal Health and Welfare (ANSES CES)

SIMV: French Union for the Veterinary Medicinal Product and Reagent Industry

S/I/R: Susceptible/Intermediate/Resistant

T: Time

WP: Withdrawal period

UMR BIPAR: Joint research unit in molecular biology and parasite and fungal immunology

VAR: Variance

VICH: Veterinary International Conference on Harmonization

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1. Background, purpose and procedure for handling the request

1.1. Background

Measure 17 of the National EcoAntibio Plan emphasises the need to maintain older antibiotic compounds. At the same time, feedback from use in the field (primarily in food-producing animals), both in France and at European level, has shown that the dosages determined when the MAs were granted 30 or 40 years ago seem ill suited to certain bacterial populations encountered in the targeted diseases. In addition, knowledge of the pharmacokinetics and pharmacodynamics of antibiotics, and their relationships, has evolved, as has the way in which the risk of selection of resistance is taken into account in the choice of dosages.

Today, it is important to have doses that are effective for the purposes of animal health, but that also help limit the selection of resistant bacteria, for public health purposes. This has led to a need to reassess these older compounds on the basis of a benefit-risk ratio that encompasses their efficacy, animal tolerance and consumer safety, but also their impact on commensal flora and the environment.

1.2. Purpose of the request

After selecting the candidate compounds for revision, the first part of the expert appraisal involved analysing the literature data relating to the pharmacokinetic profiles of older antibiotic compounds. It should be noted that the older MA dossiers provide little information, and the work was essentially based on scientific publications.

For the antibiotics that were sufficiently documented, and after defining the objectives to be achieved depending on the therapeutic indications and the pathogens' levels of susceptibility, the objective was to propose a methodology for reassessment based on the pharmacokinetic/pharmacodynamic (PK/PD) approach applied to the antibiotics. The major advantage of this approach is that, when determining the doses, it can take into account the variability of the susceptibility of the pathogen strains, and the inter-animal and inter-species variability of the pharmacokinetics of the antibiotics.

Distributing antibiotics by means of collective feed or watering systems (collective oral routes) is a source of additional variability, leading to a dispersion of the doses actually ingested by the animals. This variability was not taken into account at this stage in the dose calculations but is addressed in the discussion.

The methodology for reassessing the dosages will be presented to the European bodies. Revision of the dosages by species, type of infection and antibiotic should be carried out at the European level.

1.3. Procedure: means implemented and organisation

ANSES entrusted examination of this request to the Working Group (WG) on the "Methodology for revising the dosages of older antibiotics", reporting to the Expert Committee (CES) on "Animal Health and Welfare". This WG met seven times between November 2014 and October 2016; a hearing with the SIMV, as the leader of Measure 17 of the National EcoAntibio Plan, took place at the meeting of 6 May 2015.

The methodological and scientific aspects of this group's work were submitted to the CES. The report issued by the WG takes into account the comments and additional information provided by the members of the CES.

This work was therefore conducted by a group of experts with complementary skills.

The expert appraisal was carried out in accordance with French Standard NF X 50-110 "Quality in Expert Appraisals – General Requirements of Competence for Expert Appraisals (May 2003)".

1.4. Prevention of risks of conflicts of interest

ANSES analyses the links of interest declared by the experts prior to their appointment and throughout the work, in order to avoid potential conflicts of interest with regard to the matters dealt with as part of the expert appraisal.

The experts' declarations of interests are made public *via* the ANSES website (www.anses.fr).

2. Preliminary considerations when determining a dose

The aim of a revising dosages is to contribute to optimising the antibacterial efficacy of older antibiotics for their use in the field, while minimising the risk of selection of antibiotic-resistant bacteria.

The steps described in this report for establishing the optimisation methods are:

- Definition of a methodology for determining the effective doses.
- Application of this methodology to an investigative scope that is intentionally limited to a few antibiotics, selected to treat a single type of bacterial disease, and arbitrarily choosing a single causal agent to facilitate comparisons.
- Comparison of these dose calculations to the current MA doses, specifically studying the consequences of possible dose increases on the elements of the MA dossier, in addition to the wider consequences in terms of antibiotic resistance and prudent use of antibiotics.

It is important to mention at this point that, given the investigative framework that was defined and intentionally restricted in terms of bacterial disease, causal agent and antibiotic, this work was not intended to determine doses that would be directly applicable in the field.

2.1. History of the dosages

In view of the regulatory developments that have marked these last few decades and certain representative dossiers, different periods can be characterised in the history of the dosages in veterinary antibiotic therapy.

To begin with, the first period from 1979 to 1990:

The first MAs for veterinary medicinal products appeared in France in 1979, in application of the Act No. 75-409 and its implementing decrees of 1977 legislating on the requirement for an MA for each drug.

A large number of dossiers were then submitted and many MAs were issued during the 1980s. This was the case with many injectable antibiotics that are still currently in use: oxytetracycline, tylosin and amoxicillin for example, which were authorised from 1979-1980; it was necessary to wait until 1983 for gentamicin, 1984 for tiamulin, 1985 for doxycycline and neomycin, …

Most of the products were already on the market before 1975 and the pharmaco-toxicological and clinical expert appraisals contained in the MA dossiers were then often performed using bibliographical documents. Sometimes, in-house studies carried out with the pharmaceutical speciality of the laboratory were provided in addition (for example, bioavailability tests).

From the review of these initial dossiers, it seems that historically, the dosage of an antibiotic was established empirically. Experimental studies (in laboratory animals and/or in the target species) and clinical experience helped select the no observed adverse effect dose for which a therapeutic response was visible (concept of therapeutic index). The effect was measured on the basis of the favourable evolution of the clinical signs (sometimes substantiated by bacteriological results); using clinical and bacteriological criteria to assess the efficacy of antibiotics was introduced later. Several routes of administration were sometimes tested at the outset (for example, tests of amoxicillin on different infection models in mice, by the oral, subcutaneous and intraperitoneal routes). The dosage was adjusted according to the situations and field conditions (practicality, disorders encountered, severity of the clinical signs, *etc*.): double dose *versus* single dose, administration every 6, 12, or 24 hours, number of days of treatment.

In 1951, Cromley published a study (Sutherland *et al.*, 1975) on the use of oxytetracycline in animals, contained in the dossier for the first commercial product based on oxytetracycline marketed in France. The first authorised products based on oxytetracycline were injectable products (oxytetracycline is today authorised for various routes of administration). This 1951 publication describes the different possible uses of oxytetracycline in cattle, as well as in dogs and pigs, based on different clinical cases. The authors describe how, prior to conducting field trials, the intravenous dose was established experimentally by testing a single dose, which proved effective at the outset. Each clinical case described in cattle was represented by just one animal. In total, for the pneumonia indication, six clinical cases were described, and for the mastitis indication, two clinical cases. This article is a good example of the first dossiers submitted in support of an MA application with few clinical cases, or even isolated cases, and without any control, bacteriological diagnosis or, in some cases, objective clinical criteria.

Directive 81/852/EEC of 1981 (on the approximation of the laws of the Member States relating to analytical, pharmaco-toxicological and clinical standards and protocols in respect of the testing of veterinary medicinal products) was part of this context of granting MAs across Europe. It provides for the conducting of toxicological and pharmacological tests and clinical trials to *"demonstrate or to ascertain the therapeutic effect of the medicinal product, to specify its indications and contra-indications according to species, age, its directions for use, any side effects which it may have and its safety under normal condition of use".* The concept of determining the optimal dosage is not mentioned.

To conclude this 1st period: although this new directive provided that, "as far as possible",

clinical trials were to be carried out with control animals (concept of "controlled trials" to "compare the (therapeutic) effect obtained both with a 'placebo' and with absence of treatment and/or with the effect of a medicinal product of known therapeutic value which has already been used"), most of the trials were characterised by a failure to take statistical considerations into account (small number of animals included, absence of a statistical hypothesis, absence of randomisation, etc.). Furthermore, many aetiological uncertainties remained in the trials presented (in some cases, they related rather to digestive or respiratory tract syndromes, for example, without systematic identification of the pathogens). There was rarely any follow-up concerning relapse.

Then the second period from 1991 to 2000:

This second period began with the adoption of the first guideline on antimicrobials in 1991. The dosage (dose and duration of treatment) proposed by the MA applicant for any product containing an antibiotic henceforth had to be based on dose determination studies, PK data and clinical trials. The previous Directive 81/852/EEC had already introduced this need for sufficient toxicological and pharmacological tests prior to the establishment of clinical trials. The new guideline specific to antimicrobials retained this integrative approach, namely, selection of a dose through PK data, PD data if possible acquired using an experimental infection model in the target animal (which is not always possible), and confirmation of the dose by means of clinical trials (experimental or, more often in current practice, "field trials"). The clinical trials therefore confirmed the therapeutic regimen proposed by the applicant (validation of the duration of treatment and the rate of administration). In addition, they had to be able to justify each indication (defined by the type of infection and the germ(s) targeted). Thus, the guideline provided for as many clinical trials as there were indications. The applicant also had to justify the number of animals needed to demonstrate the efficacy of its product and the appropriate control product, if applicable.

For example, tilmicosin, enrofloxacin and florfenicol were registered during this period (1991 for enrofloxacin by the oral route and 1996 for the solution for injection, 1994 for tilmicosin and florfenicol, both in solution for injection). Difloxacin obtained a centralised MA in 1998 and valnemulin in 1999.

To conclude this 2nd period: the requirements therefore increased progressively in terms of data to be provided but no recommendation was given on how to conduct the various studies to be carried out (no details for example on the dose determination or the assessment of the results of the field trials).

Then, the third period from 2001 to 2015:

This third period was initiated by Directive 2001/82/EC and by the adoption of the new EMEA/CVMP/627/01 guideline on antibiotics at the end of 2002.

The requirements regarding preclinical and clinical trials increased sharply between the first guideline on antimicrobials in 1991 and that of 2002, both to take account of scientific advances, and to increase the level of evidence for authorising the use of the product (issuing of an MA).

The recently authorised antibiotics, as well as older compounds for which extensions of indication or animal species had been tested and validated, met the requirements of this 2002 guideline. For

example, this was the case with florfenicol in sheep (see the public assessment report for Nuflor 300 mg/ml solution for injection for cattle and sheep of 2011). This medicinal product obtained an extension of species (addition of sheep) with the indication "treatment of respiratory tract infections due to *Mannheimia haemolytica* and *Pasteurella multocida* susceptible to florfenicol". The applicant provided detailed PK and PD data to justify the dosage, as well as a dose determination study (*Mannheimia haemolytica* respiratory infection model) that confirmed the PK/PD findings. The efficacy and tolerance were then confirmed in a multicentre field trial (non-inferiority trial *versus* oxytetracycline).

To conclude this 3rd period: Recommendations were given on how to conduct the various studies to be provided. The use of appropriate statistical methods was suggested and reference was made to the first statistics guide applied to veterinary clinical trials. Demonstration of the bacteriological cure was recommended whenever possible (for certain indications). The PK/PD analysis was considered for the first time as an aid to selecting doses and administration rates.

Finally, in January 2016, the first revision of the previous guideline (EMEA/CVMP/627/01) on antibiotics was adopted (EMEA/CVMP/627/2001-Rev.1); it developed the part on the PK/PD analysis and the models. As it gives the option of substituting certain dose determination studies by robust PK/PD data, this new guideline should help decrease the number of studies needed for determining doses. To date, no medicinal product has yet been registered in application of this revised guideline.

2.2. Contribution of PK/PD relationships in dose determination

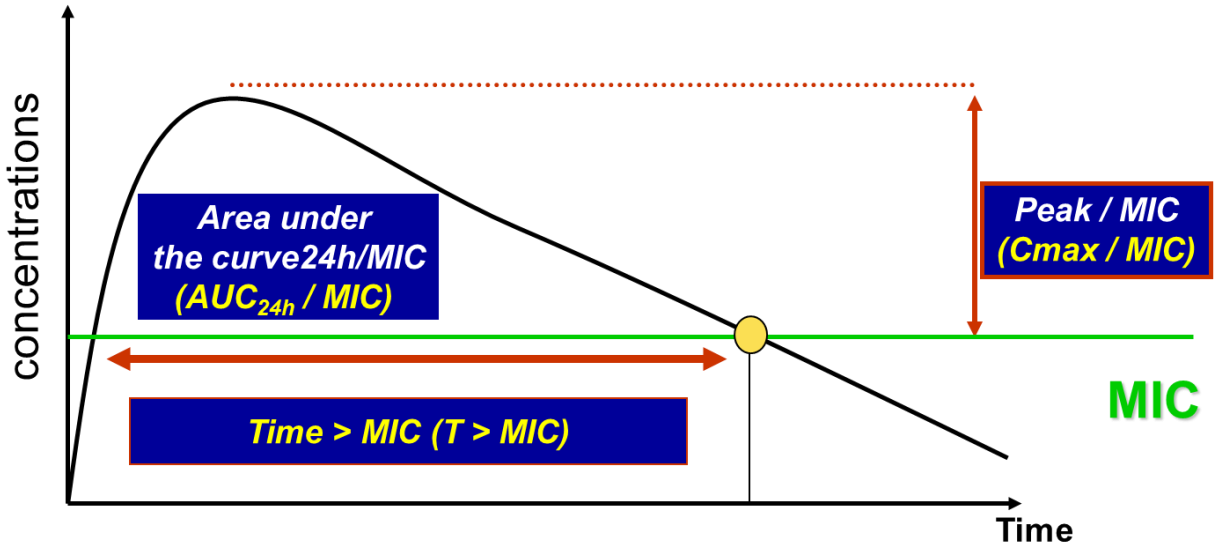
2.2.1. PK/PD indices in antibiotic therapy

The currently recognised methodology for the rational determination of an antibiotic dosage is an integrated approach that combines the pharmacokinetic and pharmacodynamic properties of the therapeutic agents, known as the pharmacokinetic/pharmacodynamic or PK/PD approach (EMEA/CVMP/627/2001-Rev.1). It involves introducing mechanistic information relating to the pharmacokinetics of the antibiotic (the result of the body's action on the antibiotic) and its pharmacodynamics (its effects), in the dosage selection process.

In the case of antibiotics, the PK/PD approach has led to "efficacy indices" or "PK/PD indices" being proposed as criteria for predicting the efficacy of treatments. These PK/PD indices reflect the characteristics of the pharmacodynamics of the antibiotics and the resulting concentration-effect relationships. The main PK/PD indices used in antibiotic therapy are: *Time>MIC*, *Cmax/MIC* and AUC_{24h}/MIC. They combine information of a pharmacokinetic nature (a parameter of internal exposure) and information of a pharmacodynamic nature, the minimum inhibitory concentration (MIC) for the pathogen in question.

These three main indicators of efficacy used in antibiotic therapy are presented in Figure 1. The pharmacokinetic parameters describe exposure in terms of duration (the time during which the plasma concentrations are above a threshold) or intensity (the peak plasma concentration known as Cmax, the area under the plasma concentration-time curve known as AUC_{24h} and measured over 24 hours). These three indices (*Time>MIC*, *Cmax/MIC* and AUC_{24h}/MIC) therefore correspond to a standardisation of plasma exposure to the antibiotic, expressed relative to the MIC, used as an indicator of the pathogen's susceptibility to the antibiotic tested.

Figure 1: The PK/PD indices for antibiotics



2.2.2. PK/PD indices and clinical efficacy

For human antibiotic therapy, the correlations between the PK/PD indices and the clinical efficacy of different classes of antibiotics have been determined from experimental infection models developed in rodents (rats, mice). Clinical studies in humans, whether prospective or retrospective, have helped quantify their levels of correlation with efficacy (clinical, microbiological) and propose threshold values (or critical values) for these indices associated with high cure probabilities (> 80-90%).

For the beta-lactams, which are qualified as time-dependent antibiotics, values have been proposed for the *Time>MIC* equal to at least 40% of the interval between two administrations for Gram-positive pathogenic bacteria, and 80% for Gram-negative pathogenic bacteria.

For the aminoglycosides, which are qualified as concentration-dependent, the *Cmax/MIC* index is most strongly correlated with efficacy, with a threshold value of 10-12.

For the quinolones, which are concentration-dependent for Gram-negative bacteria, the AUC_{24h}/MIC index has proved to be the most predictive of efficacy, with threshold values of the order of 100-125 hours, which correspond to mean concentrations over 24 hours equal to 4-5 times the MIC (i.e. 100-125/24).

Many studies carried out subsequently have shown, for the classes of antibiotics concerned, the existence of significant variations in the critical value (i.e. associated with a high probability of success) of the AUC_{24h}/MIC ratio according to the clinical characteristics of the infection (neutropenic or non-immunocompromised animals or patients) and the stage of the infection at the time of treatment (early or late).

Conversely, all other factors being equal (antibiotic-bacterium combination, type and stage of infection, immunity of the host), the threshold values of the PK/PD indices are relatively similar between the species (laboratory animals, domestic species, humans).

This means that it is possible to extrapolate therapeutic regimens to the minor species, for the same type of infection and pathogen, on the basis of the pathogen's level of susceptibility (MIC) and the pharmacokinetic characteristics of the antibiotic in the species in question.

Since the inter-species differences are primarily apparent at the pharmacokinetics stage, it will be necessary, to reach the same PK/PD index value, for example, to increase the dose for species that eliminate the antibiotic to a high degree or to decrease it for species that eliminate it only slightly. It should be noted that these indices are expressed for free concentrations of the antibiotic in plasma, and corrections will be needed in the event of inter-species variability in the binding to plasma proteins.

More recently, mathematical physiological models (taking the dynamics of bacterial growth into account) explored *in silico* the correlations between the PK/PD indices and antibacterial activity for the beta-lactams. The data obtained provide a perspective on the historical results obtained from murine experimental infection models. Indeed, the work on benzylpenicillin (Nielsen *et al.*, 2011) and more recently on meropenem (Kristoffersson *et al.*, 2016) has shown that when the half-life of the antibiotic is longer, the AUC_{24h}/MIC index is at least as effective as the *Time>MIC* index for predicting antibacterial activity. More specifically, the models confirm that the *Time>MIC* index is more efficient when the half-lives are of the order of half an hour (30 minutes), i.e. those obtained in rodents, whereas the best results with the AUC_{24h}/MIC index are obtained when the simulations are carried out with longer half-lives, such as those observed in humans (1.5-3.5 hours).

Table 1: Classification of antibiotic classes according to their method of bacterial eradication and PK/PD indices correlated to efficacy

Typical actions	Chemical groups	Examples of drugs	PK/PD indices correlated to efficacy
Eradication concentration-dependent, usually with a strong post-antibiotic effect	Fluoroquinolones	Enrofloxacin, Danofloxacin, Marbofloxacin, Difloxacin, Ibafloxacin	AUC/MIC C_{max}/MIC
	Aminoglycosides	Streptomycin, Neomycin, Gentamicin, Amikacin, Tobramycin	C_{max}/MIC
	Nitroimidazoles	Metronidazole	AUC/MIC C_{max}/MIC
	Polymyxins	Colistin	AUC/MIC
Eradication time-dependent with a limited or zero post-antibiotic effect	Penicillins	Benzylpenicillin, Cloxacillin, Ampicillin, Amoxicillin, Carbenicillin	T>MIC AUC/MIC *
	Cephalosporins	Ceftiofur, Cefalexin, Cefapirin	T>MIC
	Macrolides and Triamilides	Aivilosin, Tylosin, Erythromycin, Tilmicosin, Tulathromycin	T>MIC
	Lincosamides	Clindamycin	T>MIC
	Phenicols	Chloramphenicol, Florfenicol	T>MIC AUC/MIC *
	Sulfonamides	Sulfadoxine, Sulfadiazine	T>MIC
	Diaminopyrimidines	Trimethoprim	T>MIC
Eradication dependent on both the duration of exposure and the level of concentration	Tetracyclines	Oxytetracycline, Chlortetracycline, Doxycycline	AUC/MIC
	Ketolides	Azithromycin, Clarithromycin	AUC/MIC
	Glycopeptides	Vancomycin	AUC/MIC

Amended table from Lees *et al.* (2006). The indices with an asterisk * were proposed after publication of the table (see below).

Comments:

- The three PK/PD indices are correlated in that Cmax/MIC describes an intensity, T>MIC describes a duration, and AUC/MIC is a combination of intensity/duration.
- For each action type, the studies compared the relationship between efficacy and each of the three PK/PD indices.

The three PK/PD indices are always tested because it is easy to calculate them from the pharmacokinetic data. Depending on the compound, within the same class, AUC or C_{max} may perform better. In addition to the work on the beta-lactams, studies subsequent to the publication of Table 1 have established that the AUC_{24h}/MIC index could also be used for time-dependent antibiotics from the class of phenicols (Manning *et al.*, 2011; Maaland *et al.*, 2015).

2.2.3. PK/PD indices and prevention of resistance

Within a bacterial population susceptible to an antibiotic, resistant clones can appear continuously following a spontaneous mutation on the bacterial genome. In the absence of selection pressure related to an antibiotic treatment, these resistant mutants generally remain largely in the minority within the inoculum. Selection is exercised when the antibiotic concentrations reduce the wild majority population while at the same time not affecting the mutant sub-population.

The description of this phenomenon for the fluoroquinolones led to the concepts of mutant prevention concentration (MPC) and mutant selection window (MSW), which are illustrated by Figure 2 and described below. This phenomenon is applicable to antibiotics in which resistance appears by mutation. The fluoroquinolones are a typical example in veterinary medicine.

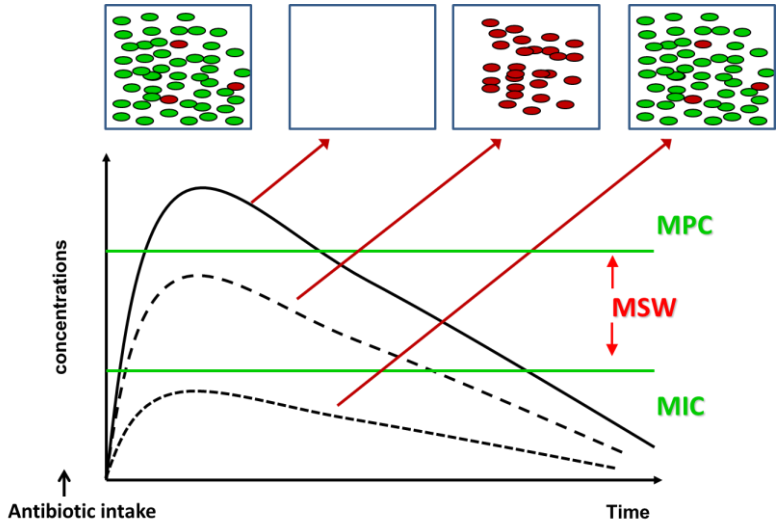


Figure 2: Concept of resistant mutant selection window from Canton and Morosini (2011)

A large enough population (10^8-10^{10} bacteria) contains very small minorities of resistant mutant sub-populations (square on the left: majority susceptible bacteria in green and minority resistant mutants in red). When the antibiotic concentrations are below the threshold of susceptibility (MIC) of the majority wild population, there is no selection (square on the right). When the concentrations progress above the MIC of the wild population and below the threshold of susceptibility of the resistant mutants (MPC: mutant prevention concentration), the latter are selected (square with red bacteria only). In this simplified ideal diagram, the concentrations of the antibiotic need to be higher than the MPC (at the threshold of susceptibility of the least susceptible sub-populations) to obtain a reduction in the bacterial load enabling the host's immune defences to eliminate all the remaining bacteria (empty square).

When the antibiotic reaches concentrations that eliminate the majority wild population within a bacterial population containing a resistant sub-population, the only way to avoid selection of the latter is to obtain concentrations capable of eliminating the mutants, i.e. higher than the MPC (mutant prevention concentration). As it is inevitable that the concentrations of the antibiotic will "cross" the mutant selection window during the course of its elimination phase, it is crucial that the intensity and/or duration of exposure above the MPC are sufficiently strong and early to rapidly eliminate the mutants.

The concept of selection window was originally developed around mechanisms of resistance generated by spontaneous mutations of the bacterial genome and transmitted vertically to successive generations. However, the concept may also be applied to mechanisms of resistance involving the horizontal transfer of resistance genes (Canton and Morosini, 2011). The concept of

selection window can be applied when a bacterial population is heterogeneous in terms of susceptibility to the antibiotic, i.e. a susceptible majority sub-population and one or more minority sub-populations with lower levels of susceptibility coexist within this population.

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

Prevention of resistance among the pathogenic bacteria targeted by the antibiotic therapy goes hand in hand with the therapeutic objective, because the focus is on the same bacterial species located in the same biophase (the infectious site). This is why the PK/PD indices described previously are used to predict both the efficacy and the prevention of resistance. Studies on animal models, confirmed by clinical trials in humans, have shown that the values of the PK/PD indices must be higher when the objective is to maximise prevention of resistance. This amounts to saying that faced with high bacterial loads, higher doses that lead to concentrations well above the MIC of the majority wild population are needed to reduce the probability of selection of resistance in the pathogenic bacteria to be eradicated (Figure 2).

2.3. **Link between exposure and antibiotic resistance**

Exposing an inoculum of pathogenic bacteria and/or commensal bacteria constituting the microbiota to anti-infectious compounds can lead to the selection of bacteria possessing one or more mechanisms of resistance.

On the basis of their genetic support (chromosome, plasmid for the largest), these mechanisms can disseminate with different degrees of efficiency within bacterial ecosystems. The intestinal microbiota is the main source of amplification and dissemination in the environment, *via* faecal elimination of resistant bacteria and antibiotic resistance genes, as well as the site of subsequent transmission of these genes to pathogenic bacteria, whether or not they are enteric pathogens, during their passage through the digestive tract.

In addition to purely veterinary concerns about the pathogens, bacteria involved in zoonotic infections may be concerned by this issue of exposure to antibiotics and antibiotic resistance. This is the case with asymptomatic carriage of zoonotic bacteria in animals treated for a concomitant disease.

Many antibiotics, some of which are considered critical for human medicine, such as beta-lactams, fluoroquinolones and even macrolides, have a varying degree of intestinal elimination that is responsible for collateral selection pressure on the host's intestinal microbiota. Thus, the use of an antibiotic to treat bacterial infections will also, in an indirect and non-controlled manner, impact the main microbiota, that of the intestinal flora. It is therefore important to consider both the impact of the antibiotic therapy on the pathogen, in order to ensure therapeutic success, and its impact on the commensal microbiota.

Many studies have shown a link between the level of exposure of the intestinal tract and the dynamics of amplification of resistance, in particular within the population of faecal Enterobacteriaceae in treated animals (ANSES 2014 report on Antimicrobial Resistance). A practical example of the final selection of resistant strains in the intestinal microbiota is presented in the RESAPATH report of 2014. In this example, it is shown that the use of florfenicol in cattle, primarily for treating respiratory infections, leads to an increase in florfenicol-resistant strains in the *E. coli* population of digestive origin. Antibiotic usage is therefore never innocuous in terms of selection of bacterial resistance. The impact on the intestinal microbiota may, however, differ depending on the class of the antibiotic, the route of administration used (oral or injectable), the kinetics of exposure of the distal segments of the intestinal tract and the level of susceptibility of the bacterial populations concerned.

The main challenge therefore is still to control the dissemination of resistance factors in the environment, mainly *via* the intestinal microbiota. Dosage optimisation should therefore aim for lower exposure of the treated animals over time.

The PK/PD methodology used in this report is suited to determining doses that are effective against the bacterial populations targeted by the antibiotic therapy, but in the current state of knowledge it is unable to simultaneously incorporate collateral impacts such as previously defined.

Given the issues related to the ecology of antibiotic resistance, and in particular the central role of the intestinal microbiota in the amplification and circulation of resistance, it is important to rationalise this aspect when choosing an antibiotic and a route of administration. Narrow-spectrum antibiotics with low intestinal elimination, and with high bioavailability when the oral route is required, should therefore be preferred to broad-spectrum antibiotics with high intestinal elimination.

3. Scope and choice of compounds of interest

3.1. Selected species

The species selected following the Working Group's deliberations were cattle and pigs. They are major food-producing species that seem to be among those most exposed to antibiotic therapy. For non-weaned pigs and cattle, use of the oral route with collective distribution is common, and is characterised by variability in the individual doses ingested and incomplete bioavailabilities resulting in increased exposure of the digestive flora.

Having made this choice, it should not be forgotten that this is a problem that affects all species, with constraints in the use of antibiotic therapy not necessarily being identical.

For food-producing species, efficacy, cost of treatment, withdrawal period and ease of implementation are factors with varying degrees of influence on the use of antibiotic therapy.

For species of pets, or sport or leisure animals, a number of characteristics distinguishing them from food-producing species should be emphasised:

- the proximity and multiplicity of contacts between humans and animals, which favour the sharing of bacterial ecosystems within the general population;
- the importance of the emotional valence, which makes owners more ready to seek treatments in general, even for chronic or recurrent conditions: owners are above all concerned with the welfare of their animals to the potential detriment of collective issues such as antibiotic resistance;
- use of the oral route is common, but the practice of collective treatment is exceptional.

3.2. Choice of indications and compounds

In both selected species, bacterial respiratory and digestive disorders are the two main reasons for the use of antibiotic therapy. Regarding the digestive sphere, the non-absorbed fraction of antibiotics administered orally has a local action whose efficacy cannot be predicted by the PK/PD approach based on plasma concentrations. For this reason, only respiratory disorders have been selected.

Among the aetiological agents responsible for these disorders, we decided to select *Pasteurella multocida*, a pathogenic bacteria in both animal species, even though it is not the major pathogen

in swine diseases. The main criterion for this choice was the availability in the literature of MIC distributions of the selected antibiotics with regard to this bacterial species. Thus, the doses established in this report according to the PK/PD approach should not be extrapolated to other pathogens. Respiratory disorders have a relatively broad therapeutic arsenal, including both older and more recent compounds, common to both species. The five selected antibiotics are:

- three tetracyclines: tetracycline, oxytetracycline, doxycycline,
- amoxicillin, and
- florfenicol.

To consolidate this choice, the antibiotics sales and exposure data were analysed.

Monitoring of sales of antibiotics in veterinary medicine at national level is based on the annual compilation of sales data collected from pharmaceutical companies (MA owners or distributors). This macroscopic monitoring cannot distinguish between the uses of antibiotics according to the indications or production stages, but can be used to identify the most widely used classes.

A classification of the most commonly sold antibiotics (in tonnage of active ingredient), taking into account only the oral routes of administration in order to limit the scope, is presented in the following table.

Table 2: The most commonly sold oral antibiotics in terms of tonnage in 2015 (oral route: medicated premixes, oral powders, oral solutions, tablets, etc.)

	ACTIVE INGREDIENT	TONNAGE OF ACTIVE INGREDIENT SOLD	PERCENTAGE OF TOTAL TONNAGE
1	**OXYTETRACYCLINE**	**117.84**	**29.26%**
2	SULFADIAZINE	42.33	10.51%
3	CHLORTETRACYCLINE	38.52	9.56%
4	SULFADIMETHOXINE	35.08	8.71%
5	COLISTIN	28.66	7.12%
6	**AMOXICILLIN**	**22.47**	**5.58%**
7	TYLOSIN	17.26	4.29%
8	TRIMETHOPRIM	13.70	3.40%
9	**DOXYCYCLINE**	**13.05**	**3.24%**
	...		
37	**FLORFENICOL**	**0.15**	**0.02%**

The tonnage of active ingredients sold or used is the indicator commonly used. However, exposure to antibiotics can be assessed by other more relevant indicators. Indeed, to take account of the diversity of antibacterial activities of the antibiotics and therefore the dosages (dose in mg/kg, frequency and duration of administration), composite indicators (in particular the number of ACDkg) have been developed to enable a comparison in terms of exposure to different classes. The ACDkg (Animal Course Dose) is the dose required to treat one kilogram of body weight over the entire duration of treatment. The number of ACDkg is calculated by dividing the quantity of active ingredient by the value selected for the ACDkg (daily dosage and duration of treatment from the MA according to the national monitoring of antibiotic sales). The number of ACDkg estimates the quantity of body weight treated by each active ingredient. A classification of the most

commonly used antibiotics (in tonnage of body weight treated) for the oral route is presented in the table below.

Table 3: The most commonly sold oral antibiotics in terms of body weight treated (number of ACDkg) in 2015 (oral route: medicated premixes, oral powders, oral solutions, tablets, etc.)

	ACTIVE INGREDIENT	TONNAGE OF BODY WEIGHT TREATED (NUMBER OF ACDKG)	CORRESPONDING PERCENTAGE
1	COLISTIN	1,185,932	32.93%
2	**OXYTETRACYCLINE**	**759,612**	**21.10%**
3	**AMOXICILLIN**	**278,969**	**7.75%**
4	**DOXYCYCLINE**	**251,845**	**6.99%**
5	SULFADIAZINE	235,962	6.55%
	...		
34	**FLORFENICOL**	**3013**	**0.08%**

It appears that oxytetracycline, amoxicillin and doxycycline are among the five most frequently used antibiotics in oral form.

In addition, these antibiotics are among the oldest authorised. Indeed, regarding antibiotics administered by the oral route for the pig sector, doxycycline was first authorised in 1985 (RONAXAN PS 5%), amoxicillin in 1989 (AXILLIN, SURAMOX, 10% ORAL POWDER) and oxytetracycline in 1989 (NEOXYNE and CONCENTRAT VO).

Florfenicol is used far less and accounts for only 0.08% of the tonnage of body weight treated by oral powders and solutions. However, including in the scientific analysis a more recent antibiotic with a therapeutic regimen harmonised at European level helps strengthen the methodology proposed in this report. This is one of the reasons why florfenicol was selected.

4. Review of the literature search and analysis

Fifty scientific articles from international peer-reviewed journals and two reports from industry were collected and analysed in order to document the pharmacokinetic parameters of the five antibiotics in the two selected species. The details on the number of scientific articles and reports from industry by species and by antibiotic are given in Table 33 in the Annex.

The year of publication of the articles was noted in order to assess the confidence that could be attributed to the data collected, related to the progress over the years of performance in analytical techniques. The publication dates are spread over more than 40 years. The breakdown of these articles according to their year of publication is given in Table 34 in the Annex.

In the selected articles or reports, the antibiotics were administered by the oral or intravenous route. Collection of data obtained for both routes of administration (the route of interest: oral, and the reference route: intravenous) was essential for calculating certain parameters, including clearance, and for consolidating the data collected. The doses administered and the route of administration (intravenous or oral) in the different studies are listed in Table 4 (data for calves) and in Table 5 (data for pigs).

Table 4: Routes of administration and doses of antibiotics used in calves

Intravenous route		**Oral route**	
Articles/Reports	**Dose (mg/kg)**	**Articles/Reports**	**Dose (mg/kg)**
Tetracycline			
Ziv and Sulman (1974) *Rodrigues et al. (2001)*	20 10	-	-
Oxytetracycline			
Pilloud (1973) *Ziv and Sulman (1974)* *Schifferli et al. (1982)* *Ames et al. (1983)* *Nouws and Vree (1983)* *Nouws et al. (1983)* *Toutain and Raynaud (1983)* *Xia et al. (1983a)* *Nouws et al. (1985)* *Mevius et al. (1986a)* *Burrows et al. (1987)* *Ucelli et al. (1988)* *Sanders and Guillot (1990)* *Meijer et al. (1993a)* *Errecalde et al. (1997)* *Kumar and Malik (1998)* *Singh et al. (1998)* *Kumar and Malik (1999)* *Kumar and Malik (2001)*	2.5 20 10 11 17 03/7/17 10/20 10 5 5 10 5 10 40 20 20 10 20 20	*Schifferli et al. (1982)*	50
Doxycycline			
Riond et al. (1989) *Meijer et al. (1993b)* *Vargas et al. (2008)*	20 5 10	*Meijer et al. (1993b)*	10
Amoxicillin			
-	-	*Soback et al. (1987)*	10/20
Florfenicol			
Varma et al. (1986) *Adams et al. (1987)* *Bretzlaff et al. (1987)* *de Craene et al. (1997)*	22 11 50 20	*Varma et al. (1986)* *Adams et al. (1987)*	22 11 (/12h)

Table 5: Routes of administration and doses of antibiotics used in pigs

Intravenous route		**Oral route**	
Articles/Reports	**Dose (mg/kg)**	**Articles/Reports**	**Dose (mg/kg)**
Tetracycline			
Kniffen et al. (1989) *Nielsen and Gyrd-Hansen (1996)*	11 10	*Kniffen et al. (1989)* *Nielsen and Gyrd-Hansen (1996)*	22 45
Oxytetracycline			
Xia et al. (1983b) *Mevius et al. (1986b)* *Pijpers et al. (1990)* *Pijpers et al. (1991)* *Nielsen and Gyrd-Hansen (1996)*	20 20 10/50 10 10	*Mevius et al. (1986b)* *Pijpers et al. (1991)* *Nielsen and Gyrd-Hansen (1996)*	20/30 50 45
Doxycycline			
Dossier 9601 (1985) *Riond and Riviere (1990)* *Dossier Veprol (1995)* *Yang et al. (2012)* *Gutiérrez et al. (2013)* *del Castillo et al. (2014)*	5/10 20 5 10 20 10	*Dossier 9601 (1985)* *Dossier Veprol (1995)* *Bousquet et al. (1998)* *Prats et al. (2005)* *Goossens et al. (2012)* *Gutiérrez et al. (2013)* *del Castillo et al. (2014)*	5/10 2.7/4 6 10 10 20 10
Amoxicillin			
Agersø and Friis (1998a) *Agersø and Friis (1998b)* *Martínez-Larrañaga et al. (2004)* *Hernandez et al. (2005)* *Reyns et al. (2008)* *Godoy et al. (2010)*	9 9 20 15 20 15	*Agersø and Friis (1998a)* *Martínez-Larrañaga et al. (2004)* *Hernandez et al. (2005)* *Reyns et al. (2008)* *Godoy et al. (2010)* *Krasucka and Kowalski (2010)*	10 20 15 20 5/9/10/15/18 28
Florfenicol			
Liu et al. (2003)	20	*Voorspoels et al. (1999)* *Liu et al. (2003)*	15 20

Depending on the scientific articles, the plasma concentrations were determined either by microbiological method (diffusion in agar medium), spectrofluorimetry or HPLC (Table 35 in the Annex). Microbiological methods usually have higher limits of quantification (LOQ) than those of spectrofluorimetry or HPLC methods. No information was available to us on the analytical method used to determine plasma concentrations in the dossiers from industry.

The pharmacokinetic parameters extracted from this literature review were clearance, "apparent" clearance (Clearance/F) and bioavailability (F) of the oral route. The numbers of animals of each species, for each pharmacokinetic parameter of each antibiotic, are presented in Table 36 and Table 37 in the Annex.

The values of the pharmacokinetic parameters clearance, clearance/F and bioavailability were:
- either directly extracted from the articles or reports,
- or calculated:
  - → from other available pharmacokinetic parameters,
  - → from a pharmacokinetic analysis of the plasma concentration profiles (individual or mean), when these were available.

Several values were therefore obtained for the same pharmacokinetic parameter, from which we calculated the mean values (± SD) for clearance, clearance/F and bioavailability for each species and each antibiotic. They are presented in Table 6 and Table 7.

The standard deviations associated with these means were calculated according to Equation 1:

$$SD_{"review"} = \sqrt{Var_{"review"}} = \sqrt{\frac{\sum[Var_i \times (n_i - 1)]}{\sum(n_i - 1)}} \qquad \textit{Equation 1}$$

Where $Var_{"review"}$ is the combined variance calculated from the variances from the bibliographic references, Var_i, in which i is the number of the reference considered, and n_i is the number of animals in the group in reference i.

Although the three parameters presented in the tables below are related, the lack of homogeneity of the bibliographic sources and the diversity of the methods for obtaining the parameter values explains the lack of exact correspondence between these different values. In any event, the differences observed are part of the variability classically encountered for these types of parameters.

Table 6: Mean value (± SD) for each antibiotic of the pharmacokinetic parameters clearance (ml/min/kg), clearance/F (ml/min/kg, for oral administrations) and bioavailability (%) by the oral route in calves

Tetracyclines		**Mean**	**SD**	**CV (%)**
Tetracycline	**Cl/F**	-	-	-
	Cl	1.86	-	-
	F	-	-	-
Oxytetracycline	**Cl/F**	5.81	-	-
	Cl	2.11	0.61	28.77
	F	46.35	24.00	51.78
Doxycycline	**Cl/F**	3.96	-	-
	Cl	1.89	0.62	32.80
	F	69.00	12.00	17.39
Amoxicillin		**Mean**	**SD**	**CV (%)**
Amoxicillin	**Cl/F**	15.56	-	-
	Cl	5.99	-	-
	F	39.63	7.86	19.83
Florfenicol		**Mean**	**SD**	**CV (%)**
Florfenicol	**Cl/F**	3.32	0.73	21.97
	Cl	2.89	0.47	16.42
	F	82.63	19.17	23.20

CV (%): coefficient of variation expressed as a percentage

Table 7: Mean value (± SD) for each antibiotic of the pharmacokinetic parameters clearance (ml/min/kg), clearance/F (ml/min/kg, for oral administrations) and bioavailability (%) by the oral route in pigs

Tetracyclines		**Mean**	**SD**	**CV (%)**
Tetracycline	**Cl/F**	33.87	11.92	35.20
	Cl	3.29	0.38	11.60
	F	15.33	5.09	33.21
Oxytetracycline	**Cl/F**	71.44	-	-
	Cl	3.60	0.37	10.26
	F	4.72	0.93	19.78
Doxycycline	**Cl/F**	8.74	2.89	33.03
	Cl	2.35	0.33	13.90
	F	30.60	9.22	30.12
Amoxicillin		**Mean**	**SD**	**CV (%)**
Amoxicillin	**Cl/F**	34.04	11.11	32.62
	Cl	8.18	3.20	39.17
	F	34.59	14.17	40.97
Florfenicol		**Mean**	**SD**	**CV (%)**
Florfenicol	**Cl/F**	3.41	0.74	21.81
	Cl	5.25	0.86	16.37
	F	106.24*	15.02	14.14

CV (%): coefficient of variation expressed as a percentage
**: must be rounded to 100%*

Summary/discussion

Following this literature search, a certain amount of pharmacokinetic data was collected that could be used to calculate dosages. The quantity and quality of the data collected for tetracycline in calves proved insufficient for enabling the dosage revision methodology to be applied to them.

It should be noted that in pigs, the oral bioavailabilities of oxytetracycline and tetracycline are very low, and those of doxycycline and amoxicillin are intermediate. Only florfenicol has very good bioavailability in both species.

5. Methodology for revising the dosages of antibiotics

5.1. Construction of the methodology

5.1.1. The PK/PD approach applied to antibiotics

The PK/PD approach makes it possible to calculate a dose taking into account in combination the pharmacokinetic and pharmacodynamic properties of a medicinal product. The relationship between the dose and the PK/PD parameters is given by Equation 2:

$$\text{Dose}_{\text{per unit of time}} = \frac{\text{Plasma clearance}}{\text{Bioavailability}} \times \text{Target concentration} \qquad \textit{Equation 2}$$

where $Dose_{per\ unit\ of\ time}$ is the dose of the antibiotic expressed per unit of time, *Clearance* and *Bioavailability* are the pharmacokinetic parameters controlling the blood concentrations of the antibiotic, and *Target concentration* is the mean plasma concentration associated with the desired therapeutic effects.

Equation 2 can be used for all drug classes but in the case of antibiotics, the target concentration must make it possible to reach the threshold value (or critical value) of the PK/PD index correlated with their efficacy.

It has been shown that the AUC_{24h}/MIC index can be used for the antibiotics studied in this report, namely the tetracyclines (Andes and Craig, 2007), amoxicillin (Lees *et al.*, 2015) and florfenicol (Sidhu *et al.*, 2013).

When the efficacy of the antibiotic is correlated with the AUC_{24h}/MIC index, the following equation gives the relationship between the target concentration of Equation 2 and the threshold value of the PK/PD index:

$$\text{Target concentration} = \frac{\left(AUC/MIC\right)_{\text{critical value}}}{24h} \times \frac{\text{MIC}}{\text{fu}} \qquad \textit{Equation 3}$$

where $\left(\text{AUC}/\text{MIC}\right)_{\text{critical value}}$ is the critical value of the PK/PD index expressed in hours (as a reminder, the AUC_{24h} is calculated over a 24h interval), *fu* is the free fraction of the antibiotic in plasma, and *MIC* is the minimum inhibitory concentration of the antibiotic for the bacterium in question.

By incorporating Equation 3 in Equation 2, an equation is obtained for calculating the daily dose (over 24h) needed to obtain the level of plasma exposure targeted by the PK/PD index:

$$\text{Dose}_{\text{daily}} = \frac{\text{Plasma clearance}}{\text{Bioavailability}} \times \frac{\text{MIC}}{\text{fu}} \times \left(AUC/MIC\right)_{\text{critical value}} \qquad \textit{Equation 4}$$

Determining a dose from Equation 4 requires the values of parameters derived from three distinct components to be documented:

1) The threshold value of the PK/PD index (here AUC_{24h}/MIC), which sets a goal of plasma exposure to the antibiotic, relative to the susceptibility of the pathogen;
2) The MIC value of the pathogen, which converts the previous objective to the absolute level of plasma exposure;

3) The values of the pharmacokinetic parameters (clearance, fu, bioavailability), which determine the link between plasma exposure and the dose.

5.1.2. Selection of the parameter values used

5.1.2.1. Threshold values of the PK/PD index

The value of the PK/PD index associated with a high cure probability plays a vital role in determining the dose, whose value will be proportional to that of the index in the case of the AUC_{24h}/MIC ratio (see Equation 4). The relationship between the efficacy of the antibiotic and the PK/PD index is very strongly impacted by the clinical context, the infection site, the capacity of the immune defences, etc. For this reason, the published studies can present very different threshold values of the AUC_{24h}/MIC index depending on the type of antibacterial activity targeted (bacteriostasis, bactericidal activity, etc.) and the clinical context (severity, immunosuppression, etc.). The different threshold values tested in this study for each antibiotic are presented in Table 8. They are derived from the data available in the literature for the antibiotic/bacterium combinations tested in the study.

Table 8: Threshold values of the PK/PD index AUC_{24h}/MIC for the tetracyclines (tetracycline, oxytetracycline and doxycycline), amoxicillin and florfenicol, and the corresponding mean steady-state concentrations

TETRACYCLINES (tetracycline, oxytetracycline, doxycycline)			
Objective	**Bacteriostatic effect Immune defences unaltered Case 1**	**Bacteriostatic effect Immune defences altered Case 2**	**Bactericidal effect 2log inoculum reduction Case 3**
AUC_{24h}/MIC	12	24	50
C_{mean}	0.5 x MIC	1 x MIC	2 x MIC
AMOXICILLIN			
Objective	**Bacteriostatic effect Case 1**	**Bactericidal effect 2log inoculum reduction Case 2**	**Bactericidal effect 4log inoculum reduction Case 3**
AUC_{24h}/MIC	28	45	60
C_{mean}	1.2 x MIC	2 x MIC	2.5 x MIC
FLORFENICOL			
Objective	**Bacteriostatic effect Case 1**	**Bactericidal effect 2log inoculum reduction Case 2**	**Bactericidal effect 4log inoculum reduction Case 3**
AUC_{24h}/MIC	8	18	25
C_{mean}	0.33 x MIC	0.75 x MIC	1 x MIC

Ref.: Andes and Craig (2007) for the tetracyclines; Lees et al. (2015) for amoxicillin; Sidhu et al. (2013) for florfenicol.

The threshold value of the AUC_{24h}/MIC index can be converted into a mean concentration that must be reached (C_{mean}) using Equation 3 presented above. This concentration can be expressed as a multiple of the MIC, with the multiplier coefficient corresponding to the value of the PK/PD index divided by 24h (fu is considered to be equal to 1 for simplicity). Thus, the multiples of the MIC have values close to 0.5, 1 or 2 for AUC_{24h}/MIC indices equal to 12h, 24h or 50h.

The available studies lack homogeneity in terms of the antibacterial activity sought (bacteriostasis, bactericidal activity) and experimental context (*in vitro* or *in vivo*, status of the immune defences).

From these data, we defined three situations (Cases 1, 2 and 3) representing three levels of increasing requirement with regard to the action of the antibiotic.

5.1.2.2. The pharmacodynamics component (MIC)

The methodology for calculating dosages for the five selected antibiotics was developed in the framework of indications relating to respiratory illnesses in calves and pigs. The MIC values used in the dose calculation are derived from the species *Pasteurella multocida*.

For each antibiotic, the diversity of susceptibility of the *Pasteurella multocida* strains is described by a MIC distribution.

In order to assess the influence of the diversity of strain susceptibility on the antibiotic doses, the MIC values were introduced into the dose calculation in two main ways:

1) Calculation using a MIC point value leading to a single dose

***1.1) Use of the critical concentration value that defines the "Susceptible" categorisation of an antibiogram for* Pasteurella multocida**

The data come from the document of the Veterinary Committee (CA-SFM-Vet) of the CA-SFM (French Microbiology Society's Antibiogram Committee, Report 2016).

1.2) Use of the epidemiological cut-off value derived from the distribution of the MICs (ECOFF)

The data were extracted from the EUCAST (European Committee on Antimicrobial Susceptibility Testing; http://mic.eucast.org/Eucast2/) database.

The critical MIC values for tetracycline, oxytetracycline, doxycycline, amoxicillin and florfenicol for *Pasteurella multocida* are presented in Table 9.

Table 9: Critical MIC values for the antibiotics tested for *Pasteurella multocida* (critical MIC from the CA-SFM and ECOFF from EUCAST)

MIC (µg/ml)	CA-SFM-Vet	ECOFF
Tetracycline	4	2
Oxytetracycline	4	1*
Doxycycline	4	1
Amoxicillin	4	1
Florfenicol	2	1

* As there is no epidemiological cut-off for oxytetracycline, the WG made the assumption that the ECOFF value for oxytetracycline was the same as that for doxycycline.

2) Calculation using the MIC distribution leading to a dose distribution

The MIC distributions used came from the EUCAST database.

The MIC distributions for each antibiotic are presented in Figure 3 (tetracycline), Figure 4 (oxytetracycline, EUCAST data), Figure 5 (doxycycline), Figure 6 (amoxicillin) and Figure 7 (florfenicol):

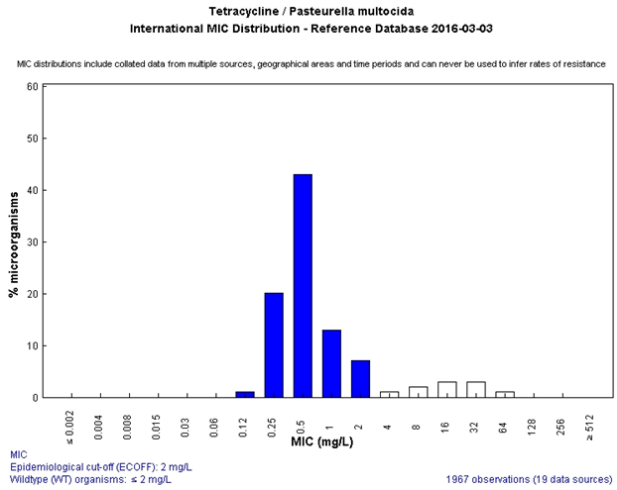


Figure 3: Tetracycline MIC distribution for *Pasteurella multocida* (EUCAST data, 1967 observations)

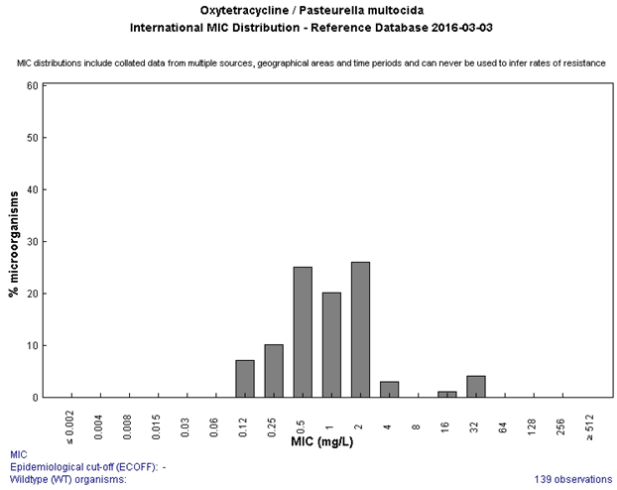


Figure 4: Oxytetracycline MIC distribution for *Pasteurella multocida* (EUCAST data, 139 observations)

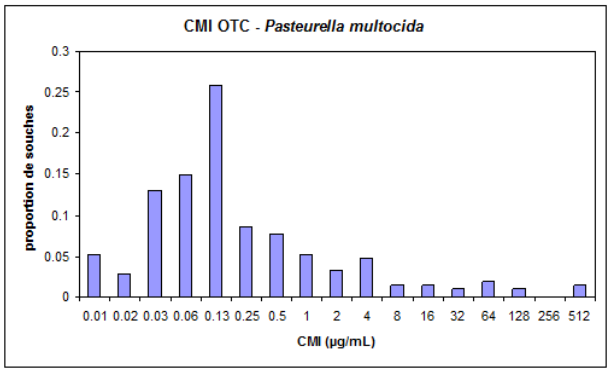


Figure 5: Doxycycline MIC distribution for *Pasteurella multocida* (EUCAST data, 338 observations)

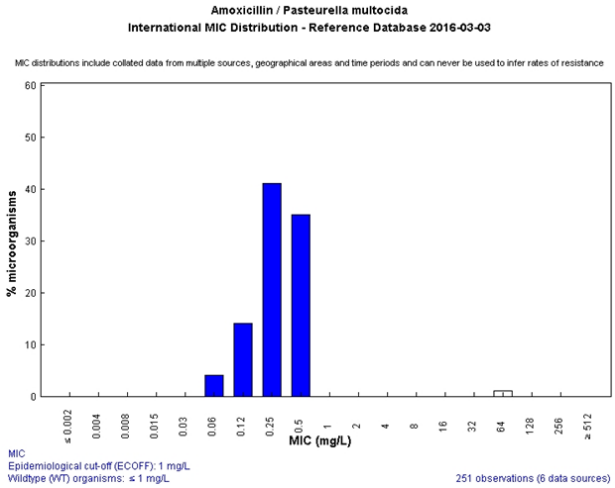


Figure 6: Amoxicillin MIC distribution for *Pasteurella multocida* (EUCAST data, 251 observations)

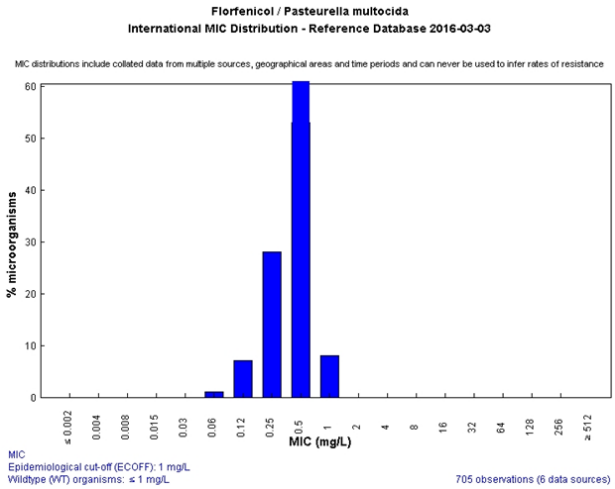


Figure 7: Florfenicol MIC distribution for *Pasteurella multocida* (EUCAST data, 705 observations)

5.1.2.3. The pharmacokinetics component (PK parameters)

The literature search on the pharmacokinetic parameters of the antibiotics enabled the WG to estimate their mean value, as well as their dispersion (described by the variance) in the animal populations in question (pigs and calves).

To take into account the influence of the interindividual dispersion of the pharmacokinetic parameters, the dose calculation was carried out according to the following methods:

1) Using a point value, corresponding to the mean value of each pharmacokinetic parameter, leading to the calculation of a single dose

2) Using a distribution for each pharmacokinetic parameter, leading to a dose distribution

The parameter distributions were generated by simulation based on the mean values and variances, assuming a log-normal distribution for the pharmacokinetic parameters clearance and bioavailability, and a uniform distribution for the free fraction. The values used came from:

2.1) means and variances obtained directly from the literature data

2.2) or from modelling of the population pharmacokinetics applied to plasma concentration profiles from the literature data.

Method 2.2 (non-linear mixed-effects modelling) theoretically leads to a more accurate estimate of the variances compared to Method 2.1 ("two-stage" method). On the other hand, it is more complicated to implement, because it requires sufficiently documented databases and extensive modelling expertise, and can be much more time-consuming.

5.2. Application of the methodology to the revision of the doses

The doses of antibiotics calculated from the methodology are reported in this Section 5.2. The doses obtained depending on the values selected for the AUC_{24h}/MIC index, the MICs (point values or distributions) and the PK parameters (mean values or distributions) have been divided into the sub-sections 5.2.1, 5.2.2 and 5.2.3.

In addition to the dose calculation, the methodology was used to calculate the threshold MICs (PK/PD cut-offs) that could be reached with the current MA doses (see Equation 6). The calculated threshold MICs are reported in Section 5.3. The MICs obtained depending on the values selected for the AUC_{24h}/MIC index and the PK parameters (mean values or distributions) have been divided into the sub-sections 5.3.1 and 5.3.2.

The following table summarises the different combinations used to calculate the doses or threshold MICs.

Table 10: Presentation of the different combinations used to calculate the doses or MICs

Section	AUC_{24h}/MIC	MIC	PK parameters	Dose
5.2.1.[a]	Fixed value	Point value	Point value (mean)	*Calculated*
5.2.2.1.[a]	Fixed value	Point value	Distribution (literature data)	*Calculated*
5.2.2.2.[b]	Fixed value	Point value	Distribution (PK population analysis)	*Calculated*
5.2.3.1.[a]	Fixed value	Distribution	Distribution (literature data)	*Calculated*
5.2.3.2.[b]	Fixed value	Distribution	Distribution (PK population analysis)	*Calculated*
5.3.1.[a]	Fixed value	*Calculated*	Point value	MA
5.3.2.1.[a]	Fixed value	*Calculated*	Distribution (literature data)	MA
5.3.2.2.[b]	Fixed value	*Calculated*	Distribution (PK population analysis)	MA

[a] there are no data for tetracycline in calves
[b] only amoxicillin in pigs

5.2.1. Dose calculations with MIC point and mean values for the PK parameters

The doses were calculated from Equation 4 using the following values:
- for clearance and bioavailability: the mean values obtained from the literature review (Table 6 and Table 7),
- for the free fraction: 0.8 for tetracycline, oxytetracycline, and florfenicol, 0.1 for doxycycline and 0.7 for amoxicillin (Bretzlaff *et al.*, 1987; Lobell *et al.*, 1994; Papich and Rivière, 2009; Villa *et al.*, 1994),
- for the MICs: the critical concentrations from the CA-SFM and the ECOFFs with regard to *Pasteurella multocida* (Table 9),
- for the PK/PD index: the threshold values presented in Table 8.

The results are presented in Table 12 (calves) and Table 13 (pigs).

Table 11: Doses from the SPC for the five antibiotics administered by the oral route in calves and pigs

Dose (mg/kg)	Calves	Pigs
Tetracycline	20	20
Oxytetracycline	20	20
Doxycycline	20	[10-20]
Amoxicillin	10	[10-20]
Florfenicol	20* (IM route)	10

* There is no oral route for florfenicol authorised in calves

Colour code applied for all the presentation tables for the calculated doses below:

- Blue : the calculated dose is close to the dose from the MA (in the range [-25%; +25%]),
- Green : the calculated dose is lower than the dose from the MA,
- Red : the calculated dose is higher than the dose from the MA.

Table 12: Calculated doses in calves with the mean values of the PK parameters

CRITICAL CONCENTRATION			
Dose (mg/kg)	AUC_{24h}/MIC no. 1 (Bacteriostatic)*	AUC_{24h}/MIC no. 2 (Bacteriostatic/-2log)*	AUC_{24h}/MIC no. 3 (-2log/-4log)*
Tetracycline	-	-	-
Oxytetracycline	16	33	68
Doxycycline	79	158	329
Amoxicillin	145	233	311
Florfenicol	4	9	13
ECOFF			
Dose (mg/kg)	AUC_{24h}/MIC no. 1 (Bacteriostatic)*	AUC_{24h}/MIC no. 2 (Bacteriostatic/-2log)*	AUC_{24h}/MIC no. 3 (-2log/-4log)*
Tetracycline	-	-	-
Oxytetracycline	4	8	17
Doxycycline	20	39	82
Amoxicillin	36	58	78
Florfenicol	2	5	7

* see Table 8

Table 13: Calculated doses in pigs with the mean values of the PK parameters

CRITICAL CONCENTRATION			
Dose (mg/kg)	AUC_{24h}/MIC no. 1 (Bacteriostatic)*	AUC_{24h}/MIC no. 2 (Bacteriostatic/-2log)*	AUC_{24h}/MIC no. 3 (-2log/-4log)*
Tetracycline	77	155	322
Oxytetracycline	275	549	1144
Doxycycline	221	442	922
Amoxicillin	227	365	486
Florfenicol	6	13	19
ECOFF			
Dose (mg/kg)	AUC_{24h}/MIC no. 1 (Bacteriostatic)*	AUC_{24h}/MIC no. 2 (Bacteriostatic/-2log)*	AUC_{24h}/MIC no. 3 (-2log/-4log)*
Tetracycline	39	77	161
Oxytetracycline	69	137	286
Doxycycline	55	111	230
Amoxicillin	57	91	122
Florfenicol	3	7	9

* see Table 8

Conclusions

For florfenicol in calves and in pigs, the doses calculated according to the PK/PD methodology were of the same order of magnitude as the doses from the MA.

Most of the doses calculated according to the PK/PD methodology for tetracycline, oxytetracycline, doxycycline and amoxicillin in both species were far higher than the doses from the MA, regardless of the targeted effect (bacteriostatic or bactericidal) or the targeted MIC (critical MIC from the CA-SFM or ECOFF). The ratios [calculated dose/MA dose] were between 2 and 60 depending on the targeted effects. They differed greatly depending on whether the value considered was the critical MIC from the CA-SFM or the ECOFF, which varied by a factor of 2 or 4 depending on the antibiotic.

The tetracycline doses were systematically higher in pigs due to lower bioavailabilities than in calves (by a factor of 2.5 for doxycycline and a factor of 10 for oxytetracycline)

For oxytetracycline in calves, the calculated doses for targeting pathogenic bacteria with a MIC of 1 µg/ml (ECOFF) were of the same order of magnitude as the dose from the MA.

5.2.2. Taking the interindividual variability of the PK parameters into account

5.2.2.1. Variability estimated from the literature

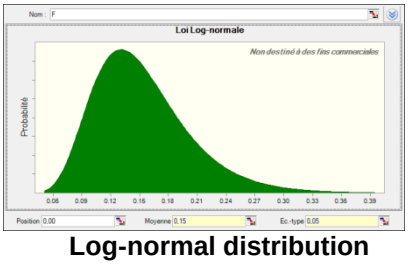
The doses were calculated from Equation 4 using the following values:
- distributions of values for clearance and bioavailability obtained by simulations from a log-normal distribution (Figure 8) and mean values and standard deviations from the literature review (Table 6 and Table 7),
- distributions of values for the free fraction obtained by simulation from a uniform distribution (Figure 88) in the range 0.7-0.9 for tetracycline, oxytetracycline and florfenicol, 0.05-0.2 for doxycycline, and 0.6-0.8 for amoxicillin,
- for the MICs: the critical concentrations from the CA-SFM and the ECOFFs with regard to *Pasteurella multocida* (Table 9),
- for the PK/PD index: the threshold values presented in Table 8.

The distributions of individual values of the pharmacokinetic parameters (clearance, bioavailability and free fraction) were generated for each antibiotic using Crystal Ball software (Oracle Crystal Ball®, Version 11.1.2.4). For example, Table 14 shows the values of the pharmacokinetic parameters for tetracycline in pigs, which were used to generate these distributions.

Ultimately, a sample of 5000 triplets of individual values (of Cl, F, fu) was generated by simulation. This sample simulates 5000 "individuals" characterised by their own individual values for the parameters (Cl, F, fu).

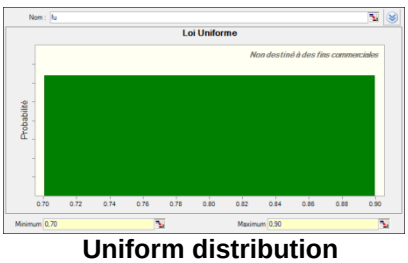
Table 14: Mean, standard deviation and coefficient of variation (CV) values (or mean, minimum and maximum) of the pharmacokinetic parameters used to simulate the individual values (example here for tetracycline in pigs)

	Mean	SD	CV(%)
Cl/F	33.87	11.92	35.20
Cl	3.29	0.38	11.60
F	15.33	5.09	33.21
	Mean	**Min**	**Max**
fu	0.8	0.7	0.9



Log-normal distribution

Simulation of individual values of Cl and F



Uniform distribution

Simulation of individual values of fu

Figure 8: Graphical representation of the log-normal distribution and the uniform distribution

Figure 9 shows the example of a distribution of 5000 individual doses calculated from the previous sample.

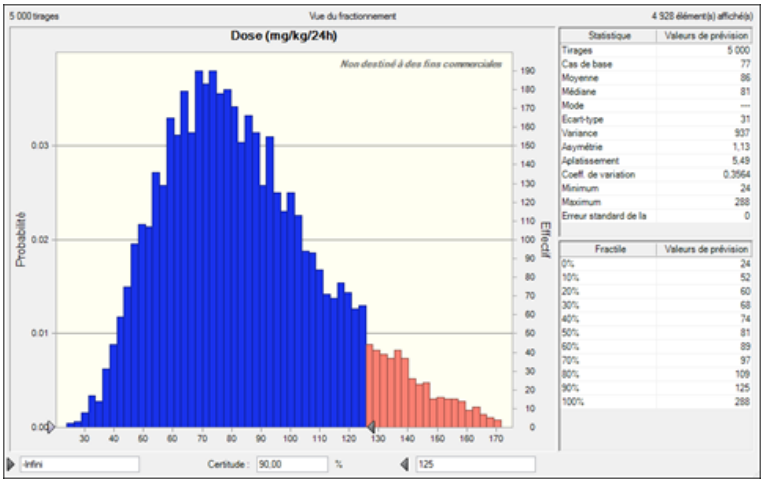


Figure 9: Histogram of distribution of individual doses of tetracycline in pigs corresponding to the least constraining value of the PK/PD index (see Table 8) and to the critical MIC from the CA-SFM

From the sample of 5000 individual doses, the dose enabling the threshold value of the PK/PD index to be reached in 90% of the animals was selected. In Figure 9, the effective dose in 90% of individuals is delineated by the blue part of the histogram. The doses corresponding to the various antibiotics tested and the different thresholds of efficacy are reported in calves in Table 15 and in pigs in Table 16.

Table 15: Calculated doses in calves with the distributions of the PK parameters. The values reported correspond to the effective dose in 90% of calves.

CRITICAL CONCENTRATION			
Dose (mg/kg)	AUC_{24h}/MIC no. 1 (Bacteriostatic)*	AUC_{24h}/MIC no. 2 (Bacteriostatic/-2log)*	AUC_{24h}/MIC no. 3 (-2log/-4log)*
Tetracycline	-	-	-
Oxytetracycline	37	73	153
Doxycycline	131	262	545
Amoxicillin	198	319	425
Florfenicol	6	14	19
ECOFF			
Dose (mg/kg)	AUC_{24h}/MIC no. 1 (Bacteriostatic)*	AUC_{24h}/MIC no. 2 (Bacteriostatic/-2log)*	AUC_{24h}/MIC no. 3 (-2log/-4log)*
Tetracycline	-	-	-
Oxytetracycline	9	18	38
Doxycycline	33	65	136
Amoxicillin	50	80	106
Florfenicol	3	7	10

* see Table 9

Table 16: Calculated doses in pigs with the distributions of the PK parameters. The values reported correspond to the effective dose in 90% of pigs

CRITICAL CONCENTRATION			
Dose (mg/kg)	AUC_{24h}/MIC no. 1 (Bacteriostatic)*	AUC_{24h}/MIC no. 2 (Bacteriostatic/-2log)*	AUC_{24h}/MIC no. 3 (-2log/-4log)*
Tetracycline	125	251	523
Oxytetracycline	374	748	1558

Doxycycline	381	762	1588
Amoxicillin	465	747	996
Florfenicol	8	18	25
ECOFF			
Dose (mg/kg)	AUC_{24h}/MIC no. 1 (Bacteriostatic)*	AUC_{24h}/MIC no. 2 (Bacteriostatic/-2log)*	AUC_{24h}/MIC no. 3 (-2log/-4log)*
Tetracycline	63	125	261
Oxytetracycline	93	187	389
Doxycycline	95	191	397
Amoxicillin	116	187	249
Florfenicol	4	9	12

* see Table 9

Conclusions

Taking the interindividual variability of the pharmacokinetic parameters into account led to calculated doses that were higher than those obtained with the mean values of the pharmacokinetic parameters (previous section) and therefore higher than the MA doses.

The conclusions were similar to the previous ones, namely the doses calculated from the PK/PD methodology were of the same order of magnitude as the doses from the MA for florfenicol in calves and in pigs, and for oxytetracycline in calves, when targeting a bacteriostatic effect on pathogenic bacteria with a MIC of 1 µg/ml (ECOFF).

5.2.2.2. Variability estimated from a population analysis

This analysis was only carried out for amoxicillin in pigs.

The dose calculation was carried out in the same way as in the previous section (5.2.2.1.).

However, the pharmacokinetic data were generated using a pharmacokinetic population analysis on a sample of individual concentration profiles over time.

In total, we had 43 individual plasma concentration profiles over time (i.e. 441 concentrations above the limit of quantification), obtained after oral administration of an amoxicillin bolus in pigs. As the administered doses differed, the plasma concentrations were normalised to a dose of 20 mg/kg (assuming the linearity of the pharmacokinetics of amoxicillin in pigs). These data have been described elsewhere (Rey *et al.*, 2014).

The analysis was carried out using Phoenix software® (Phoenix NLME, Version 6.3, Certara L. P. (Pharsight), St. Louis, MO). The evolution over time of plasma amoxicillin concentrations was described using a one-compartment model with absorption compartment. The variance model chosen for the residual errors was a log-additive model. The interindividual variability was described assuming a log-normal distribution of the pharmacokinetic parameters. The apparent clearance (Cl/F) was modelled according to Equation 5:

$$(Cl/F)_i = \theta_{Cl} \times \exp\left(\eta_{Cl,i}\right) \qquad \textit{Equation 5}$$

where $(Cl/F)_i$ is the apparent clearance of the i^{th} animal, θ_{Cl} is the geometric mean of the population, and $\eta_{Cl,i}$ is a random variable following a normal distribution of mean 0 and variance ω^2_{Cl}.

The mean value and the interindividual dispersion of the apparent clearance (Cl/F) of amoxicillin administered orally in pigs obtained with this population analysis are presented in Table 17 and compared with the values from the literature search.

Table 17: Mean values and coefficients of variation (CV %) of the apparent clearance (Cl/F) of amoxicillin by the oral route in pigs

Amoxicillin		**Mean**	**CV (%)**
Bibliographic analysis	**Cl/F**	34.04	33
Population analysis	**Cl/F**	38.78	52

The doses were calculated from Equation 4 according to the same methods and with the same parameter values as in Section 5.2.2.1., with the exception of the values for apparent clearance (Cl/F), which were obtained by simulations from a log-normal distribution and the mean and dispersion values derived from the population analysis (Table 17).

From the sample of 5000 individual doses, the dose enabling the threshold value of the PK/PD index to be reached in 90% of the animals was selected. The amoxicillin doses corresponding to the different thresholds of effectiveness are reported in Table 18.

Table 18: Calculated doses in pigs with the distributions of the PK parameters obtained after the population analysis. The values reported correspond to the effective dose in 90% of pigs.

CRITICAL CONCENTRATION			
Dose (mg/kg)	AUC_{24h}/MIC no. 1 (Bacteriostatic)*	AUC_{24h}/MIC no. 2 (-2log)*	AUC_{24h}/MIC no. 3 (-4log)*
Amoxicillin	422	678	904
ECOFF			
Dose (mg/kg)	AUC_{24h}/MIC no. 1 (Bacteriostatic)*	AUC_{24h}/MIC no. 2 (-2log)*	AUC_{24h}/MIC no. 3 (-4log)*
Amoxicillin	106	170	226

* see Table 8

Conclusions

The dose calculations achieved with the distribution of the apparent clearance (Cl/F) derived from the population analysis (Table 18) give very similar results to those obtained with the previous methodology (Table 16): they are systematically lower, with deviations not exceeding 10%.

5.2.3. Taking the MIC distributions and the interindividual variability of the PK parameters into account

5.2.3.1. Variability estimated from the literature

The doses were calculated from Equation 4 using the following values:
- distributions of values for clearance and bioavailability obtained by simulations from a log-normal distribution (Figure 8) and mean values and standard deviations from the literature review (Table 6 and Table 7),
- distributions of values for the free fraction obtained by simulation from a uniform distribution (Figure 8) in the range 0.7-0.9 for tetracycline, oxytetracycline and florfenicol, 0.05-0.2 for doxycycline, and 0.6-0.8 for amoxicillin,
- MIC values obtained from a random selection in the MIC distributions of strains of *Pasteurella multocida* from the EUCAST collection (see Section 5.1.2.2.); the MICs higher than the critical concentration from CA-SFM-Vet were excluded from the selection to avoid calculating doses on scenarios for which the bacterial strain would appear to be resistant with a susceptibility test, therefore ruling out the use of the antibiotic,
- for the PK/PD index: the threshold values presented in Table 8.

The distributions of individual values of the pharmacokinetic parameters (clearance, bioavailability and free fraction) were generated for each antibiotic using Crystal Ball software (Oracle Crystal Ball®, Version 11.1.2.4).

Ultimately, a sample of 5000 triplets of individual values (of Cl, F, fu) was generated by simulation. This sample simulates 5000 "individuals" characterised by their own individual values for the parameters (Cl, F, fu).

From the previous sample, 5000 individual doses were calculated and the dose enabling the threshold value of the PK/PD index to be reached in 90% of the animals was determined.

The doses corresponding to the various antibiotics tested and the different thresholds of efficacy are reported in Table 19 (calves) and Table 20 (pigs).

Table 19: Calculated doses in calves taking into account the MIC distributions and the distributions of the PK parameters. The values reported correspond to the effective dose in 90% of calves.

Dose (mg/kg)	AUC_{24h}/MIC no. 1 (Bacteriostatic)*	AUC_{24h}/MIC no. 2 (Bacteriostatic/-2log)*	AUC_{24h}/MIC no. 3 (-2log/-4log)*
Tetracycline	-	-	-
Oxytetracycline	13	27	55
Doxycycline	13	26	54
Amoxicillin	22	36	48
Florfenicol	2	4	5

* see Table 8

Table 20: Calculated doses in pigs taking into account the MIC distributions and the distributions of the PK parameters. The values reported correspond to the effective dose in 90% of pigs.

Dose (mg/kg)	AUC_{24h}/MIC no. 1 (Bacteriostatic)*	AUC_{24h}/MIC no. 2 (Bacteriostatic/-2log)*	AUC_{24h}/MIC no. 3 (-2log/-4log)*
Tetracycline	32	63	132
Oxytetracycline	168	337	701
Doxycycline	38	75	157
Amoxicillin	45	73	97
Florfenicol	2	5	7

* see Table 8

Conclusions

Taking into account the MIC distributions with regard to *Pasteurella multocida* led to calculated doses that were generally lower than those obtained with the MIC point values (critical concentration or ECOFF).

The calculated doses were of the same order of magnitude as the doses from the MA in calves and pigs for florfenicol; and in calves only for oxytetracycline and doxycycline, when targeting a bacteriostatic effect in the presence of unaltered immune defences (see Table 8).

5.2.3.2. Variability estimated from a population analysis

This analysis was only carried out for amoxicillin in pigs.
The doses were calculated from Equation 4 using the following values:

- distributions of values for apparent clearance (Clearance/F) obtained by simulations from a log-normal distribution (Figure 8) and data from the population analysis (Table 7),

- the free fraction simulated from a uniform distribution between 0.6 and 0.8 for amoxicillin,
- MIC values obtained from a random selection in the MIC distributions of strains of *Pasteurella multocida* from the EUCAST collection (see Section 5.1.2.2.); the MICs higher than the critical concentration from CA-SFM-Vet were excluded from the selection for the same reason as in the section above,
- for the PK/PD index: the threshold values presented in Table 8.

From the 5000 individual doses calculated as indicated previously, the dose enabling the threshold value of the PK/PD index to be reached in 90% of the animals was determined.

The amoxicillin doses in pigs corresponding to the different thresholds of efficacy are reported in Table 21.

Table 21: Calculated doses in pigs taking into account the MIC distribution and the distributions of the PK parameters obtained after the population analysis. The values reported correspond to the effective dose in 90% of pigs.

Dose (mg/kg)	AUC_{24h}/MIC no. 1 (Bacteriostatic)*	AUC_{24h}/MIC no. 2 (-2log)*	AUC_{24h}/MIC no. 3 (-4log)*
Amoxicillin	51	82	109

* see Table 8

Conclusions

All other things being equal (on the pharmacokinetics component), taking into account the MIC distributions with regard to *Pasteurella multocida* led to calculated doses that were only about half those obtained with the ECOFF (Table 18).

In addition, the dose calculations achieved with the distribution of the apparent clearance (Cl/F) derived from the population analysis gave very similar results to those obtained with the previous methodology (Table 20): they were systematically higher, with deviations of 11-12%.

5.3. Application of the PK/PD approach to determining the threshold MICs (PK/PD cut-offs) for the doses derived from the current MAs

Selection of the critical concentrations delineating the I/S/R categories of antibiotic susceptibility tests (antibiograms) requires the determination of three threshold MICs, respectively known as the epidemiological cut-off, the clinical cut-off and the PK/PD cut-off. Calculating the latter calls on the principles of the PK/PD approach used for determining a dose. The threshold MIC according to the PK/PD approach (PK/PD cut-off) can be obtained from the following equation, which is directly derived from Equation 4:

$$MIC_{PK/PD} = Dose_{daily} \times \frac{Bioavailability}{Plasma\ clearance} \times \frac{fu}{\left(AUC/MIC\right)_{critical\ value}} \qquad \textit{Equation 6}$$

Using the same pharmacokinetic data as previously, we calculated for each antibiotic the PK/PD cut-offs corresponding to the doses recommended by the MA.

5.3.1. Calculations of PK/PD cut-offs with mean values for the PK parameters

The MICs were calculated from Equation 6 using the following values:
- for clearance and bioavailability: the mean values obtained from the literature review (Table 6 and Table 7),
- for the free fraction, 0.8 for tetracycline, oxytetracycline, and florfenicol, 0.1 for doxycycline, and 0.7 for amoxicillin,
- the reference dose presented in Table 11,
- for the PK/PD index: the threshold values presented in Table 8.

The results are presented in Table 23 (calves) and Table 24 (pigs). For both tables, the calculated MICs are in red if at least a twofold dilution lower, in green if at least a twofold dilution higher, or blue if found in the interval between the two dilutions immediately below and above, compared to the critical values (breakpoints) of the *Susceptible* category from the CA-SFM or the CLSI (Clinical

and Laboratory Standards Institute), and also compared to the ECOFF values provided by EUCAST (Table 22).

Table 22: Critical MIC values of the *Susceptible* category for the antibiotics tested with regard to *Pasteurella multocida* according to the CA-SFM or the CLSI, and the ECOFF from EUCAST

MIC (µg/ml)	Critical value CA-SFM	Critical value CLSI		ECOFF
		Calves	Pigs	
Tetracycline	4	2	0.5	2
Oxytetracycline	4	2	0.5	1
Doxycycline	4	2	0.5	1
Amoxicillin	4	0.25	0.5	1
Florfenicol	2	2	4	1

Table 23: PK/PD cut-offs obtained in calves with the dose from the MA

Comparison with the critical concentration from the CA-SFM-Vet			
MIC (µg/ml)	AUC_{24h}/MIC no. 1 (Bacteriostatic)*	AUC_{24h}/MIC no. 2 (Bacteriostatic/-2log)*	AUC_{24h}/MIC no. 3 (-2log/-4log)*
Tetracycline	-	-	-
Oxytetracycline	5	2	1
Doxycycline	1	0.5	0.2
Amoxicillin	0.3	0.2	0.1
Florfenicol	10	4	3
Comparison with the critical concentration of the CLSI			
MIC (µg/ml)	AUC_{24h}/MIC no. 1 (Bacteriostatic)*	AUC_{24h}/MIC no. 2 (Bacteriostatic/-2log)*	AUC_{24h}/MIC no. 3 (-2log/-4log)*
Tetracycline	-	-	-
Oxytetracycline	5	2	1
Doxycycline	1	0.5	0.2
Amoxicillin	0.3	0.2	0.1
Florfenicol	10	4	3
Comparison with the ECOFF			
MIC (µg/ml)	AUC_{24h}/MIC no. 1 (Bacteriostatic)*	AUC_{24h}/MIC no. 2 (Bacteriostatic/-2log)*	AUC_{24h}/MIC no. 3 (-2log/-4log)*
Tetracycline	-	-	-
Oxytetracycline	5	2	1
Doxycycline	1	0.5	0.2
Amoxicillin	0.3	0.2	0.1
Florfenicol	10	4	3

* see Table 8

Table 24: PK/PD cut-offs obtained in pigs with the dose from the MA

Comparison with the critical concentration from the CA-SFM-Vet			
MIC (µg/ml)	AUC_{24h}/MIC no. 1 (Bacteriostatic)*	AUC_{24h}/MIC no. 2 (Bacteriostatic/-2log)*	AUC_{24h}/MIC no. 3 (-2log/-4log)*
Tetracycline	1	0.5	0.2
Oxytetracycline	0.3	0.15	0.07
Doxycycline	0.18	0.09	0.04
Amoxicillin	0.4	0.22	0.16
Florfenicol	5	2.2	1.6
Comparison with the critical concentration of the CLSI			
MIC (µg/ml)	AUC_{24h}/MIC no. 1 (Bacteriostatic)*	AUC_{24h}/MIC no. 2 (Bacteriostatic/-2log)*	AUC_{24h}/MIC no. 3 (-2log/-4log)*
Tetracycline	1	0.5	0.2
Oxytetracycline	0.3	0.15	0.07
Doxycycline	0.18	0.09	0.04
Amoxicillin	0.4	0.22	0.16
Florfenicol	5	2.2	1.6
Comparison with the ECOFF			
MIC (µg/ml)	AUC_{24h}/MIC no. 1 (Bacteriostatic)*	AUC_{24h}/MIC no. 2 (Bacteriostatic/-2log)*	AUC_{24h}/MIC no. 3 (-2log/-4log)*
Tetracycline	1	0.5	0.2
Oxytetracycline	0.3	0.15	0.07
Doxycycline	0.18	0.09	0.04
Amoxicillin	0.4	0.22	0.16
Florfenicol	5	2.2	1.6

* see Table 8

Conclusions

For florfenicol in calves and pigs, most of the calculated PK/PD cut-offs are of the same order of magnitude as or higher than the critical MICs from the CA-SFM-Vet or the CLSI, as well as the ECOFFs.

In calves, the PK/PD cut-offs calculated for amoxicillin were far lower than the critical MICs from the CA-SFM-Vet. The situation is more varied with the critical concentration from the CLSI or with the other antibiotics.

In pigs, the PK/PD cut-offs calculated for tetracycline, oxytetracycline, doxycycline and amoxicillin were far lower than the critical MICs from the CA-SFM-VET, as well as the ECOFFs. The situation is more varied with the critical MICs from the CLSI.

5.3.2. Taking the interindividual variability of the PK parameters into account

5.3.2.1. Variability estimated from the literature

The MICs were calculated from Equation 6 using the following values:
- distributions of values for clearance and bioavailability obtained by simulations from a log-normal distribution (Figure 8) and mean values and standard deviations from the literature review (Table 6 and Table 7),
- distributions of values for the free fraction obtained by simulation from a uniform distribution (Figure 8) in the range 0.7-0.9 for tetracycline, oxytetracycline and florfenicol, 0.05-0.2 for doxycycline, and 0.6-0.8 for amoxicillin,
- the MA dose (Table 11),
- for the PK/PD index: the threshold values presented in Table 8.

For each condition (antibiotic, species), a sample of 5000 triplets (Cl, F, fu) corresponding to 5000 "individuals" was created, and used to calculate the MICs for which the threshold value of the PK/PD index is reached. Figure 10 presents the sample of the 5000 MICs obtained for tetracycline in pigs with the lowest value of the PK/PD index (Case 1).

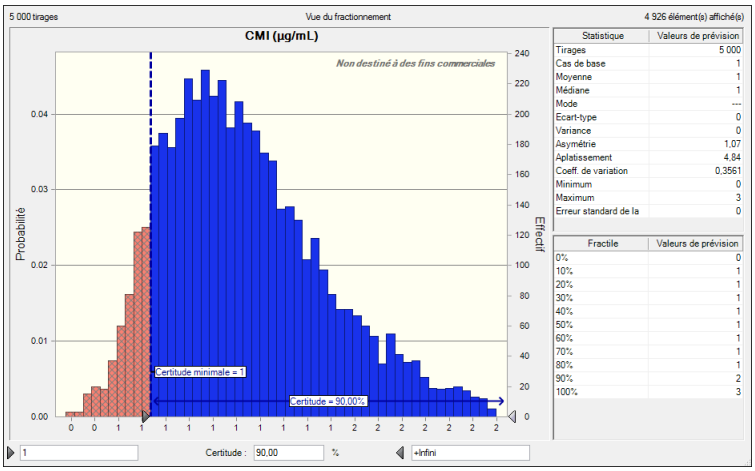


Figure 10: Histogram of distribution of MICs for which the MA dose of tetracycline in pigs gives the least constraining value of the PK/PD index (see Table 8)

From the sample of 5000 individual MICs, the MIC (PK/PD cut-off) for which the dose from the MA gives the threshold value of the PK/PD index in 90% of the animals was selected. The PK/PD cut-offs corresponding to the various antibiotics tested and the different thresholds of effectiveness are reported in Table 25 (calves) and Table 26 (pigs).

Table 25: PK/PD cut-offs obtained in calves with the MA dose after integration of pharmacokinetic variability

Comparison with the critical concentration from the CA-SFM-Vet			
MIC (µg/ml)	AUC_{24h}/MIC no. 1 (Bacteriostatic)*	AUC_{24h}/MIC no. 2 (Bacteriostatic/-2log)*	AUC_{24h}/MIC no. 3 (-2log/-4log)*
Tetracycline	-	-	-
Oxytetracycline	2	1	1
Doxycycline	1	0.3	0.1
Amoxicillin	0.2	0.1	0.1
Florfenicol	7	3	2
Comparison with the critical concentration of the CLSI			
MIC (µg/ml)	AUC_{24h}/MIC no. 1 (Bacteriostatic)*	AUC_{24h}/MIC no. 2 (Bacteriostatic/-2log)*	AUC_{24h}/MIC no. 3 (-2log/-4log)*
Tetracycline	-	-	-
Oxytetracycline	2	1	1
Doxycycline	1	0.3	0.1
Amoxicillin	0.2	0.1	0.1
Florfenicol	7	3	2
Comparison with the ECOFF			
MIC (µg/ml)	AUC_{24h}/MIC no. 1 (Bacteriostatic)*	AUC_{24h}/MIC no. 2 (Bacteriostatic/-2log)*	AUC_{24h}/MIC no. 3 (-2log/-4log)*
Tetracycline	-	-	-
Oxytetracycline	2	1	1
Doxycycline	1	0.3	0.1
Amoxicillin	0.2	0.1	0.1
Florfenicol	7	3	2

* see Table 8

Table 26: PK/PD cut-offs obtained in pigs with the MA dose after integration of pharmacokinetic variability

Comparison with the critical concentration from the CA-SFM-Vet			
MIC (µg/ml)	AUC_{24h}/MIC no. 1 (Bacteriostatic)*	AUC_{24h}/MIC no. 2 (Bacteriostatic/-2log)*	AUC_{24h}/MIC no. 3 (-2log/-4log)*
Tetracycline	1	0.3	0.2
Oxytetracycline	0.2	0.1	0.05
Doxycycline	0.1	0.05	0.03
Amoxicillin	0.2	0.1	0.08
Florfenicol	4	1.7	1.2
Comparison with the critical concentration of the CLSI			
MIC (µg/ml)	AUC_{24h}/MIC no. 1 (Bacteriostatic)*	AUC_{24h}/MIC no. 2 (Bacteriostatic/-2log)*	AUC_{24h}/MIC no. 3 (-2log/-4log)*
Tetracycline	1	0.3	0.2
Oxytetracycline	0.2	0.1	0.05
Doxycycline	0.1	0.05	0.03
Amoxicillin	0.2	0.1	0.08
Florfenicol	4	1.7	1.2
Comparison with the ECOFF			
MIC (µg/ml)	AUC_{24h}/MIC no. 1 (Bacteriostatic)*	AUC_{24h}/MIC no. 2 (Bacteriostatic/-2log)*	AUC_{24h}/MIC no. 3 (-2log/-4log)*
Tetracycline	1	0.3	0.2
Oxytetracycline	0.2	0.1	0.05
Doxycycline	0.1	0.05	0.03
Amoxicillin	0.2	0.1	0.08
Florfenicol	4	1.7	1.2

* see Table 8

Conclusions

Taking into account the interindividual variability in the pharmacokinetic parameters led to PK/PD cut-offs that were lower than those obtained with the mean values of the pharmacokinetic parameters. This result was expected, since within the simulated distribution of individuals, some were found with a higher clearance and/or a lower bioavailability than the population means, leading to lower antibiotic concentrations than the mean for the same dose. The consequence of these reduced concentrations is that the threshold value of the PK/PD index can only be reached with a lower MIC.

5.3.2.2. Variability estimated from a population analysis

This analysis was only carried out for amoxicillin in pigs.
The MICs were calculated from Equation 6 using the following values:
- the clearance/F simulated from the population analysis (Table 17),
- the free fraction simulated from a uniform distribution between 0.6 and 0.8 for amoxicillin,
- the MA dose for amoxicillin in pigs (Table 11),
- for the PK/PD index: the threshold values presented in Table 8.

The PK/PD cut-offs corresponding to the MA dose of amoxicillin in pigs and to the different thresholds of efficacy are reported in Table 27.

Table 27: PK/PD cut-offs obtained in pigs with the MA dose of amoxicillin after integration of pharmacokinetic variability via a population analysis

Comparison with the critical concentration from the CA-SFM-Vet, the CLSI or the ECOFF			
MIC (µg/ml)	AUC_{24h}/MIC no. 1 (Bacteriostatic)*	AUC_{24h}/MIC no. 2 (Bacteriostatic/-2log)*	AUC_{24h}/MIC no. 3 (-2log/-4log)*
Amoxicillin	0.2	0.1	0.1

* see Table 8

Conclusions

When the pharmacokinetic parameter clearance/F is simulated from the population analysis, the calculated PK/PD cut-offs are identical to those obtained when the pharmacokinetic parameters clearance and bioavailability are simulated from the literature analysis.

The PK/PD cut-offs calculated for amoxicillin in pigs are far lower than the critical concentrations from the CA-SFM-Vet and the CLSI, or the ECOFFs, regardless of the effect sought (bacteriostatic, or 2log or 4log inoculum reduction).

5.4. Discussion on the elements of the methodology

The following discussion focuses on the various elements of the PK/PD methodology.

The PK/PD index

The value of the PK/PD index associated with a high cure probability plays a vital role in determining the dose, whose value will be proportional to that of the index in the case of the AUC_{24h}/MIC ratio. The relationship between the antibiotic's efficacy and the PK/PD index is very strongly impacted by the clinical context, the infection site, and the capacity of the immune defences.

From the literature, we had various threshold values for the PK/PD index AUC_{24h}/MIC, depending on the objective assigned to the antibacterial activity (bacteriostasis, bactericidal activity), the experimental framework (*in vitro* or *in vivo*) and the status of the immune defences for the *in vivo* models (with a contrast between preserved immune defences *versus* experimental neutropaenia).

To attempt to make a link with the clinical contexts encountered in the field, and bearing in mind that the experimental models cannot reproduce the diversity of spontaneous disorders, we can establish the following associations:
- the doses established with the most constraining objectives for the PK/PD index (bactericidal activity with 4log reduction of the bacterial inoculum) seem to correspond to the treatment of bacterial infections with proven, even severe clinical signs,
- the doses established with the least constraining objectives for the PK/PD index (bacteriostasis with maintenance of the immune defences) could correspond to the early treatment of recently-evolved bacterial infections, for example in the framework of interventions such as metaphylaxis.

Given the pivotal role of the PK/PD index in the relationship between dose and efficacy, conducting clinical field trials combined with pharmacokinetic studies, able to describe this relationship, is essential for determining realistic doses using the PK/PD approach.

Variability of pharmacokinetic and pharmacodynamic origin

The major advantage of the PK/PD approach is that it makes it possible, when determining the doses, to simultaneously take into account the variability of the susceptibility of the pathogen strains and the inter-animal variability of the pharmacokinetics of the antibiotics.

Prior to this, we performed an intermediate calculation step which involved establishing the MIC value, in order to unequivocally describe the impact of the pharmacokinetic variability on the dose calculation.

The interindividual variability of the pharmacokinetic parameters

Taking into account the interindividual variability of the pharmacokinetic parameters within the animal populations gives the calculated dose the status of random variable. From this point, each dose can be associated with the percentage of individuals in the population that will be treated "correctly" by this dose. We chose to retain an objective of 90% "coverage" of the population (i.e. the Dose90%). Indeed, the 90% threshold is classically used in the literature in studies of this type.

Thus, each Dose90% established in this study can be interpreted as guaranteeing either a 90% probability of success for an individual treatment, or therapeutic success in 90% of individuals for a collective treatment, making the assumption that all individuals in the treated batch ingest the same dose.

Selection of a Dose90% mechanically leads to doses that are higher than those calculated from the mean values of the pharmacokinetic parameters, with the latter corresponding to Dose50% values, which only correctly treat "around" 50% of the individuals in the population.

The interindividual variability of the pharmacokinetic parameters was included in the dose calculation by generating distributions of these parameters through simulations from the mean values and variances, assuming a log-normal distribution of the parameters.

We compared two methods for determining the means and variances: Method 1, which consisted in calculating them from the means and variances derived from the available studies in the literature; Method 2, which consisted in performing non-linear mixed-effects modelling (known as population pharmacokinetics) of the plasma concentration profiles available in the literature. Method 2 theoretically leads to a more accurate estimate of the variances compared to Method 1 ("two-stage" method). On the other hand, it is more complicated to implement, because it requires sufficiently documented databases and extensive modelling expertise, and can be much more time-consuming. Ultimately, the doses obtained with these two methods proved to be very similar, with deviations of less than 12%.

The value of a population pharmacokinetics analysis can therefore be questioned, particularly in the case of older compounds for which the available databases document relatively few raw data (individual profiles of concentrations over time). This does not call into question the value of population pharmacokinetics when developing new compounds, including new antibiotics.

Variability in the susceptibility of the bacterial strains to antibiotics

Dose calculation according to the PK/PD approach takes the bacterial susceptibility to antibiotics into account *via* the MIC parameter.

As indicated above, we determined the doses initially using a MIC point value, in order to study the influence of pharmacokinetic variability alone in the dose calculation.

The MIC point values used were:
- the critical concentration ("breakpoint") provided by the Veterinary Committee of the CA-SFM-Vet, which delineates the *Susceptible* category of the antibiotic susceptibility tests (antibiograms),
- the epidemiological cut-off, or ECOFF, provided by EUCAST.

- for the antibiotics tested and the bacterium considered (*Pasteurella multocida*), the critical concentrations from the CA-SFM-Vet were systematically higher than the ECOFFs derived from the MIC distributions from EUCAST. The result is that given the relationship of proportionality between the dose and the MIC (Equation 4), the antibiotic doses were systematically higher for the critical concentrations from the CA-SFM-Vet than for the ECOFFs.

We then calculated the doses by integrating all the MIC distributions of the strains of *Pasteurella multocida*. In this case, the distribution of individual doses obtained takes into account the probability that the pathogen strain has a (sometimes far) lower MIC than the selected MIC point value. The result is that the Dose90% values calculated for all antibiotics (with the exception of oxytetracycline) were always lower than those obtained with the selected MIC point values. In other words, the dose calculated to cover 90% of possible situations (Dose90%) was lower than the more cautious one obtained while assuming that 100% of the strains have a MIC equal to the selected MIC point.

To conclude, the method of choice for calculating the doses integrates both the variability of pharmacokinetic origin and the MIC distribution of the pathogens.

Determination of critical MICs from the current doses recommended by the MA

The same PK/PD methodology can be used to address the issue of antibiotic dosages in terms of the indications.

The method consists in determining for the current doses new critical concentrations for the *Susceptible* category of bacterial strains during antibiogram tests, aiming for a probability of therapeutic success greater than 80-90%.

The methods for calculating doses or MICs, which use the same theoretical bases and the same experimental data (PK/PD approach, pharmacokinetic parameters and PK/PD indices), will always yield concordant results. For example, if the first method leads to an increase in dose being advocated to maintain the same indications, the second method will lead to a decrease in the critical MICs being recommended for unchanged doses. Comparing the two approaches did not fall within the scope of the internal request and will not be developed in this report.

6. Limitations to the proposed method

6.1. Limitations to the use of the MIC as a PD indicator

Among the limitations of the PK/PD approach, some are inherent in the use of MICs as indicators of antibiotic activity.

First of all, the MIC is determined *in vitro* in a standardised environment that is not always representative of the environment of the bacterium *in situ*.

In a number of situations, the MICs are not predictive of antibacterial activity, for example for intracellular pathogens or in a biofilm environment (Ferran *et al*., Frontiers in Microbiology, 2016).

In addition, the MIC does not take into account other modes of action of certain antibiotics: anti-inflammatory and immunomodulatory activities (Fischer *et al.*, 2011).

6.2. Duration of treatment

One of the limitations of the PK/PD methodology applied to the revision of the dosages of older antibiotics is that it helps determine a dose but does not give any information on the duration of treatment.

In veterinary medicine, as in human medicine, the treatment durations are not based on a solid scientific rationale. Most of the recommendations mention durations in the form of extended intervals that can vary by up to 100%, for example from 3 to 6 weeks for acute pyelonephritis in dogs (GRAM 2010) or from 3 to 5 days for respiratory infections in cattle (Marbocyl 10%, doxyval 20%, etc.)(ANMV). They may also mention maximum treatment durations (for example, commercial colistin products for the oral route).

In veterinary medicine, to our knowledge there are no published data from randomised double-blind clinical studies comparing two treatment durations with the same antibiotic. In the absence of reliable data and due to a fear of therapeutic failure, the advocated treatment durations are probably longer than necessary. Indeed, many studies in humans have shown that shorter treatment durations than those traditionally used were possible without compromising efficacy, for different categories of infections such as urinary tract, lung, osteoarticular or intra-abdominal infections (Dinh, Bouchand *et al.* 2016; Rice 2008). Establishing clinical trials assessing the efficacy of shorter treatments in veterinary medicine should be an objective. It should be pointed out that if such trials are carried out in the field, they should probably include relatively high numbers of animals, in order to control for the many sources of bias. In the absence of comparative trials, it is also possible to directly encourage the clinician to shorten treatment durations through the clinical follow-up of treated animals. Among the options that could be considered, cessation of treatment could be decided on the basis of the disappearance of symptoms (el Moussaoui, de Borgie *et al.* 2006) or on the determination of a serum marker such as procalcitonin or the C-reactive protein used in human medicine (Bouadma, Luyt *et al.* 2010; Schuetz, Christ-Crain *et al.* 2009). Carrying out scientific studies establishing the value of a high probability of therapeutic success for such markers is an essential prerequisite to formulating this type of recommendation. To do this, it will be necessary to overcome the fear of therapeutic failure among prescribers and animal owners. For example, in human medicine, it seems that "shorter" treatments have been readily applied to ENT infections and lower urinary tract infections, mainly because of the absence of severity in these disorders.

Limiting the durations of antibiotic treatment to the minimum necessary can help reduce costs and adverse effects, but the main benefit is to reduce the duration of exposure of the commensal

microbiota to antibiotics, which is an essential element in preventing the emergence, amplification and circulation of bacterial resistance. In human medicine, the French National Authority for Health (HAS) issued recommendations on the proper use of antibiotics in April 2008, specifying that prolonged antibiotic therapy led to exposure to an unfavourable risk-benefit ratio with higher bacterial resistance and increased toxicity (HAS 2008).

In vitro, it been shown that a longer duration of exposure to a fluoroquinolone facilitated the emergence of resistant mutants, and that it was therefore necessary to increase the concentrations to eradicate these mutants (Tam, Louie *et al.* 2007). A number of studies have assessed the impact of the duration of an antibiotic treatment on the amplification of resistance within the commensal flora. For example, it has been shown in children treated with beta-lactam antibiotics that the risk of isolation of resistant *Streptococcus pneumoniae* in the rhinopharyngeal flora was significantly increased for treatment durations longer than 5 days (Guillemot, Carbon *et al.* 1998). The same finding was made during the comparison of two amoxicillin treatments in children: the risk of isolation of resistant *Streptococcus pneumoniae* was higher with 40 mg/kg for 10 days than with 90 mg/kg for 5 days (Schrag, Peña *et al.* 2001). In this last study, a shorter treatment duration was combined with an increased dose (in other words, exposure to the antibiotic was more intense but briefer). This strategy has been proposed in other studies conducted in humans (Dunbar, Wunderink *et al.* 2003) and it could also contribute to improving the risk-benefit ratio for the use of antibiotics in animals.

Conclusions

The PK/PD approach provides no insight on the duration of the treatment. A moderate increase in the daily dose could, however, be at least partially offset by a decrease in the duration of treatment.

While the clinical efficacy will have to be verified (see 6.3), such an approach would limit the need for other studies (ecotoxicity, withdrawal period, etc.).

6.3. **Need for confirmation in the field**

As concluded previously in Section 2.1 History of the dosages, the requirements regarding preclinical and clinical trials to be carried out to demonstrate the efficacy of new antibiotics, at the doses and administration rates proposed by the MA applicant, increased sharply between 1991 and 2016, both to take account of scientific advances and to increase the level of evidence for validating the use of the product for the claimed indications. These requirements have led to an obvious restriction on the indications (mass of data to be provided, and therefore large number of studies to be produced), compared to the "older" products.

However, it is difficult to conceive that, for products that are now widely used in the field (so-called "older" products) and have proven their clinical benefit, new efficacy studies should be required under the regulations and according to current requirements. The clinical field studies as laid down in the guideline on antibiotics are cumbersome to put in place, with regard to both selection of candidates and follow-up. To be able to reach a conclusion in this type of study, the studies must be multicentre, randomised, blind, controlled and conducted with a sufficient number of animals. For certain indications, obtaining sufficient clinical field data can be difficult (complex diagnosis, rare infections).

Nevertheless, in some cases it may be necessary to turn to the users, and the scope of this option still needs to be defined.

6.4. **Methods of administration**

The method proposed thus far considers the intake of the medicinal product to be "perfect". For injection routes, this is hardly a problem, provided that good hygiene measures are followed and needles and syringes suited to the dosage are used.

In contrast, bioavailability studies by the oral route are all based on the forced drenching of animals. While pets receive their antibiotic by drenching, oral treatments of food-producing animals are most often collective and based on "voluntary" intake by the animals, either by a solid medium *via* medicated feed, or by a liquid medium *via* drinking water.

This induces a new individual variability that the method presented here cannot take into account.

6.4.1. Administration *via* feed

In France, medicated feeds are manufactured by approved plants, capable of providing guarantees on the dosage in active ingredient, and on the homogeneity of the premix/feed mixtures.

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

Revising the dosage upwards could lead to a decline in the palatability of the medicated feed manufactured and therefore a reduction in the dose ingested. However, in view of the usual rate of incorporation for medicated premixes (around 5g/kg) and their media (cereals, calcium carbonate), such an impact is highly unlikely.

6.4.2. Administration *via* drinking water

Drinking water has become the main vector for the administration of antibiotics in the form of oral powders and solutions: 51% in 2013 in the pig sector (compared with 21.9% of treatments in 1999) and up to 90% in poultry farming (ANMV, 2014).

Compared to feed, administration *via* drinking water has several advantages that are consistent with the objectives of the EcoAntibio 2017 Plan:

- Treatment durations are usually shorter than *via* feed. Exposure of commensal flora is thus lower.
- It is easier to target a smaller batch of animals (1 room only, rather than the entire herd) and thus to reduce overall exposure.
- Treatment can be started more quickly: the bacterial inoculum will be lower, meaning that the dosage and duration can be adapted accordingly.

In the case of water, the limitations and uncertainties are rather linked to the compliance of the dosage finally administered to the animals: accuracy of the dosage, quality of the medicated water and homogeneity.

The new MA dossiers should provide data on maximum solubility in the event that a metering pump is used (with a stock solution up to 20 times more concentrated) in two types of water, with different pH and hardness values, and at two different temperatures (4°C and 20°C):

- Soft water/low pH: pH from 5.0 to 7.0 and 60 mg/L or less of calcium carbonate (0 - 6°F)
- Hard water/high pH: pH from 8.0 to 9.0 and 180 to 350 mg/L of calcium carbonate (18 - 35°F).

Regarding the stability of the medicated water, this should be studied at the lowest nominal concentration on batches of different ages, in the two water qualities and at the two temperatures. Lastly, the impact of exposure to light should be studied but, in any case, regardless of the results, acceptable expiration in drinking water must not exceed 24 hours.

These new elements are important but do not however reflect the great variability encountered in the field. Many data are lacking: impact of the physico-chemical and bacteriological quality of the water, interaction with the various biocidal products used in animal husbandry, efficacy in the presence of biofilms, etc.

There are also many questions on the equipment used: several studies (Hémonic *et al.,* 2010) have shown possible differences between the different pumps as to the accuracy of the dosing. These differences are accentuated with lower flow rates (young animals and small numbers treated). This equipment should be regularly maintained and monitored to ensure distribution of the correct dosage.

Conclusions

The limitations and questions on administration *via* drinking water are a major issue for the sectors concerned. They are currently the subject of numerous debates and studies that go far beyond the context of the method proposed here.

In our case, at the very least, studies should be conducted, or reviewed if they exist already, on the solubility and stability in stock solution while complying with the new dosage adopted.

7. Consequences on animal health and public health

7.1. Animal tolerance

Applying the methodology presented may lead to an increase in dosages. This therefore raises the question of safety in the treated animals.

According to the information found in the SPCs, it should be noted that for three out of the four antibiotics families targeted, adverse effects are reported in the event of overdose. For example:

- oxytetracycline can cause growth delay, including delays in bone healing in pigs and calves;
- cardiotoxic effects have been reported with doxycycline in calves;
- florfenicol can cause reduced body weight gain in pigs.

The safety of amoxicillin is confirmed by the absence of adverse effects at five times the current therapeutic dose, which constitutes an acceptable safety margin for use of this antibiotic.

Revising the dosages could therefore directly affect the acceptability of the medicinal products concerned from the tolerance point of view. In the current MA revision process, tolerance should therefore be reassessed according to the methodology described in the VICH 43 guideline ("Target Animal Safety for Veterinary Pharmaceutical Products").

The most recent MA dossiers assess this tolerance in tolerance studies carried out on healthy animals. Generally, tolerance is tested for groups of animals receiving 3 and 5 times the maximum dose selected for the MA. When available, the results of these studies enable a first approximation to be made of the expected tolerance to the new dosage selected, provided that the increase in dose is still lower than the tests already performed.

For the oldest MAs, these studies may not exist. As laid down by the Guideline VICH 43, the literature review can provide some clarification, as well as an analysis of any pharmacovigilance cases. The clinical signs reported, along with their severity and frequency of occurrence, could contribute to the analysis of the "expected" tolerance of the new dosage selected. To date, the low volume of pharmacovigilance cases recorded for these antibiotics in the species studied means that these data cannot be exploited.

For an injection product, particular attention should be paid to possible reactions at the injection site.

Conclusions

If the revision of the dosage implies an increase in the dose, a re-assessment of the tolerance will be essential.

It could be done, for example:

from available data, if they exist,

where appropriate, according to the VICH 43 guideline, mainly by conducting studies,

and in all cases, with reinforced monitoring of the animals treated by the new defined dosages.

The three options presented are explained by the fact that some dossiers contain toxicology studies with doses far higher than the dose from the MA, and already provide data on the available therapeutic margin, as well as pharmacovigilance data, whereas in others, the available data on tolerance are insufficient for a re-assessment following an increased dose.

7.2. **Environment**

Assessment of the environmental risk for veterinary medicinal products is a mandatory step in the MA dossier. The different steps are described in a guideline that was reviewed in 2008 (EMEA/CVMP/ERA/418282/2005-Rev.1). It is carried out in two stages.

- **The first stage, or phase I** is purely theoretical and constitutes selecting drugs that are eligible for the second phase. It includes statutory exceptions (pets, for example). It is then necessary to determine a Predictable Environmental Concentration (PEC) for food-producing animals according to predefined production scenarios (intensive, indoor, in pasture farming, etc.) and taking into account the type of production and the use of the drug. The calculation method is based on a default percentage of the numbers treated according to the drugs and the uses considered. Thus, for antibiotics, the following values are used:

- 100% of the animals treated for administration in water or feed
- 100% of the animals treated for drying-off products
- 100% of the animals treated for foot rot in sheep
- 50% of the animals treated for injectable antibiotics (pigs, respiratory infections in cattle)
- 30% of the animals treated for products for the treatment of diarrhoea in calves.

  An essential point of this risk assessment is that it must be applied for each dossier, regardless of the active substance. This approach is very different from the one applied for plant protection products, for which one monograph per active substance is available.

  In this first step, calculation of the PEC considers the total release of active substance residues in the environment, without taking into account factors of biotransformation by the animal or degradation in manure, or possible change in the toxicity to non-target organisms. This calculation is dependent on the dose, the duration and the species considered (all these parameters are available in the SPC) as well as the maximum quantity of nitrogen that could be spread per hectare (by convention, in Europe, the value is 170 kg of nitrogen per hectare). The calculation results are then compared to the threshold value of 100 µg/kg of soil. If the calculated values are lower than the limit value, it is considered that the product will not have an unacceptable environmental impact and the environmental risk assessment is ended.

- **If this is not the case, the assessment passes to the second stage, or phase II**, which includes an assessment of the physico-chemical properties of the active substance, its behaviour in the environment and its biological effects.

  However, if a dossier provides acceptable studies demonstrating rapid and total biodegradability of the active substance in manure, the environmental risk assessment can be ended there, even if the threshold is exceeded. The guideline (EMA/CVMP/ERA/430327/2009) describes the experimental conditions for the studies to be performed, as well as how to interpret these studies. However, it should be noted that very few compounds are likely to undergo complete degradation and therefore be exempted from phase II in the event that the threshold is exceeded.

  The table below summarises the PEC calculation for amoxicillin administered orally at the dosage of 10 mg/kg for 5 days in cattle, poultry and pigs, and the consequences if the dosage is revised to 15 mg/kg/d and 48 mg/kg/d by reducing the duration of use from 5 days to 3.

Table 28: Theoretical expected changes to the PEC (in µg/kg soil) according the dosages claimed (in bold: PEC > 100 µg/kg)

Antimicrobial agent	Dosage	Calf	Dairy cow	Bovine >2yrs	Piglet
Amoxicillin	10 mg/kg/d, 5d	**285.6**	**160.6**	**291.4**	**443.5**
Amoxicillin	15 mg/kg/d, 5d	**428.4**	**240.8**	**437.1**	**651.7**
Amoxicillin	48 mg/kg/d, 3d	**822**	**462.4**	**839.3**	**1251.2**

In stage I, as the risk assessment is based on a total quantity used (dose x no. of days), any increase in the doses that is not offset by a decrease in the duration of treatment will immediately result in a proportional increase in the PECs and therefore a higher probability of exceeding the threshold value of 100 µg/kg soil, therefore leading to a refined risk assessment for many older products that have never previously undergone this analysis. At this stage, however, the pharmacokinetic data or data on degradation of antibiotics in manure can be used to refine this first step and thus avoid going to phase 2. This second step is today required for each MA dossier, which implies, in particular for older substances such as ampicillin or amoxicillin, numerous re-assessments for the revision of the dosages. The above example shows that for a substance such as amoxicillin, a revision of the dosages involves a refined analysis of the environmental risk for all the scenarios proposed.

At this stage, the Predictable No Effect Concentration (PNEC) will depend on the compound tested and the taxon considered. This PNEC is established from experimental data that are assigned a safety factor. However, as each industrial company has to provide a dossier to support its MA application, the results may differ from one dossier to another for the same active substance.

For antibiotics, it is generally accepted that cyanobacteria are more relevant than green algae, because they are generally more susceptible (Wollenberg *et al.*, 2000/Halling-Sorensen *et al.*, 2000). A recent study proposed standardising the tests for the use of different strains of cyanobacteria for this purpose. For example, amoxicillin presents very different toxicity values with high susceptibility of the cyanobacteria strains for which the EC_{50} (threshold of acute toxicity or modelled concentration for which effects are expected to be observed on 50% of the population of a species) values vary from 0.0037 mg/L to 56.3 mg/L (Diass *et al.,* 2015). On the other hand, the available data on green algae (*Pseudokirchneriella subcapitata*) give EC_{50} values > 2000 mg/L. Although the subject is important, very few published data are available on the susceptibility of micro- and macro-organisms in soil or water to older antibiotics.

Table 29: Published EC_{50} values in mg/L for several older antibiotics in the environment

	Cyanobacteria	Green alga	*Daphnia*/Shellfish*	Earthworm	Fish
Amoxicillin	0.037-56.3 (PNEC)	>2000*	3108		
Ampicillin		>2000*			
Doxycycline			5790 (PNEC)		
Oxytetracycline	0.21	0.342-11.18	22.64-805-870*	>2000	>1000 62-200*
Tetracycline	0.4-1.6	2.2-3.1**	>340	>2000	220
Trimethoprim	>1.6	16-110*	123	>2000	>100
Sulfamethoxazole		0.146*-1.53	123-205	>4000	>1000
Florfenicol		2.5-6.06	337-889*		

Crustaceans: *Daphnia magna* or **Artemia* spp., Green alga: *Lemna gibba* or **Pseudokirchneriella* sp., Fish: *Onchorhynchus mykiss* or **Danio rerio*

It is clear that the available data are partial, produced in very varied experimental conditions (EC_{50} values ranging from 24 to 96 h in *Daphnia* can be found) and do not meet current testing conditions. On the basis of these few values, however, it can be seen that certain compartments or organisms are highly susceptible (cyanobacteria), while others (earthworms, fish) appear less so.

Conclusion

An environmental risk assessment should therefore be proposed for many older medicinal products by refining stage I (pharmacokinetic data) or even by passing to stage II by providing experimental data to define the PNECs and probably also data for calculating a refined PEC. Indeed, it can be seen that certain species (cyanobacteria in particular) have a high susceptibility to antibiotics. The PECs originally proposed by the standard scenarios can be high depending on the antibiotic and the dosage selected, leading to calculations of the RQ (Risk Quotient = PEC/PNEC) >1, indicating a potentially high risk to the environment. It would then be necessary to provide additional studies, firstly to refine the PEC, taking into account data on the degradation of antibiotics in livestock manure, and secondly to refine the PNEC by conducting chronic toxicity studies.

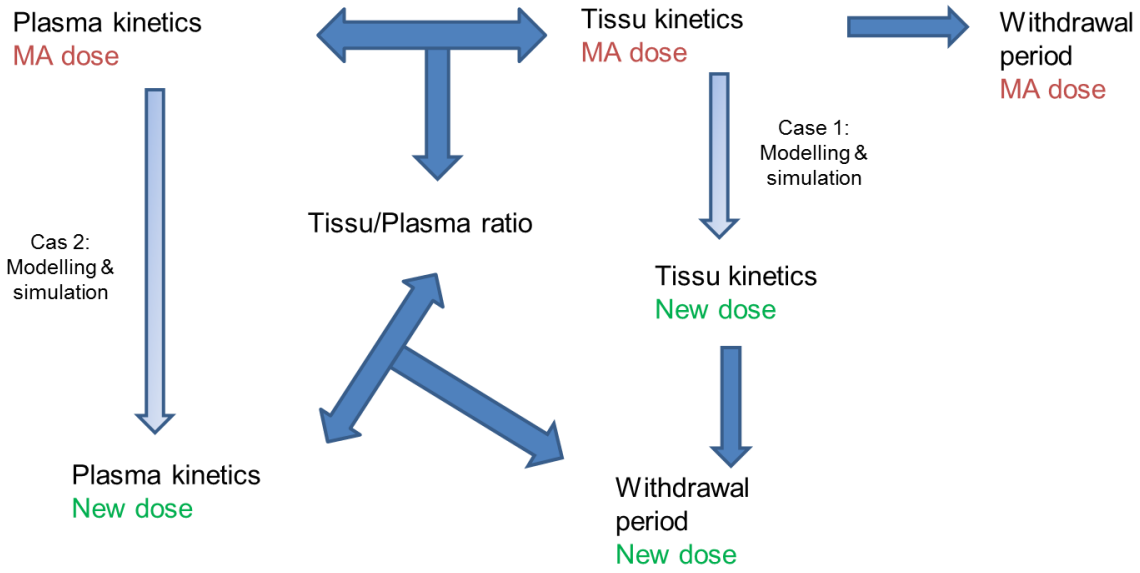
7.3. **Consumer**

A drug's withdrawal period is closely linked to its dosage.
By definition, the **withdrawal period (WP)** (Directive 2001/82/EC) is the period necessary between the last administration of the veterinary medicinal product to animals, under normal conditions of use, and the production of foodstuffs from such animals, in order to protect public health by ensuring that such foodstuffs do not contain residues in quantities in excess of the maximum residue limits for active substances laid down pursuant to Regulation (EC) No 470/2009.
The WP is determined from a **depletion study,** carried out in the target species at the **maximum dose recommended in the MA,** according to the VICH guidelines GL48 and GL49.
When the dose is changed, there may be an impact on the WP of the drug, and in any case these changes must be documented.
If the **dose is reduced**, the WP currently notified for a higher dose is still safe for the consumer. On the other hand, it may be unfavourable to the farmer (economic constraint).
If the **dose is increased,** it is necessary to check whether the withdrawal period established for the currently notified dose is still safe enough for the consumer. If this is not the case, a new WP needs to be established.

Methodology
The figure below explains the various possibilities for calculating the WP for a new dose.
Case 1: The tissue kinetics are known
A depletion model is established for the residual levels for the "MA" dose (data from the original dossier). The residual levels corresponding to the new dose are determined by simulation and the WP is calculated.
Case 2: The tissue kinetics are not known but the tissue/plasma ratios are known
The plasma kinetics of the active ingredient and/or its metabolites are known and available. A depletion model is established for the plasma concentrations for the "MA" dose. The plasma concentrations for the new dose are determined by simulation.
The WP can be calculated if the tissue/plasma ratios have been determined.

Limiting factors
To carry out the modelling and simulations, it is necessary to comply with the linearity hypothesis (proportionality of the pharmacokinetic processes).
It is also assumed that there is no accumulation, i.e. the impact of the duration of treatment on the establishment of the withdrawal period is probably very limited.
It should be noted that for injectable drugs, for which the injection site is very often the limiting tissue for determining a withdrawal period, it is not possible to estimate the impact of a change of dose on this withdrawal period.

Figure 11: Determination of the WP, according to whether the plasma or tissue data are known for a drug with an SPC and for non-injectable formulations



To illustrate these cases, two examples are presented.

Example 1, corresponding to Case 1
Active substance: florfenicol
Oral route: drinking water
Species: pigs
Dose: 10 mg/kg bw per day for 5 consecutive days
WP: 20 days

The depletion kinetics in the kidney and the liver (limiting tissues) were modelled using the data provided in the original dossier. Tissue concentrations were then simulated for different doses of florfenicol.
By comparing these simulated concentrations to the MRLs of florfenicol for the kidney and liver in pigs (500 and 2000 µg/kg, respectively), the first time point after the end of treatment, when all concentrations are below the MRLs, was then identified. The data thus obtained are summarised in the table below.

Table 30: First depletion kinetics time point, after the end of treatment (days), when the simulated tissue concentration is below the MRLs for the kidney and liver for the different doses tested

	Dose tested (mg/kg bw)				
	3	5	10[a]	20	30
Kidney Time point (days)	NC	4	9	14	17
Liver Time point (days)	2	5	11	16	19

NC: cannot be calculated
[a]: MA dose

This time point does not correspond to the WP. Indeed, a safety factor (safety span) of 30% may have been added because the study presents biases (number of time points, dose, weight of the

animals). In addition, the calculation procedure used (pragmatic method) is based on the time point when all residual concentrations are below the MRL, which may explain the difference between the calculated WP and that of the MA.
Conclusion:
On the basis of the tissue depletion kinetics, it is possible to calculate the WP corresponding to the new dosage. However the existence of a safety factor must also be taken into account when determining the value of this withdrawal period.

Example 2, corresponding to Case 2
Active substance: Amoxicillin
Oral route: drinking water
Species: pigs
Dose: 20 mg/kg bw per day for 5 consecutive days
WP: 14 days

The tissue/plasma ratios were those calculated at the 24-hour time point of the depletion study.. The mean of these ratios were determined and are presented in the table below.

Table 31: Calculation of the mean tissue/plasma ratios of amoxicillin in pigs following oral administration *via* drinking water at the dose of 20 mg of amoxicillin/kg bw

	Biological matrices				
	Muscle (µg/g)	Liver (µg/g)	Kidney (µg/g)	Fat + skin (µg/g)	Plasma (µg/ml)
Mean levels	0.0430	0.0425	0.8930	0.0363	0.0801
Tissue/plasma ratios	0.54	0.53	11.15	0.45	NC

NC: cannot be calculated

As the kidney is the limiting tissue, the tissue/plasma ratio defined in the kidney was selected i.e. 11.

A pharmacokinetic model was established for the plasma concentrations obtained for the "MA" dose.
The plasma concentrations were then simulated for different doses of amoxicillin.
Using the tissue/plasma ratio established for the limiting tissue and its MRL, it was possible to establish the virtual plasma value corresponding to the MRL in the kidney.
In our case, the MRL in the kidney is 50 µg/kg, and as the tissue/plasma ratio in the kidney is 11, the corresponding virtual plasma concentration is 0.0045 µg/ml. The simulated data for the new dose were compared to this value. The first due date after the end of treatment when the concentration is lower was selected.
The data thus obtained are summarised in the table below.

Table 32: First depletion kinetics time point when the plasma concentration simulated from the plasma model is below the virtual plasma value for different doses tested

	Doses tested (mg/kg bw)					
	20[a]	50	100	200	350	500
1st due date (days)	12	15	18	20	22	24

[a]: MA dose

This time point does not correspond to the WP. Indeed, according to the calculation procedure used in the initial MA dossier, a safety factor (safety span) of 30% may have been added.

Knowledge of the plasma kinetics and the tissue/plasma ratio enables a WP corresponding to the new dosage to be estimated. This time point needs to be corrected by a safety factor if the calculation procedure used in the original dossier integrates this factor.

Conclusion
The withdrawal period for new dosages can be estimated from tissue or plasma data provided that they were supplied in the original dossier. In addition, the quality of these data must be sufficient to carry out modelling and simulations.
All of these calculations are based on the linearity hypothesis.
According to the calculation procedure used in the original dossier, a safety factor may be added to the time point chosen as the withdrawal period for the new dosage.
However if the quality of the data is insufficient, confirmation of the value of the withdrawal period should be considered.
The withdrawal period for injectable commercial products cannot be estimated using these processes because the injection site is the limiting factor.

7.4. **Antimicrobial resistance**

The importance of revising the dosages is based on a need to optimise the doses of older antibiotics because repeated exposure to inappropriate concentrations represents a major risk in terms of antibiotic resistance. Alongside recommendations on good use, it is therefore necessary to rationalise the effective dose in order to optimise the dosage guaranteeing a clinical benefit for the animal while reducing the risks associated with the use of antibiotics.

An optimal dosage must be determined to ensure the efficacy of the treatment, but also to prevent the emergence, selection and/or dissemination of resistant micro-organisms in a bacterial population. Interindividual variability, in terms of exposure to the antibiotic, is certainly one of the risk factors with the greatest influence on the emergence of antimicrobial-resistant organisms. Accordingly, a dosage should be based on a PK/PD approach and should take the interindividual variability into account, regarding both pharmacokinetics and pharmacodynamics.

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

The current doses of "older" antibiotics generally provide a clinical benefit without this being optimised with regard to the risk of antibiotic resistance, whether it concerns the pathogenic bacteria targeted or the commensal microbiota.

8. Conclusions

In the framework of the WG's work on the methodology for revising the dosages of older antibiotics, several points were highlighted.

The analysis of the successive guidelines on the "Efficacy of veterinary antibiotics" published since the 1980s has shown a refinement of the regulatory framework in terms of data to be provided and how to conduct and exploit the studies, with a significant contribution made by PK/PD relationships in determining the doses, prior to their validation by clinical trials.

The PK/PD approach for determining doses was applied in the specific framework of antibiotic therapy for respiratory diseases in cattle and pigs. Tetracycline, oxytetracycline, doxycycline and amoxicillin were chosen because of their widespread use, as well as a more recent antibiotic, florfenicol.

It was found that the florfenicol doses determined according to the PK/PD approach are closer to the recommended doses than for the other, older, antibiotics tested. It is likely that alongside other more specific factors (see below), the increased regulatory requirements at the time florfenicol was placed on the market contributed to an improvement in the quality of the original dossier.

The PK/PD approach requires consolidated data to be available on two components, the pharmacokinetics of the antibiotics in the species considered, and the pathogens' susceptibility to antibiotics, in the form of MIC distributions.

The literature search carried out prior to the WG's work attempted to collect as exhaustively as possible relevant pharmacokinetic data (on clearance and bioavailability) for the antibiotic/animal species combinations in question. In the end, only the tetracycline/calf combination was excluded from the analyses due to a lack of usable data.

The effectiveness indices (PK/PD indices) are central to the PK/PD methodology applied to antibiotics, whether in the area of human or animal antibiotic therapy, because they are required to be predictive of a high probability of therapeutic success, in potentially varying clinical situations. The WG worked with the PK/PD index values available in the literature, and obtained from *in vitro* or *in vivo* models that were as relevant as possible with respect to the animal species (pigs, cattle), bacteria (*P. multocida*) and antibiotics (tetracyclines, amoxicillin, florfenicol) studied. There were few available data however, and besides the issue of older antibiotics, major progress will be made in animal antibiotic therapy when these PK/PD indices (and their threshold values) are determined from controlled clinical trials performed in the target species.

The major advantage of the PK/PD approach is that it makes it possible, when determining the doses, to take into account the variability of the susceptibility of the pathogenic bacterial strains and the inter-animal variability of the pharmacokinetics of the antibiotics. The different technical options that we tested enabled us to reach a conclusion on the optimal options:

- for the pharmacokinetic component, the interindividual variability of the processes of absorption and elimination can be taken into account through a classic literature analysis, without having to turn to analyses of population pharmacokinetics,
- for the pharmacodynamic component, the most rational approach involves integrating in the dose calculation the MIC distributions relating to the antibiotic/pathogenic bacterium combination.

The doses calculated according to the PK/PD approach mostly proved to be far higher than the current recommended doses, with the notable exception of florfenicol, the most recent antibiotic among the compounds studied (see above). The explanations for these much increased doses are:

- firstly, incomplete, or very low bioavailabilities (florfenicol had the highest bioavailability, with 80-100%),
- secondly, simultaneously taking into account the interindividual variability of the pharmacokinetics of the antibiotics and the dispersion of the MICs of the pathogens.

Given the greater dispersion of the MIC values of the bacterial strains compared with the individual values of the pharmacokinetic parameters, it is this first source of variability (the susceptibility of the pathogens) that has the greatest impact on the dispersion of the individual doses calculated. It will therefore be crucial to have databases (MIC distributions) that are as large and unbiased as possible.

Implementing the methodology will therefore involve collecting MICs that are representative of the bacteria potentially targeted by the antibiotic in the different geographical areas, farming systems, etc. Assuming that the disparity of the MICs obtained in the different conditions could lead to very large differences in doses, proposed doses adapted to specific epidemiological situations could be considered. Lastly, the identification of any change over time in the MICs of the pathogens should also lead to periodic dose re-assessments. It is also possible that the years of use of the older antibiotics have contributed to a gradual increase in the MICs to their current values, which is responsible for the large increase in doses calculated for these antibiotics.

Ultimately, MIC values obtained with validated methods and available within a reasonable timeframe would make the methodology for calculating individual doses directly accessible to the prescriber *via* searchable online expert systems. In most cases, the doses thus calculated would be lower than the corresponding Dose90% values.

Limitations to the proposed method were also identified by the WG:

- In the PK/PD methodology applied by the WG, the MIC was the only PD parameter included, because in most of the infections, it is representative of the activity of the antibiotic at the infectious site. The methodology does not therefore apply to cases where the MIC is not representative of the *in vivo* activity (intracellular bacterium, biofilm, etc.) or to cases where the antibiotic has an activity other than antibacterial.
- The methodology proposed by the WG draws on robust scientific bases. However, the efficacy results and thus the doses obtained when applying this methodology will need to be confirmed in the field under conditions that remain to be defined.
- The PK/PD approach cannot currently be used to propose an optimal duration for an antibiotic therapy. The increase in certain limits for daily doses could, however, be offset at least partially by a decrease in the treatment durations, if current durations allow. In this regard, the increase in individual doses could be largely offset by a decrease in the number of animals treated, through the generalisation of targeted intervention strategies, based on the stalls or pens occupied by the sick animals and the animals immediately around them, instead of treating a whole room or building.
- The PK variability introduced in the dose calculation only represented the interindividual variability for clearance and bioavailability. Integrating this variability had less influence on the doses than the introduction of MIC distributions. However, in the field, when antibiotics are administered by drinking water or in feed, there is another far greater source of variability: the quantity of antibiotic ingested. This variability should be quantified and integrated in order to assess its impact on the dose calculation.
- The limitations and questions on administration *via* drinking water are a major issue for the sectors concerned. They are currently the subject of numerous debates and studies that go well beyond the context of the method proposed here. In our case, at the very least, studies should be conducted, or reviewed if they exist already, on the solubility and stability in stock solution while complying with the new dosage adopted.

It should be pointed out that in spite of current doses sometimes being far lower than the calculated doses, reports of therapeutic failures remain rare in practice. This apparent contradiction could be related to field uses such as metaphylaxis that are more favourable to the antibiotic, a high proportion of spontaneous cures (studies of efficacy compared with a placebo are non-existent for older antibiotics), or the existence of effects other than antibacterial ones (immunomodulation, for example) that underlie the therapeutic efficacy of the antibiotics and are not taken into account by the PK/PD approach.

The consequences in terms of animal tolerance, the environment and consumer protection (residues and withdrawal period) were addressed by the WG:

- If the revision of the dosage implies an increase in the dose, a re-assessment of the tolerance will be essential. It could be done, for example:
    - from available data,
    - where appropriate, according to the VICH 43 guideline, mainly by conducting studies,
    - and with reinforced monitoring of the animals treated by the new defined dosages.
- It is highly likely that the proposed doses for the older antibiotics will lead to the threshold of 100 µg/kg for the PEC being exceeded. An environmental risk assessment should probably therefore be proposed by refining stage I (pharmacokinetic data) or even by passing to stage II by providing experimental data to define the PNECs and probably also data for calculating a refined PEC. Indeed, it can be seen that certain species (cyanobacteria in particular) have a high susceptibility to antibiotics. The PECs originally proposed by the standard scenarios can be high depending on the antibiotic and the dosage selected, leading to calculations of the RQ (Risk Quotient = PEC/PNEC) >1, thus indicating a potentially high risk to the environment. It would then be necessary to provide additional studies, firstly to refine the PEC, taking into account data on the degradation of antibiotics in livestock manure, and secondly to refine the PNEC by conducting chronic toxicity studies.
- Revision of the dosages would have to lead to revision of the withdrawal periods. The withdrawal period for new dosages can be estimated from tissue or plasma data provided that they were supplied in the original dossier. In addition, the quality of these data must be sufficient to carry out modelling and simulations. All of these calculations are based on the linearity hypothesis. According to the calculation procedure used in the original dossier, a safety factor may be added to the time point chosen as the withdrawal period for the new dosage. However if the quality of the data is insufficient, confirmation of the value of the withdrawal period should be considered. The withdrawal period for injectable commercial products cannot be estimated using these processes because the high values of residues at the injection site now need to be taken into account.

The dosage calculation methodology does not directly take into account the component on exposure of the commensal microbiota, in particular digestive, known to be one of the main gateways for transmission to humans of risks of antibiotic resistance. The upward re-assessment of the daily doses of antibiotics could lead to an increase in the quantities of antibiotics consumed, which is unacceptable in the current context. For this reason, this re-assessment should be accompanied by measures to offset, or even reverse, its effect on consumption.

In the area of antibiotic therapy, these measures should propose:

- A decrease in the treatment durations, if current durations allow,

- A drastic change in the methods of therapeutic intervention in farming, by seeking to reduce the numbers of animals treated when infectious episodes occur (increasingly early detection and diagnosis, "targeting" of treated animals, etc.).

However, the previous proposals are part of a broader context of optimised animal health management, with the establishment of actions seeking to optimise farming conditions and the robustness/resilience of the animals when faced with disease, and to develop alternatives in the areas of prevention or therapy.

Date of validation of the collective expertise appraisal report by the Working Group and the Expert Committee: 7 February 2017.

9. References

9.1. Publications

Adams, P. E., Varma, K. J., Powers, T. E., and Lamendola, J. F. 1987. Tissue concentrations and pharmacokinetics of florfenicol in male veal calves given repeated doses. American Journal of Veterinary Research 48: 1725-1732

Agersø, H., and Friis, C. 1998a. Bioavailability of amoxycillin in pigs. Journal of Veterinary Pharmacology and Therapeutics 21: 41-46

Agersø, H., and Friis, C. 1998b. Penetration of amoxycillin into the respiratory tract tissues and secretions in pigs. Research in Veterinary Science 64: 245-250

Ames, T. R., Larson, V. L., and Stowe, C. M. 1983. Oxytetracycline concentrations in healthy and diseased calves. American Journal of Veterinary Research 44: 1354-1357

Andes, A., and Craig, W. A. 2007. Pharmacokinetics and Pharmacodynamics of Tetracyclines. In: Antimicrobial Pharmacodynamics in Theory and Clinical Practice. Ed: Charles H. Nightingale *et al.*, 2nd edition

Baguer A.J., Jensen J., Henning Krogh P. 2000. Effects of the antibiotics oxytetracycline and tylosin on soil fauna. Chemosphere 40: 751-757

Bousquet, E., Nouws, J., Terlouw, P., and de Kleyne, S. 1998. Pharmacokinetics of doxycycline in pigs following oral administration in feed. Veterinary Research 29: 475-485

Bretzlaff, K. N., Neff-Davis, C. A., Ott, R. S., Koritz, G. D., Gustafsson, B. K., and Davis, L. E. 1987. Florfenicol in non-lactating dairy cows: pharmacokinetics, binding to plasma proteins, and effects on phagocytosis by blood neutrophils. Journal of Veterinary Pharmacology and Therapeutics 10: 233-240

Burrows, G. E., Barto, P. B., and Martin, B. 1987. Comparative pharmacokinetics of gentamicin, neomycin and oxytetracycline in newborn calves. Journal of Veterinary Pharmacology and Therapeutics 10: 54-63

Cantón, R., and Morosini, M.I. 2011. Emergence and spread of antibiotic resistance following exposure to antibiotics. FEMS Microbiology Reviews, 35: 977-991

Cromley CW, Hagely JM. Clinical studies on the effectiveness of terramycin in large and small animals. Veterinary medicine. June 1951. 219-221.

del Castillo, J. R. E., Laroute, V., Pommier, P., Zémirline, C., Keïta, A., Concordet, D., and Toutain, P.-L. 2006. Interindividual variability in plasma concentrations after systemic exposure of swine to dietary doxycycline supplied with and without paracetamol: a population pharmacokinetic approach. Journal of Animal Science 84: 3155-3166

de Craene, B., Deprez, P., d'Haese, E., Nelis, H. J., van den Bossche, W., and de Leenheer, A. P. 1997. Pharmacokinetics of florfenicol in cerebrospinal fluid and plasma of calves. Antimicrobial Agents and Chemotherapy 41: 1991-1995

Dias E., Oliveira M., Jones-Dias D., Vasconcelos V., Ferreira E., Manageiro V., Canica M. 2015. Assessing the antibiotic susceptibility of freshwater Cyanobacteria spp. Frontiers in Microbiology 6: 1-11

Eguchi K., Nagase H., Ozawa M., Endoh Y.S., Goto K., Hirata K., Miyamoto K., Yoshimura H. 2004. Evaluation of antimicrobial agents for veterinary use in the ecotoxicity test using microalgae. Chemosphere 57: 1733-1738

EMA/CVMP/261180/2012. Guideline for the demonstration of efficacy for veterinary medicinal products containing antimicrobial substances. Committee for Medicinal Products for Veterinary Use (CVMP).

Errecalde, J. O., Mestorino, N., and Mariño, E. L. 1997.The effects of the method of calculation on the evaluation of the pharmacokinetic parameters of oxytetracycline after intravenous administration to calves. Veterinary Research Communications 21: 273-281

Ferran A., Liu JJ, Toutain PL, Bousquet-Mélou A. 2016. Frontiers in Microbiology. Comparison of the In vitro Activity of Five Antimicrobial Drugs against Staphylococcus pseudintermedius and Staphylococcus aureus Biofilms. Front. Microbiol., 02 August 2016

Fischer CD, Beatty JK, Zvaigzne CG, Morck DW, Lucas MJ, Buret AG. 2011. Anti-Inflammatory benefits of antibiotic-induced neutrophil apoptosis: tulathromycin induces caspase-3-dependent neutrophil programmed cell death and inhibits NF-kappaB signaling and CXCL8 transcription. Antimicrob Agents Chemother. 55(1):338-48.

Godoy, C., Castells, G., Martí, G., Capece, B. P. S., Colom, H., and Cristòfol, C. 2010. Influence of a pig respiratory disease on the pharmacokinetic behaviour of amoxicillin after oral ad libitum administration in medicated feed. Journal of Veterinary Pharmacology and Therapeutics 34: 235-276

Goncalves Ferreira C.S., Nunes B.A., Henriques-Almeida J.M., Guilhermino L. 2007. Acute toxicity of oxytetracycline and florfenicol to the microalgae tetraselmis chuii and to the crustacean Artemia parthenogenetica. Ecotoxicology and Environemntal Safety 67: 452-458

Gonzalez-¨leiter M., Gonzalo S., Rodea-Palomares I., Leganes F., Rosal R., Boltes K., Marco E., Fernandez-Piñas F. 2013. Toxicity of five antibiotics and their mixtures towards photosynthetic aquatic organisms: implications for environmental risk assessment. Water Research 47: 2050-206

Goossens, J., Vandenbroucke, V., Pasmans, F., de Baere, S., Devreese, M., Osselaere, A., Verbrugghe, E., Haesebrouck, F., de Saeger, S., Eeckhout, M., Audenaert, K., Haesaert, G., de Backer, P., and Croubels, S. 2012. Influence of mycotoxins and mycotoxin adsorbing agent on the oral bioavailability of commonly used antibiotics in pigs. Toxins 4: 281-295

Gutiérrez, L., Ocampo, L., Espinosa, F., and Sumano, H. 2013. Pharmacokinetics of an injectable long-acting parenteral formulation of doxycycline hyclate in pigs. Journal of Veterinary Pharmacology and Therapeutics 37: 83-89

Halling-Sorensen B., Lützhoft H.C.H., Anderse H.R., Ingerslev F. 2000. Environmental risk assessment of antibiotics: comparison of mecillinam, trimethroprim and ciprofloxaci. Journal of Antimicrobial Therapy. 46: 53-58

Hernandez, E., Rey, R., Puig, M., Garcia, M. A., Solans, C., and Bregante, M. A. 2005. Pharmacokinetics and residues of a new oral amoxicillin formulation in piglets: a preliminary study. The Veterinary Journal 170: 237-242

Hong-Thih L., Jung-Hsin H., Chyong-Ing S., Chun-Lang C. 2009. Effetcs of chloramphenicol, flrofenicol and thiamphenicol on growth of algae Chlorella pyrenoidosa, Isochrysis galbana and tetraselmis chui. Ecotoxicology and Enevironmental Safety 72: 329-334

Isidori M., Lavorgna M., Nardelli A., Pascarella L., Parrella A. 2005. Toxic and genotoxic evaluation of six antibiotics on non-target organisms. Science of the Total Environment 346: 87-98

Kniffen, T. S., Bane, D. P., Hall, W. F., Koritz, G. D., and Bevill, R. F. 1989. Bioavailability, pharmacokinetics, and plasma concentration of tetracycline hydrochloride fed to swine. American Journal of Veterinary Research 50: 518-521

Kolodziejska M., Maszkowska J., Bialk-Bielinska A., Steudte S., Kumirska J., Stepnowski P., Stolte S. 2013. Aquatic toxicity of four veterinary drugs commonly applied in fish farming and animal husbandry. Chemosphere 92: 1253-1259

Krasucka, D., and Kowalski, C. J. 2010. Pharmacokinetic parameters of amoxicillin in pigs and poultry. Acta Poloniae Pharmaceutica. 6: 729-732

Kristoffersson, A.N., David-Pierson, P., Parrott, N.J., Kuhlmann, O., Lave, T., Friberg, L.E., and Nielsen, E.I. 2016. Simulation-Based Evaluation of PK/PD Indices for Meropenem Across Patient Groups and Experimental Designs. Pharmaceutical Research 33: 1115-1125

Kumar, R., and Malik, J. K. 1998. Some pharmacokinetic parameters and dosage regimens for a long-acting formulation of oxytetracycline in 6- to 8-month-old male calves. Veterinary Research Communications 22: 533-544

Kumar, R., and Malik, J. K. 1999. Influence of experimentally induced theileriosis (Theileria annulata) on the pharmacokinetics of a long-acting formulation of oxytetracycline (OTC-LA) in calves. Journal of Veterinary Pharmacology and Therapeutics 22: 320-326

Kumar, R., and Malik, J. K. 2001. Effects of multiple injections of Escherichia coli endotoxin on the pharmacokinetics and dosage regimens of a long-acting formulation of oxytetracycline (OTC-LA) in cross-breed calves. Veterinarski Arhiv. 71: 245-263

Lees, P., Concordet, D., Aliabadi F.S., and Toutain, P.-L. 2006. Drug selection and optimization of dosage schedules to minimize antimicrobial resistance. In: Antimicrobial Resistance in Bacteria of Animal Origin. Ed. by Frank M. Aarestrup, ASM Press, Washington, D.C.

Lees, P., Pelligand, L., Illambas, J., Potter, T., Lacroix, M., Rycroft, A., and Toutain P.-L. 2015. Pharmacokinetic/pharmacodynamic integration and modelling of amoxicillin for the calf pathogens Mannheimia haemolytica and Pasteurella multocida. Journal of Veterinary Pharmacology and Therapeutics 38: 457-470

Liu, J., Fung, K.-F., Chen, Z., Zeng, Z., and Zhang, J. 2003. Pharmacokinetics of florfenicol in healthy pigs and in pigs experimentally infected with Actinobacillus pleuropneumoniae. Antimicrobial Agents and Chemotherapy 47: 820-823

Lobell RD, Varma KJ, Johnson JC, Sams RA, Gerken DF, Ashcraft SM. 1994. Pharmacokinetics of florfenicol following intravenous and intramuscular doses to cattle. Journal of Veterinary Pharmacology and Therapeutics 17: 253-8

Maaland, M. G., Mo, S. S., Schwarz, S., and Guardabassi, L. 2015. In vitro assessment of chloramphenicol and florfenicol as second-line antimicrobial agents in dogs. J. Vet. Pharmacol. Therap. 38: 443–450.

Magdaleno A., Saenz M.E., Juarez A.B., Moretton J. 2015. Effects of six antibiotics and their ninary mixtures on growth of Pseudokirchneriella subcapitata. Ecotoxicology and Environmental Safety 113: 72-78

Manning, L., Laman, M., Greenhill, A.R., Michael, A., Siba, P., Mueller, I. & Davis, T.M.E. 2011. Increasing chloramphenicol resistance in Streptococcus pneumoniae isolates from Papua New Guinean children with acute bacterial meningitis. Antimicrobial Agents and Chemotherapy, 55: 4454–4456.

Martínez-Larrañaga, M. R., Anadón, A., Martínez, M. A., Díaz, M. J., Frejo, M. T., Castellano, V. J., Isea, G., and de la Cruz, C. O. 2004. Pharmacokinetics of amoxicillin and the rate of depletion of its residues in pigs. Veterinary Record 154: 627-632

Meijer, L. A., Ceyssens, G. F., de Jong, W. Th., de Grève, B. I. J. A. C. 1993a. Three phase elimination of oxytetracycline in veal calves; the presence of an extended terminal elimination phase. Journal of Veterinary Pharmacology and Therapeutics 16: 214-222

Meijer, L. A., Ceyssens, K. G. F., de Grève, B. I. J. A. C., de Bruijn, W. 1993b. Pharmacokinetics and bioavailability of doxycycline hyclate after oral administration in calves. Veterinary Quarterly 15: 1-5

Mevius, D. J., Vellenga, L., Breukink, H. J., Nouws, J. F. M., Vree, T. B., and Driessens, F. 1986b. Pharmacokinetics and renal clearance of oxytetracycline in piglets following intravenous and oral administration. Veterinary Quarterly 8: 274-284

Mevius, D. J., Nouws, J. F. M., Breukink, H. J., Vree, T. B., Driessens, F. , and Verkaik, R. 1986a. Comparative pharmacokinetics, bioavailability and renal clearance of five parenteral oxytetracycline-20% formulations in dairy cow. Veterinary Quarterly 8: 285-294

Nielsen, P., and Gyrd-Hansen, N. 1996. Bioavailability of oxytetracycline, tetracycline and chlortetracycline after oral administration to fed and fasted pigs. Journal of Veterinary Pharmacology and Therapeutics 19: 305-311

Nielsen, E.I., Cars, O., and Friberg, L.E. 2011. Pharmacokinetic/pharmacodynamic (PK/PD) indices of antibiotics predicted by a semimechanistic PKPD model: a step toward model-based dose optimization. Antimicrobial Agents and Chemotherapy 55: 4619-4630

Nouws, J. F. M., van Ginneken, C. A. M., and Ziv, G. 1983. Age-dependent pharmacokinetics of oxytetracycline in ruminants. Journal of Veterinary Pharmacology and Therapeutics 6: 59-66

Nouws, J. F. M., and Vree, T. B. 1983. Effect of injection site on the bioavailability of an oxytetracycline formulation in ruminant calves. Veterinary Quarterly 5: 165-170

Nouws, J. F. M., Vree, T. B., Termond, E., Lohuis, J., van Lith, P., Binkhorst, G. J., and Breukink, H. J. 1985. Pharmacokinetics and renal clearance of oxytetracycline after intravenous and intramuscular administration to dairy cows. Veterinary Quarterly 7: 296-305

Papich and Riviere. 2009. Tetracyclin antibiotics. In: Veterinary Pharmacology & Therapeutics 9th Edition. Ed: Jim E. Riviere, Marc G. Papich.

Park S., Choi K. 2008. Hazard assessment of commonly used agricultural antibiotics on aquatic ecosystems. Ecotoxicology 17: 526-538

Pijpers, A., Schoevers, E. J., van Gogh, H., van Leengoed, L. A. M. G., Visser, I. J. R., van Miert, A. S. J. P. A. M., and Verheijden, J. H. M. 1990. The pharmacokinetics of oxytetracycline following intravenous administration in healthy and diseased pigs. Journal of Veterinary Pharmacology and Therapeutics 13: 320-326

Pijpers, A., Schoevers, E. J., van Gogh, H., van Leengoed, L. A. M. G., Visser, I. J. R., van Miert, A. S. J. P. A. M., and Verheijden, J. H. M. 1991. The influence of disease on feed and water consumption and on pharmacokinetics of orally administered oxytetracycline in pigs. Journal of Animal Science 69: 2947-2954

Pilloud, M. 1973. Pharmacokinetics, plasma protein binding and dosage of oxytetracycline in cattle and horses. Research in Veterinary Science 15: 224-230

Prats, C., el Korchi, G., Giralt, M., Cristòfol, C., Peña, J., Zorilla, I., Saborit, J., and Pérez, B. 2005. PK and PK/PD of doxycycline in drinking water after therapeutic use in pigs. Journal of Veterinary Pharmacology and Therapeutics 28: 525-530

Publicly available assessment report for a veterinary medicinal product NUFLOR 300 mg/ml solution for injection for cattle and sheep

ANSES 2014 report on Antimicrobial Resistance

RESAPATH 2014 report

Rey, J. F., Laffont, C. M., Croubels, S. C., de Backer, P., Zemirline, C., Bousquet, E., Guyonnet, J., Ferran, A. A., Bousquet-Mélou, A., Toutain, P.-L. 2014. Use of Monte Carlo simulation to determine pharmacodynamic cutoffs of amoxicillin to establish a breakpoint for antimicrobial susceptibility testing in pigs. American Journal of Veterinary Research 75: 124-131

Reyns, T., de Boever, S., Schauvliege, S., Gasthuys, F., Meissonnier, G., Oswald, I., de Baecker, P., and Croubels, S. 2008. Influence of administration route on the biotransformation of amoxicillin in the pig. Journal of Veterinary Pharmacology and Therapeutics 32: 241-248

Riond, J.-L., Tyczkowska, K., and Riviere, E. 1989. Pharmacokinetics and metabolic inertness of doxycycline in calves with mature or immature rumen function. American Journal of Veterinary Research 50: 1329-1333

Riond, J.-L., and Riviere, J. E. 1990. Pharmacokinetics and metabolic inertness of doxycycline in young pigs. American Journal of Veterinary Research 51: 1271-1275

Rodrigues, C. A., Hussni, C. A., Nascimento, E. S., Esteban, C. , and Perri, S. H. V. 2009. Pharmacokinetics of tetracycline in plasma, synovial fluid and milk using single intravenous and single intravenous regional doses in dairy cattle with papillomatous digital dermatitis. Journal of Veterinary Pharmacology and Therapeutics 33: 363-370

Rosa Pino M., Val J., Mainar A.M., Zuriaga E., Español C., Langa E. 2015. Acute toxicological effects on the earthworm Eisenia fetida of 18 common pharmaceuticals in artificial soil. Science of the Total Environment 518-519: 225-237

Sanders, P., and Guillot, P. 1990. Etude de la bioéquivalence des voies d'administration intramusculaire et sous-cutanée pour une formulation d'oxytétracycline chez le taurillon. Annales de Recherche Vétérinaire 21,57s-65s

Schifferli, D., Galeazzi, R. L., Nicolet, J., and Wanner, M. 1982. Pharmacokinetics of oxytetracycline and therapeutic implications in veal calves. Journal of Veterinary Pharmacology and Therapeutics 5: 247-257

Sidhu, P., Rassouli, A., Illambas, J., Potter, T., Pelligand, L., Rycroft, A., and Lees, P. 2013. Pharmacokinetic-pharmacodynamic integration and modeling of florfenicol in calves. Journal of Veterinary Pharmacology and Therapeutics 37: 231-242

Singh, R. P., Srivastava, A. K., Sharma, S. K., and Nauriyal, D. C. 1998. Influence of Escherichia coli endotoxin induced fever on the pharmacokinetics and dosage regimen of oxytetracycline in cross-bred calves. Acta Veterinaria Hungarica 46: 95-100

Soback, S., Bor, A., Kurtz, B., Paz, R., and Ziv, G. 1987. Clavulanate-potentiated amoxycillin: in vitro antibacterial activity and oral bioavailability in calves. Journal of Veterinary Pharmacology and Therapeutics 10: 105-113

Soraci, A.L., 2014, Exposure variability of fosfomycin administered to pigs in food or water: Impact of social rank. Research in Veterinary Science; Volume 96, Issue 1, February 2014, Pages 153-159

Sutherland R, Comber KR, Osborne CD. Activité in vivo de l'amoxicilline. La nouvelle presse médicale. 15 October 1975. 4, n°34

Tam, V.H., Louie, A., Fritsche, T.R., Deziel, M., Liu, W., Brown, D.L., Deshpande, L., Leary, R., Jones, R.N., Drusano, G.L. . 2007. Impact of drug-exposure intensity and duration of therapy on the emergence of Staphylococcus aureus resistance to a quinolone antimicrobial. Journal of Infectious Diseases 195 (12), pp. 1818-1827

Toutain, P.-L., and Raynaud, J.-P. 1983. Pharmacokinetics of oxytetracycline in young cattle: comparison of conventional vs long-acting formulations. American Journal of Veterinary Research 44: 1203-1209

Ucelli, V., Deleforge, J., and Boisrame, B. 1988. Pharmacocinétique et tolérance locale comparées de deux formulations d'oxytétracycline chez la vache. Recueil de Médecine Vétérinaire 164: 939-943

Vargas-Estrada, D., Gracia-Mora, J., and Sumano, H. 2008. Pharmacokinetic study of an injectable long-acting parenteral formulation of doxycycline hyclate in calves. Research in Veterinary Science 84: 477-482

Varma, K. J., Adams, P. E., Powers, T. E., Powers, J. D., and Lamendola, J. F. 1986. Pharmacokinetics of florfenicol in veal calves. Journal of Veterinary Pharmacology and Therapeutics 9: 412-425

Villa, R., Prandin, E., Montesissa, C. & Silvano, C. 1994. Serum protein binding of ß-lactamine derivatives in farm and domestic animals. Journal of Veterinary Pharmacology and Therapeutics 17: 216–217

Voorspoels, J., d'Haese, E., de Craene, A., Vervaet, C., de Riemaecker, D., Deprez, P., Nelis, H., and Remon, J. P. 1999. Pharmacokinetics of florfenicol after treatment of pigs with single oral or intramuscular doses or with medicated feed for three days. Veterinary Record 145: 397-399

Wollenberger L., Halling-Sorensen B., Kusk K.O. 2000. Acute and chronic toxicity of veterinary antibiotics to Daphnia magna. Chemosphere 40: 723-730

Xia, W., Nielsen, P., and Gyrd-Hansen, N. 1983a. Oxytetracyclines in cattle. A comparison between a conventional and a long-acting preparation. Acta Veterinaria Scandinavica 24: 120-128

Xia, W., Gyrd-Hansen, N., and Nielsen, P. 1983b. Comparison of pharmacokinetic parameters for two oxytetracycline preparations in pigs. Journal of Veterinary Pharmacology and Therapeutics 6: 113-120

Yang, F., Liu, H. W., Li, M., Ding, H. Z., Huang, X. H., and Zeng, Z. L. 2012. Use of a Monte Carlo analysis within a physiologically based pharmacokinetic model to predict doxycycline residue withdrawal time in edible tissues in swine. Food Additives & Contaminants Part A 29: 73-84

Ziv, G., and Sulman, F. G. 1974. Analysis of pharmacokinetic properties of nine tetracycline analogues in dairy cows and ewes. American Journal of Veterinary Research 35: 1197-1201

9.2. **Standards**

NF X 50-110 (May 2003) Quality in expertise activities – General requirements of competence for an expertise activity. AFNOR (classification index X 50-110).

9.3. **Legislation and Regulations**

Directive 81/852/EEC of 1981 (on the approximation of the laws of the Member States relating to analytical, pharmaco-toxicological and clinical standards and protocols in respect of the testing of veterinary medicinal products)

Directive 2001/82/EC amended by Directive 2004/28/EC.

1991 Guideline on antimicrobials: Antimicrobials for general veterinary use in target species (excluding intramammary preparations) 7AE8a

2001 Guideline on antibiotics: EMEA/CVMP/627/01

2001 Guideline on antibiotics, revised in 2016: EMA/CVMP/627/2001-Rev.1

Guideline EMEA/CVMP/ERA/418282/2005-Rev.1

Guideline EMA/CVMP/ERA/430327/2009

VICH Guideline 43 "Target animal safety for veterinary pharmaceutical products"

Act no 75-409 and its implementing decrees of 1977 legislating on the requirement for an MA for each drug

Measure 17 of the national EcoAntibio plan

Ecoantibio 2017 Plan

Regulation (EC) No 470/2009 of the European Parliament and of the Council of 6 May 2009 laying down Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin, repealing Council Regulation (EEC) No 2377/90 and amending Directive 2001/82/EC of the European Parliament and of the Council and Regulation (EC) No 726/2004 of the European Parliament and of the Council

VICH GL 48 Studies to evaluate the metabolism and residue kinetics of veterinary drugs in food-producing animals: Marker residue depletion studies to establish product withdrawal periods - January 2015

VICH GL 49 Studies to evaluate the metabolism and residue kinetics of veterinary drugs in food-producing animals: Validation of analytical methods used in residue depletion studies - February 2011

ANNEXES

Annex 1: Formal request letter

French Agency for Food, Environmental and Occupational Health & Safety (ANSES)

2014-SA-0080

Decision No 2014-03-101

INTERNAL REQUEST

The Director General of the French Agency for Food, Environmental and Occupational Health & Safety (ANSES),

Having regard to the Public Health Code, and in particular its Article L. 1313-3 giving ANSES the prerogative to issue an internal request on any question with a view to accomplishing its missions,

Has decided:

Article 1 – The French Agency for Food, Environmental and Occupational Health & Safety is issuing an internal request to conduct an expert appraisal whose characteristics are listed below.

1.1 Themes and objectives of the expert appraisal

Methodology for revising the dosages of older antibiotics

1.2 Background of the internal request

Measure 17 of the National EcoAntibio Plan places an emphasis on maintaining older antibiotic compounds. At the same time, data reflecting conditions of use in the field (primarily in food-producing animals), both in France and at European level, have shown that the dosages determined when the MAs were granted 30 or 40 years ago are no longer suited to the bacterial strains encountered in the targeted diseases. In addition, knowledge of the pharmacokinetics and pharmacodynamics of antibiotics has evolved, as has the way in which the risk of selection of resistance is taken into account in the dosage regimen.

Today, it is important to ensure that antibiotics are placed on the market at effective doses, as stated in the SPCs of the MAs, for the purposes of animal health. But it is also important to guarantee doses that help limit the selection of resistant bacteria, for the purposes of public health. This has led to a need to reassess these older compounds on the basis of a benefit-risk ratio that encompasses their efficacy, animal tolerance and consumer safety, but also their impact on commensal flora and the environment.

1.3 Questions on which the expert appraisal work will focus

Phase 1 – Review of the literature data relating to the pharmacodynamic and pharmacokinetic profiles of older antibiotic compounds. Selection of candidate compounds.

This first step should lead to the selection of candidate compounds for revision: an observed lack of efficacy and/or a lowering in the susceptibility of pathogenic germs are the first elements, but it will also be necessary to take into account the impact of an increase in the dosage on the bacterial floras that are critical for antibiotic resistance, as well as the major issue for collective oral administration, with the interindividual variability of exposure within the group, inherent to this method of administration. The use observed in practice by veterinarians will also provide useful information.

Phase 2 – Determine a reassessment methodology based on the pharmacokinetic/pharmacodynamic (PK/PD) approach applied to antibiotics.

For antibiotics for which sufficient data are available, the methodology should attempt to define the objectives to be achieved: therapeutic indications and thresholds of susceptibility.

Where appropriate, define the need for external data (CRD project) relating to studies in the animal phase, in order to determine, for a given antibiotic, the most relevant substitution criteria to take into account in the reassessment methodology.

Phase 3 – Where appropriate, develop a Research and Development Agreement (CRD) in order to model a PK/PD animal profile, to quantify the variability of exposure related to collective oral routes.

Phase 4 – A methodology for reassessing dosage regimens could be proposed to the European bodies. The case-by-case revision of the dosages should be carried out at the European level.

1.4 Estimated duration of the expert appraisal

Phase 1-2-4: 2nd half of 2015.

Phase 3: CRD during 2016.

Article 2 – The proposed methodology will be submitted to the Director of the ANMV on completion of the work.

Signed at Maisons-Alfort, on 28 March 2014.

Marc MORTUREUX

[signature]

Director General

Annex 2: Tracking of report updates

Date	Version	Page	Description of the change
	01		version for meeting of 22/03/2016
	02		version for meeting of 07/06/2016
	03		version for meeting of 13/09/2016
	04		version for meeting of 26/10/2016
	05		version following meeting of 26/10/2016
	06		version integrating the conclusion
16/11/2016	07		version for provision to the CES SABA in view of the meeting of 06/12/2016
24/01/2017	08		version for provision to the CES SABA in view of the meeting of 07/02/2017
06/02/2017	09		version updated following comments from the reread of two members of the CES SABA
17/02/2017	10		version modified following the most recent comments by the CES SABA on 07/02/2017
09/03/2017	Final		Final version

Annex 3: Literature analysis tables

Table 33: Number of scientific articles and reports from industry used for the analyses

	Calves	Pigs	Total antibiotic
Tetracycline	**2** *Ziv and Sulman (1974)** *Rodrigues et al. (2001)*	**2** *Kniffen et al. (1989)* *Nielsen and Gyrd-Hansen (1996)**	**4**
Oxytetracycline	**19** *Pilloud (1973)* *Ziv and Sulman (1974)** *Schifferli et al. (1982)* *Ames et al. (1983)* *Nouws and Vree (1983)* *Nouws et al. (1983)* *Toutain and Raynaud (1983)* *Xia et al. (1983a)* *Nouws et al. (1985)* *Mevius et al. (1986a)* *Burrows et al. (1987)* *Ucelli et al. (1988)* *Sanders and Guillot (1990)* *Meijer et al. (1993a)* *Errecalde et al. (1997)* *Kumar and Malik (1998)* *Singh et al. (1998)* *Kumar and Malik (1999)* *Kumar and Malik (2001)*	**5** *Xia et al. (1983b)* *Mevius et al. (1986b)* *Pijpers et al. (1990)* *Pijpers et al. (1991)* *Nielsen and Gyrd-Hansen (1996)**	**24**
Doxycycline	**3** *Riond et al. (1989)* *Meijer et al. (1993b)* *Vargas et al. (2008)*	**9** *Dossier 9601 (1985)* *Riond and Riviere (1990)* *Dossier Veprol (1995)* *Bousquet et al. (1998)* *Prats et al. (2005)* *Goossens et al. (2012)* *Yang et al. (2012)* *Gutiérrez et al. (2013)* *del Castillo et al. (2014)*	**12**
Amoxicillin	**1** *Soback et al. (1987)*	**7** *Agersø and Friis (1998a)* *Agersø and Friis (1998b)* *Martínez-Larrañaga et al. (2004)* *Hernandez et al. (2005)* *Reyns et al. (2008)* *Godoy et al. (2010)* *Krasucka and Kowalski (2010)*	**8**
Florfenicol	**4** *Varma et al. (1986)* *Adams et al. (1987)* *Bretzlaff et al. (1987)* *de Craene et al. (1997)*	**2** *Voorspoels et al. (1999)* *Liu et al. (2003)*	**6**
Total species	**29 (28)**	**25 (24)**	**54 (52)**

The underlined references correspond to the reports from industry.

*The references followed by * are references cited twice (tetracycline and oxytetracycline in the same article). The totals in brackets are calculated by counting these articles only once.*

Table 34: Publication years of the scientific articles and reports from industry

	Calves		**Pigs**	
1971-1975	*Pilloud (1973)* *Ziv and Sulman (1974)*	OTC TC/OTC	-	
1976-1980	-		-	
1981-1985	*Schifferli et al. (1982)* *Ames et al. (1983)* *Nouws and Vree (1983)* *Nouws et al. (1983)* *Toutain and Raynaud (1983)* *Xia et al. (1983a)* *Nouws et al. (1985)*	OTC OTC OTC OTC OTC OTC OTC	*Xia et al. (1983b)* *Dossier 9601 (1985)*	OTC DOXY
1986-1990	*Mevius et al. (1986a)* *Varma et al. (1986)* *Burrows et al. (1987)* *Soback et al. (1987)* *Adams et al. (1987)* *Bretzlaff et al. (1987)* *Ucelli et al. (1988)* *Riond et al. (1989)* *Sanders and Guillot (1990)*	OTC FLOR OTC AMOX FLOR FLOR OTC DOXY OTC	*Mevius et al. (1986b)* *Kniffen et al. (1989)* *Pijpers et al. (1990)* *Riond and Riviere (1990)*	OTC TC OTC DOXY
1991-1995	*Meijer et al. (1993a)* *Meijer et al. (1993b)*	OTC DOXY	*Pijpers et al. (1991)* *Dossier Veprol (1995)*	OTC DOXY
1996-2000	*Errecalde et al. (1997)* *de Craene et al. (1997)* *Kumar and Malik (1998)* *Singh et al. (1998)* *Kumar and Malik (1999)*	OTC FLOR OTC OTC OTC	*Nielsen and Gyrd-Hansen (1996)* *Bousquet et al. (1998)* *Agersø and Friis (1998a)* *Agersø and Friis (1998b)* *Voorspoels et al. (1999)*	TC/OTC DOXY AMOX AMOX FLOR
2001-2005	*Rodrigues et al. (2001)* *Kumar and Malik (2001)*	TC OTC	*Liu et al. (2003)* *Martínez-Larrañaga et al. (2004)* *Prats et al. (2005)* *Hernandez et al. (2005)*	FLOR AMOX DOXY AMOX
2006-2010	*Vargas et al. (2008)*	DOXY	*Reyns et al. (2008)* *Godoy et al. (2010)* *Krasucka and Kowalski (2010)*	AMOX AMOX AMOX
2011-2015	-		*Goossens et al. (2012)* *Yang et al. (2012)* *Gutiérrez et al. (2013)* *del Castillo et al. (2014)*	DOXY DOXY DOXY DOXY

TC: Tetracycline/OTC: Oxytetracycline/DOXY: Doxycycline/AMOX: Amoxicillin/FLOR: Florfenicol

Table 35: Methods for determining plasma concentrations in the various scientific articles

Calves		Pigs	
Microbiological method			
Ziv and Sulman (1974)	TC/OTC	*Mevius et al. (1986b)*	OTC
Schifferli et al. (1982)	OTC	*Pijpers et al. (1990)*	OTC
Ames et al. (1983)	OTC	*Pijpers et al. (1991)*	OTC
Nouws and Vree (1983)	OTC		
Nouws et al. (1983)	OTC		
Toutain and Raynaud (1983)	OTC		
Nouws et al. (1985)	OTC		
Mevius et al. (1986a)	OTC		
Burrows et al. (1987)	OTC		
Soback et al. (1987)	AMOX		
Ucelli et al. (1988)	OTC		
Errecalde et al. (1997)	OTC		
Kumar and Malik (1998)	OTC		
Singh et al. (1998)	OTC		
Kumar and Malik (1999)	OTC		
Kumar and Malik (2001)	OTC		
Vargas et al. (2008)	DOXY		
Spectrofluorimetry			
Pilloud (1973)	OTC	*Xia et al. (1983b)*	OTC
Xia et al. (1983a)	OTC		
HPLC			
Varma et al. (1986)	FLOR	*Kniffen et al. (1989)*	TC
Adams et al. (1987)	FLOR	*Riond and Riviere (1990)*	DOXY
Bretzlaff et al. (1987)	FLOR	*Nielsen and Gyrd-Hansen (1996)*	TC/OTC
Riond et al. (1989)	DOXY	*Bousquet et al. (1998)*	DOXY
Sanders and Guillot (1990)	OTC	*Agersø and Friis (1998a)*	AMOX
Meijer et al. (1993a)	OTC	*Agersø and Friis (1998b)*	AMOX
Meijer et al. (1993b)	DOXY	*Voorspoels et al. (1999)*	FLOR
de Craene et al. (1997)	FLOR	*Liu et al. (2003)*	FLOR
Rodrigues et al. (2001)	TC	*Martínez-Larrañaga et al. (2004)*	AMOX
		Prats et al. (2005)	DOXY
		Hernandez et al. (2005)	AMOX
		Reyns et al. (2008)	AMOX
		Godoy et al. (2010)	AMOX
		Krasucka and Kowalski (2010)	AMOX
		Goossens et al. (2012)	DOXY
		Yang et al. (2012)	DOXY
		Gutiérrez et al. (2013)	DOXY
		del Castillo et al. (2014)	DOXY

TC: Tetracycline/OTC: Oxytetracycline/DOXY: Doxycycline/AMOX: Amoxicillin/FLOR: Florfenicol

Table 36: Number of studies (with numbers of animals) used to extract the pharmacokinetic parameters clearance and/or clearance/F, and oral bioavailability in calves

Pharmacokinetic parameter	Studies	Numbers of animals per study	Total numbers of animals
Tetracycline			
Clearance	*Ziv and Sulman (1974)* *Rodrigues et al. (2001)*	6 6	**12**
Clearance/F	-	-	**0**
Bioavailability	-	-	**0**
Oxytetracycline			
Clearance	*Pilloud (1973)* *Ziv and Sulman (1974)* *Schifferli et al. (1982)* *Ames et al. (1983)* *Nouws and Vree (1983)* *Nouws et al. (1983)* *Toutain and Raynaud (1983)* *Xia et al. (1983a)* *Nouws et al. (1985)* *Mevius et al. (1986a)* *Burrows et al. (1987)* *Ucelli et al. (1988)* *Sanders and Guillot (1990)* *Meijer et al. (1993a)* *Errecalde et al. (1997)* *Kumar and Malik (1998)* *Singh et al. (1998)* *Kumar and Malik (1999)* *Kumar and Malik (2001)*	5 6 6 + 8 5 + 5 6 4 + 4 + 6 + 4 + 5 6 + 8 4 5 + 10 10 4 + 4 + 4 + 4 + 4 + 4 8 6 5 6 8 5 + 5 6 5	**185**
Clearance/F	*Schifferli et al. (1982)*	4	**4**
Bioavailability	*Schifferli et al. (1982)*	4	**4**
Doxycycline			
Clearance	*Riond et al. (1989)* *Meijer et al. (1993b)* *Vargas et al. (2008)*	5 + 4 4 10	**23**
Clearance/F	*Meijer et al. (1993b)*	4	**4**
Bioavailability	*Meijer et al. (1993b)*	4	**4**
Amoxicillin			
Clearance	-	-	**0**
Clearance/F	*Soback et al. (1987)*	10 + 10 + 11 + 11	**42**
Bioavailability	*Soback et al. (1987)*	10 + 10 + 11 + 11	**42**
Florfenicol			
Clearance	*Varma et al. (1986)* *Adams et al. (1987)* *Bretzlaff et al. (1987)* *de Craene et al. (1997)*	6 6 5 6	**23**
Clearance/F	*Varma et al. (1986)* *Adams et al. (1987)*	6 + 5 5 + 6	**22**
Bioavailability	*Varma et al. (1986)* *Adams et al. (1987)*	6 + 5 5	**16**

The additions represent animals from different batches tested in the same publication.

Table 37: Number of studies (with numbers of animals) used to extract the pharmacokinetic parameters clearance and/or clearance/F, and oral bioavailability in pigs

Pharmacokinetic parameter	Studies	Numbers of animals per study	Total numbers of animals
Tetracycline			
Clearance	*Kniffen et al. (1989)* *Nielsen and Gyrd-Hansen (1996)*	4 6	**10**
Clearance/F	*Kniffen et al. (1989)* *Nielsen and Gyrd-Hansen (1996)*	4 6 + 6	**16**
Bioavailability	*Kniffen et al. (1989)* *Nielsen and Gyrd-Hansen (1996)*	4 6 + 6	**16**
Oxytetracycline			
Clearance	*Xia et al. (1983b)* *Mevius et al. (1986b)* *Pijpers et al. (1990)* *Pijpers et al. (1991)* *Nielsen and Gyrd-Hansen (1996)*	3 3 7 + 6 + 7 + 6 6 6	**44**
Clearance/F	*Mevius et al. (1986b)* *Pijpers et al. (1991)* *Nielsen and Gyrd-Hansen (1996)*	3 6 + 6 6 + 6	**27**
Bioavailability	*Mevius et al. (1986b)* *Pijpers et al. (1991)* *Nielsen and Gyrd-Hansen (1996)*	3 6 + 6 6 + 6	**27**
Doxycycline			
Clearance	*Dossier 9601 (1985)* *Riond and Riviere (1990)* *Dossier Veprol (1995)* *Yang et al. (2012)* *Gutiérrez et al. (2013)* *del Castillo et al. (2014)*	4 + 4 + 4 + 4 4 7 8 12 5	**52**
Clearance/F	*Dossier 9601 (1985)* *Dossier Veprol (1995)* *Bousquet et al. (1998)* *Prats et al. (2005)* *Goossens et al. (2012)* *Gutiérrez et al. (2013)* *del Castillo et al. (2014)*	4 + 3 + 2 + 3 + 2 + 4 + 3 + 4 + 4 + 4 7 + 3 9 + 9 + 9 12 6 + 6 + 6 + 6 + 6 + 6 + 6 + 6 12 5	**147**
Bioavailability	*Dossier 9601 (1985)* *Dossier Veprol (1995)* *Gutiérrez et al. (2013)* *del Castillo et al. (2014)*	4 + 3 + 2 + 3 + 2 + 4 + 3 + 4 + 4 + 4 7 + 3 12 5	**60**
Amoxicillin			
Clearance	*Agersø and Friis (1998a)* *Agersø and Friis (1998b)* *Martínez-Larrañaga et al. (2004)* *Hernandez et al. (2005)* *Reyns et al. (2008)* *Godoy et al. (2010)*	4 + 4 5 6 4 4 8	**35**
Clearance/F	*Agersø and Friis (1998a)* *Martínez-Larrañaga et al. (2004)* *Hernandez et al. (2005)* *Reyns et al. (2008)* *Godoy et al. (2010)* *Krasucka and Kowalski (2010)*	4 + 4 6 3 4 8 + 6 + 3 + 8 + 8 + 8 8	**70**
Bioavailability	*Agersø and Friis (1998a)* *Martínez-Larrañaga et al. (2004)* *Hernandez et al. (2005)* *Reyns et al. (2008)* *Godoy et al. (2010)*	4 + 4 6 3 4 8 + 6 + 3 + 8 + 8 + 8	**62**
Florfenicol			
Clearance	*Liu et al. (2003)*	6 + 6	**12**
Clearance/F	*Voorspoels et al. (1999)* *Liu et al. (2003)*	6 + 6 + 6 + 6 6 + 6	**36**
Bioavailability	*Liu et al. (2003)*	6 + 6	**12**

The additions represent animals from different batches tested in the same publication.

Notes

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