ERAVMIS: ENVIRONMENTAL RISK ASSESSMENT OF VETERINARY MEDICINES IN SLURRY

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A GUIDE TO RISK ASSESSMENT OF VETERINARY MEDICINAL PRODUCTS USED IN ANIMAL HUSBANDRY

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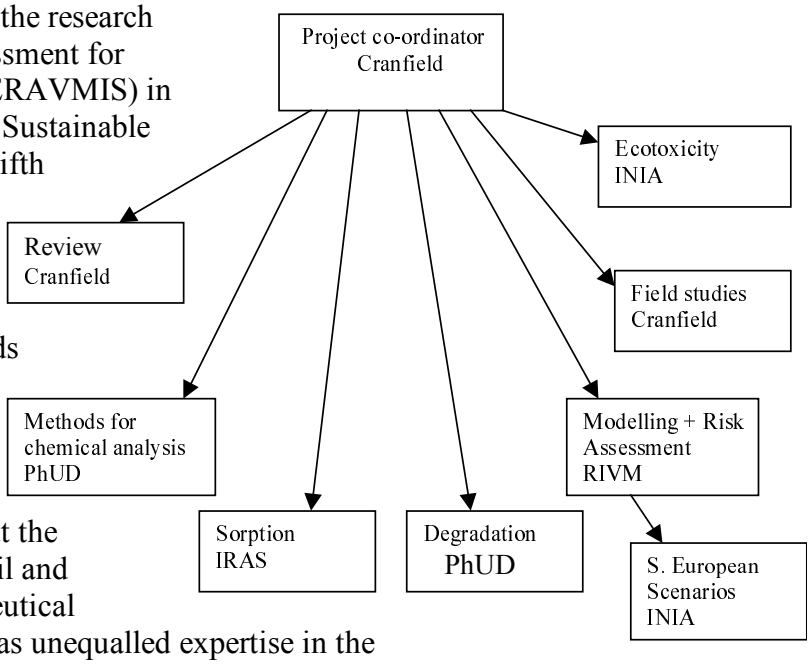
Abstract

The procedures described in this document give guidance on recommended methods for the risk assessment of veterinary medicinal products applied in slurry, for regulatory purposes.

The document gives considerations to fate and effect testing strategies in particular with respect to the hallmarks of veterinary medicinal antibiotics. It puts forward what modelling approaches are available and what considerations to scenario definitions should be given. Risk characterisation is addressed as the focal point for exposure and effect assessment. Attention is paid to risk mitigation measures and conditions under which these would be applicable.

Preface

The procedures described in this document have been compiled from a variety of sources representing research and opinions from academia, government and industry. Taken together with other guidance documents (Environmental risk assessment for veterinary medicinal products other than GMO-containing and immunological products, EMEA 1997; The available scientific approaches to assess the potential effects and risk of chemicals on terrestrial ecosystems, CSTEE 2000; Procedures for Assessing the Environmental Fate and Toxicity of Pesticides, SETAC-EUROPE 1995) they provide a suitable basis for the test strategies for the assessment of the risk of veterinary medicines applied to land in manure.



This document was developed by the research project Environmental Risk Assessment for Veterinary Medicines In Slurry (ERAVMIS) in the EU Energy, Environment and Sustainable Development programme of the Fifth Framework Programme. The project consortium brougth together organisations who are internationally recognised in their respective fields and who have complementary skills. The Cranfield Centre for Ecochemistry works for regulators and industry throughout the world on all matters relating to soil and soil contamination. The Pharmaceutical University of Denmark (PhUD) has unequalled expertise in the degradation of veterinary medicines in the environment. The Institute for Risk Assessment Studies (IRAS) is internationally recognised for their work into partitioning of substances in the environment and of predicting environmental properties and effects. The Laboratory of Ecotoxicology at INIA has considerable experience in the assessment of the ecotoxicity of veterinary medicines. The Expert Centre for Substances at RIVM has extensive experience of environmental modelling and the development of risk assessment procedures for use across Europe.

The proposed strategies and scenarios do not prejudice the authority of Member States in national authorisations, nor do they prejudice the application of other Community legislation in force. It is recommended that Member States develop their own national and regional scenarios for the assessment to ensure that the national quality standards are met. Amongst others, changes in immission standards, storage times, manure qualities, and differentiations in manure application, animal categories, soil type, land use, and crop are to be considered.

Contents

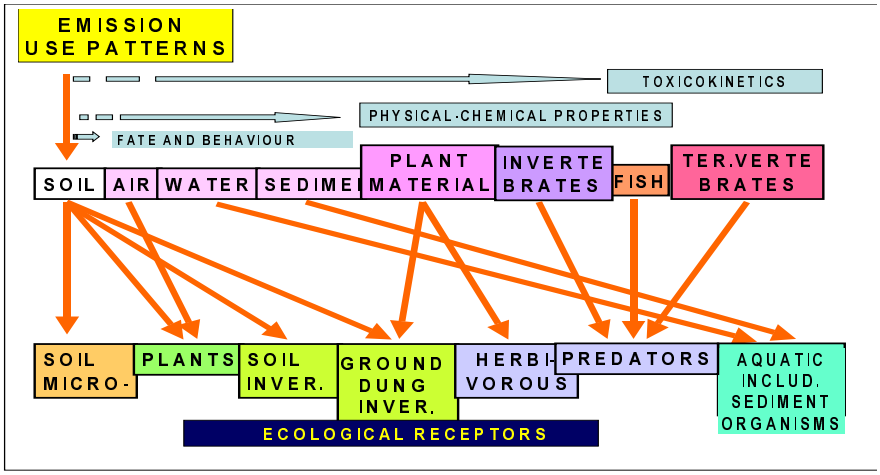
Summary

The procedures described in this document give guidance on recommended methods for the assessment of the environmental fate and effects on non-target organisms of veterinary medicinal products applied in slurry, for regulatory purposes. Veterinary medicines are regulated in order to protect animal health, consumers, professional users, the environment as well as the internal market. The document gives considerations to fate and effect testing strategies in particular with respect to the hallmarks of veterinary medicinal antibiotics. It puts forward what modelling approaches are available and what considerations to scenario definitions should be given. Risk characterisation is addressed as the focal point for exposure and effect assessment. Attention is paid to risk mitigation measures and conditions under which these would be applicable. For exposure modelling, realistic worst case conditions are proposed in a simple scenario assuming:

- single treatment per animal place,
- standard European nitrogen production values,
- a manure production volume of 1 month (30 days) containing the full residue,
- an application rate of 170 kg N/ha/year in one event into 5 cm soil,
- no dissipation during storage, and no after-treatment of slurry.

The PECsoil can be used in conjunction with screening level models that predict mass transfer to groundwater and surface water. Screening level and mechanistic models provided by FOCUS are considered as suitable for veterinary drugs as for pesticides and can be run using European scenarios for soil and weather, with and without incorporation of the substance in the topsoil. Additionally, a scenario for run-off and erosion is provided. Fate testing at lower tier levels is thus limited to soil degradation and sorption, for which standard test guidelines are generally suitable. Urgent research needs related to fate assessment of VMPs are:

- Better baseline information on environmental concentrations especially in manure.
- Improve analytical methods especially for very low concentrations in soil and manure.
- (Q)SAR development for distribution predictions and prediction of fate for homologues (tetracyclines, macrolides, sulphonamides).
- Refining of Tier A studies to relate better to more advanced Tier B studies.



The proposed effect testing strategy is described in four different levels. Levels 1 and 2 should be required for all veterinary medicines. Level 1 tries to summarise the basic profile of the molecule. The level 2 offers an innovative proposal, based on a new conceptual model for covering non-homogeneous risk assessments. Levels 3 and 4 are part of the effect assessment refinement, and therefore, are only required if the risk characterisation conducted on the basis of the information generated in levels 1 and 2 cannot exclude that the proposed use represent a low environmental risk.

1. Risk assessment and registration

1.1 Introduction to risk assessment

In general, *environmental risk assessment* is the process of estimating the probability of occurrence of an event in the environment and the probable magnitude of adverse effects over a specified time period in the environment. The questions to be answered are mainly: does the event happen and what will the effect on the environment be? This process entails some or all of the following elements:
- hazard identification,
- exposure assessment,
- effect assessment, and
- risk characterisation.

Hazard identification is concerned with the protection goals:
- what should be protected: species, functions, structures;
- where should one look for possible impact:
  - what compartment (soil, water, air, groundwater, sediment),
  - what scale (local, regional, continental, world-wide) and
  - what organisation level: e.g. cells, individuals, species, populations, communities.

Exposure assessment is the determination of emissions, pathways and rates of movement of a substance and its transformation or degradation in order to estimate the concentrations/doses to which environmental compartments are or may be exposed [1].

Effect assessment is concerned with the performance and interpretation of effect tests and the derivation of an effect value (dosage, concentration) that represents the impact on the protection goal of concern within a certain timeframe.
Exposure and effect assessment can both be performed in real life field situations and in model situations. Models can be physical (constructions, animals) or mathematical simulations (models).

Risk characterisation is the process in which exposure and effect assessments are combined as to give information on the risk: the combination of chance of occurrence and extent of effect. Risk characterisation should provide information that enables decision-making: be it on the direction of further assessment (pre- or post registration) or on risk management: e.g. risk mitigation measures, or risk elimination measures.

In order to elucidate the use of these building blocks of risk assessment for veterinary medicines, the framework in which this is applied will be discussed first.

1.2 The registration framework

Veterinary medicines are regulated in order to protect animal health, consumers, professional users, the environment as well as the internal market. The framework of the registration

procedure and assessments for both the applicant and regulator consists of a European Commission and Council directive (2001/82/EC)[2], and communautary guidance documents. As a general observation it is stated here that the primary goal of any environmental assessment is risk mitigation and risk management. The risk assessment should target the desired level of quality and define when this level is reached in terms of risks, concentrations or likelihood.
The European Agency for the Evaluation of Medicinal Products (EMEA)[1], through its scientific committee, the committee for veterinary medicinal products (CVMP), agrees opinions on the applications submitted for the authorisation of new medicines through the centralised procedure. These are the basis of decisions taken by the Commission. Disagreement between the Member States in the decentralised procedure (mutual recognition) leads to a referral to the CVMP for arbitration on any particular point in the assessment. The EMEA has published guidance on the environmental risk assessment (ERA) of VMPs, and this assessment was implemented in 1997 [3]. The assessment scheme takes the use of the product and the properties of the products into account in the assessment (Phase I or II), the emission routes (slurry-soil; water; pasture) and the data requirements. After the final draft of the EMEA (1997) guidance, an international harmonisation between the EU, USA and Japan was started by the International Co-operation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH)[2] to which both the European Commission and the EMEA are committed [4]. The guidance document on Phase I was completed and finalised (15 June 2000) for implementation by July 1st 2001 in the European Union and United States [5] and replaces the EMEA 1997 guidance on Phase I. For Phase II the EMEA guidance is still leading, but in VICH a new Phase II guidance is under preparation (August 2003).
The Directive 2001/82/EC addresses both industry and regulatory authorities: applicants and regulators. These are therefore considered the users of this guide on risk assessment. The environmental risk assessment is mandatory for new substances only. Therefore, the EMEA guidance is considered to target the central Marketing Authorisation Decision at the community level for the specific use of the product.

1.3 A tiered approach to risk assessment

The environmental risk assessment should be conducted in two phases according to the 2001/82/EC directive (see Table 1). In the first phase, the investigator shall assess the potential extent of exposure to the environment of the product, its active substances or relevant metabolites, taking into account:
- the target species, and the proposed pattern of use (for example, mass-medication or individual animal medication),
- the method of administration, in particular the likely extent to which the product will enter directly into environmental systems,
- the possible excretion of the product, its active substances or relevant metabolites into the environment by treated animals; persistence in such excreta,
- the disposal of unused or waste product.

In a second phase, having regard to the extent of exposure of the product to the environment, and the available information about the physical/chemical, pharmacological and/or toxicological properties of the compound which has been obtained during the conduct of the

[1] Commonly referred to as the European Medicines Evaluation Agency
[2] Commonly referred to as the Veterinary International Conference on Harmonisation

other tests and trials required by this Directive, the investigator shall then consider whether further specific investigation of the effects of the product on particular eco-systems is necessary. As appropriate, further investigation may be required of:
- fate and behaviour in soil,
- fate and behaviour in water and air,
- effects on aquatic organisms,
- effects on other non-target organisms.

The directive thus provided for the hazard identification and risk assessment approach. The EMEA guidance has elaborated upon these with the following tiered approach in Phase II (Table 1).

Table 1 The tiered approach in the risk assessment of veterinary medicinal products.

Stage in regulatory evaluation	**Stage in risk assessment**	**Objective**	**Method**	**Requirements**
Phase I		identify exposure	action limits	no test requirement
Phase II tier A	screening	rapid prediction of risk	risk assessment	base data set on toxicology and fate
Phase II tier B	primary	standard approach to ensure consistent decision making	risk assessment	extended data set on emission, fate and effect;
	secondary	substance and site-specific refinement		case-by-case; alternative approaches

Tier A begins an evaluation of the possible fate and effects of the drug and/or major metabolites. If within Tier A, no hazard is detected or the risk management strategy proposed by the applicant is taking care of any potential hazard, thus avoiding harmful effects of the product on the environment, there would be no need to proceed to Tier B. If the applicant is unable to demonstrate that exposure is minimised to a level of no concern to the environment, then the effects in the relevant compartment must be adequately investigated in Tier B.
It depends on the amount of information available which stage is the most appropriate for risk assessment. This guidance does not strictly adhere to the decision making scheme.

1.4 Scope of this guide and contents of the document

This guide is confined to risk assessment at pre-marketing registration of veterinary antibiotics used in intensive animal husbandry. The assessment investigates the risk of the use of a product.

The guide does not provide for the decision making scheme.

The framework set out by the EMEA for decision making and moving forward in the decision making scheme should be followed by the user. The regulatory scheme is not

discussed here in terms of dealing with the substance under consideration or the application of trigger values in any stage of the scheme.

This guide provides information and considerations on key issues in the different stages of the risk assessment. It formulates definitions used and assumptions made at these stages, thus increasing the transparency of the process and the validity of the results.

The guide gives considerations to fate and effect testing strategies in particular with respect to the hallmarks of veterinary medicinal antibiotics (Chapters 2 and 3).

It puts forward what modelling approaches are available and what considerations to scenario definitions should be given (Chapter 4).

Risk characterisation is addressed as the focal point for exposure and effect assessment (Chapter 5.1). Attention is paid to risk mitigation measures and conditions under which these would be applicable (Chapter 5.2).

2. Fate testing strategies

As for other chemicals environmental chemistry is the main contributor to the fate assessment of veterinary medicinal products (VMPs). The scenarios and guidance documents needed to assess the fate of VMPs resemble the ones currently being used for pesticides. Therefore a lot of information already present in such guidelines that may be applied also for VMPs. Some specific factors related to VMPs are of course important to incorporate in the assessment. We therefore propose to take advantage of a booklet edited by SETAC-Europe [6] that describes procedure for assessing the environmental fate and ecotoxicity of pesticides. Below are therefore only stated those issues that are specifically important to the VMPs. General issues related to assessing the fate of organic pollutants or pesticides are therefore not considered in this document.

Information from medicinal science such as structure and activity relationship of the drug can help in the prediction of its environmental fate. The medicinal literature contains information on likelihood of e.g. pH dependent hydrolysis, photolysis and complexation as all these processes also will affect the bioavailable concentration of the drug in humans or animals during medical treatment. The available data may often be found in pre-1980 pharmaceutical journals for especially antibacterial agents, disinfectants, fungicides, anthelmintics and high volume drugs.

Pharmacological information may furthermore be used to predict the proportion of parent/metabolites in excreta, and the types of metabolites (phase I/II). Deconjugation processes that may convert phase II metabolites back to parent or phase I metabolites, which in theory may be as potent as the parent (e.g. metronidazole) are other areas were the pharmacological literature offers valuable information.

In the ERAVMIS project we mainly focused on anti-bacterial agents. Antibacterial agents are generally water-soluble compared with lipophilic compounds such as e.g. hormones that also are veterinary relevant compounds. The physico-chemical behaviour of hormone VMPs is therefore different to other VMP groups and the fate strategy here might therefore be limited to these compound groups due to a very limited knowledge base. This project has thus primarily been looking at the fate of compounds with a log Kow value of less than 3. Project findings may not be appropriate as generalisations.

Generally suggested testing strategies for VMPs are often based on masses rather than potency. Taking compound groups as hormones or antibacterial agents into account this strategy might not be optimal.

The persistence of a drug in a soil or manure mostly depends on its photostability, its binding and adsorption capability, its biodegradation rate, and leaching in water. Furthermore quite a number of different mechanisms are involved in drug sorption. The most important being sorption to organic matter, surface adsorption to mineral constituents, ion exchange, and complex formation with metal ions such as Ca^{2+}, Mg^{2+}, Fe^{3+}, or Al^{3+} (e.g. tetracyclines).

2.1 Toolboxes for assessment of the fate of veterinary drugs

To assess the fate of VMPs two different tools are needed: analytical methods and test-set-ups. A range of appropriated sample preparation procedures and analytical methods to generate resulting compound concentrations of the assessed VMPs in the different test set-ups in matrices such as soil, manure, soil interstitial water and surface water have been developed. Furthermore a number of test methods that may assess the fate of the compounds in relevant matrices.

Many of the tests set-ups to assess the fate are already developed under OECD, ISO or other regulatory testing bodies for pesticides but have to be evaluated in the context of VMPs. The main factor to investigate in this context is whether manure amendment to a given test set-up will increase or reduce a given fate of the VMPs. Below is listed the endpoints that these test should cover.

These test endpoints are:
- manure degradation studies under different redox conditions
- pH dependent soil photolysis
- pH dependent soil complexation
- pH dependent soil hydrolysis
- soil degradation studies under different redox conditions (with and without amendment of manure)
- field soil dissipation (with and without amendment of manure)
- soil adsorption and desorption
- aged residue studies
- soil leaching (with and without amendment of manure)
- lysimeter/field leaching
- aquatic degradation.

2.2 Analytical methods

The analysis of soils, sediment, sludge and manure for determination of pharmaceuticals is required in some EU directives (Tier B) related to the monitoring of the environmental quality. To date, studies devoted to determine pharmaceuticals in solid environmental samples are scarce in comparison with those carried out in aqueous media. The monitoring of pharmaceuticals in solid environmental samples normally requires the use of time-consuming and labour-consuming methodologies. The quality assurance of each step involved in the whole analytical procedure, including sampling and storage, is essential for the reliability of the analytical determinations that follow.

Clean up of extracts, when performed, has been carried out by solid phase extraction (SPE). SPE has been preferred in most instances because it is fast, requires low volume of organic solvent, presents low contamination risk and can be used on-line.

Except for the methods developed by Ternes [7] based on the use of GC/MS/MS, the analysis of the target pharmaceuticals in the extract obtained has been carried out with HPLC, HPLC-MS and HPLC-MS-MS. Separation has mostly been performed with reverse phase C18 columns using different mobile phases. UV detection and to a lesser extent MS, and

fluorescence have been the detection techniques used coupled to HPLC for the analysis of drugs in solid environmental matrices.

With the methodologies developed so far the limit of detection varied roughly between 10 ng/g soil and 200 ng/g soil when UV was employed for detection; between 0.2 ng/g soil and 40 ng/g soil with MS detection and, around 10 ng/g soil with fluorescence detection. With respect to the accuracy of the methods described, relatively low recoveries were occasionally reported for some drugs. These low recoveries could be explained in terms of the formation of complexes between the drug and components in the matrix. Many drug structures contain multiple functional groups resulting in poor understanding of the sorption to environmental relevant matrixes.

2.3 Fate testing

Below important parameters that may be used to model the fate of VMPs are listed.

2.3.1 Manure degradation studies under different redox conditions

No validated or standardised method for assessing the fate of VMPs in manure at either field or laboratory level exists [8]. Tests in pesticide guidelines do not cover these aspects. In terms of fate we have poor knowledge of what happens in slurry prior to soil amendment but this is probably the most important area in terms of risk management. Important issues are to relate the measured concentrations of VMPs in the manure to manure type, storage conditions in the tank, mode of medication, agricultural practise, solids concentration, OC, water content, pH, temperature, redox conditions in different layers of the tank. Disappearance of VMPs in manure should be followed under both methanogenic, denitrifying and aerobic conditions. Deconjugation rate of excreted VMPs in manure may be significant and require further study under the different above-mentioned conditions.
So far it is problematic relating laboratory degradation experiments to real situations. Degradation studies have shown large variations in estimation of half-life depending on experimental set-up. Prescriptions of VMPs are difficult to relate to expected manure concentrations.

2.3.2 pH dependent soil photolysis

VMPs often contain multiple functional groups in the structure suggesting that photolysis may be an important factor and pH depended in the abiotic degradation pathway. ISO, OECD and other standardising bodies have suggested appropriated methods.
Much information is available in the pharmaceutical literature related to photolysis of VMPs. Little knowledge exists on field scale level, as to the dominant process governing removal in the ‘real world’. Again manure amendment can affect abiotic processes in water systems – pH, solid content, etcetera.

2.3.3 pH dependent soil complexation

VMPs often contain multiple functional groups in the structure suggesting that soil complexation may be an important factor and pH depended in the abiotic degradation pathway. See aged residue studies (2.3.8). ISO, OECD and other standardising bodies have suggested appropriated methods developed for chemicals.
Much information is available in the pharmaceutical literature related to complexation of VMPs to metals etc. There is little knowledge on field scale level, as to the dominant process governing removal in the 'real world'. Again manure amendment can affect abiotic processes in water systems – pH, solid content, etcetera.

2.3.4 pH dependent soil hydrolysis

VMPs often contain multiple functional groups in the structure suggesting that hydrolysis may be an important factor and pH depended in the abiotic degradation pathway. ISO, OECD and other standardising bodies have suggested appropriated methods.
Much information is available in the pharmaceutical literature related to hydrolysis of VMPs. There is little knowledge on field scale level, uncertainty as to the dominant process governing removal in the 'real world'. Again manure amendment can affect abiotic processes in water systems – pH, solid content, etcetera.

2.3.5 Soil degradation studies with(out) amendment of manure

A number of suitable validated and guidance methods developed for pesticide scenarios exist for following the degradation of VMPs under aerobic, anaerobic and denitrifying conditions and may be suggested as starting point for assessing VMPs. Field or soil history is of utmost importance because initial studies suggests that some VMPs e.g. antibacterial agents such as tetracyclines and tylosin are persistent compounds. The main question to include is of course the role of manure in soil systems in terms of degradation pathways and removal rates. Manure amendment changes the properties of the soil system by including water, increasing OC, and modifying pH and the buffer capacity of the soil. Furthermore inclusion of manure alters the bacterial concentration and diversity in the topsoil. Initial investigations although suggests based on laboratory scale experiments that manure inclusion up to 10 % w/w is not affecting the rate of degradation. Whether changes of microbiological degradation pathways may be enforced by manure inclusion is not investigated.

Assessing antibacterial and fungicides agents at unrealistically high spiking levels of the compounds may give false data on biotic removal due to bacteriostatic or bacteriocidic effects of tested compounds. Radiolabelled antimicrobial agents are generally not commercially available because they are difficult to produce due to their semi-synthetic origin.

2.3.6 Field soil dissipation with(out) amendment of manure

Very few studies are carried out so only limited data are available on VMPs dissipation in fields. But the few data (DT50) from ERAVMIS, Hamscher, DeLiguoro and a few more appears consistent between studies but not necessarily to Tier A studies [9-11]. At this step it is important to mention that agricultural practise concerning medication of animals and

storage of manure are important factors to include in order to understand the different results. Compounds can be applied to field in manure via injection, broadcast spreading and as solid or liquid manure.

No guidance documents exist to specific assess VMPs on this level but broad knowledge and practise from similar pesticide studies may be used in this context as the scenarios to some extent are the same (see above remarks).

2.3.7 Soil adsorption and desorption

Guidelines methods applicable to VMPs are published by several regulatory bodies e.g. ISO, OECD. Few published data on sorption coefficients can be found in the open literature. Coefficients are often higher than expected from their lipophilicity e.g. tetracyclines and quinolones. These results are consistent with a review paper by Tolls [12]. Thus QSARs based on e.g. Kow can overestimate mobility. Coefficients are concentration dependent – high spiking concentrations may give unrealistic results.

To be able to model potential movement to surface water via soil or manure it is important to have realistic distribution coefficients to actual soil or manure. More detailed experiments may be appropriate with regard to spiking level, pH, manure content and ionic strength and metal content for most of the high volume antibacterial agents.

2.3.8 Aged residue studies (time dependent sorption)

Time dependent sorption appears to be a very important mechanism of removal for certain compounds e.g. tetracyclines. More detailed experiments are needed to understand these mechanisms for VMPs. Again the many functional groups on VMPs molecule make it difficult to predict.

2.3.9 Soil leaching with(out) amendment of manure

Several methods are described in the literature that may be used for assessing soil leaching of VMPs. $CaCl_2$ concentration in solutions for column leaching experiments may not be appropriate for e.g. tetracyclines because of complexation and thus overestimation of binding to soil [13].

2.3.10 Lysimeter/field leaching

No present methods or guidelines have been validated yet. Clearly this is very important for VMPs to clarify. Again the role of manure is uncertain (see above).

2.3.11 Aquatic degradation

The molecular structure of VMPs often contain multiple functional groups suggesting that aquatic degradation may be an important factor and pH depended in the abiotic degradation pathway. Both ISO, OECD and other standardising bodies have suggested appropriated methods. Much information is available in the pharmaceutical literature related to

complexation of VMPs to metals etcetera. Little knowledge exists on field scale level, as to the dominant process governing removal in the 'real world'. Again manure amendment can affect abiotic processes in water systems – pH, solid content, etcetera.

2.3.12 Aqueous hydrolysis

VMPs often contain multiple functional groups in the structure suggesting that aqueous hydrolysis may be an important factor and pH depended in the abiotic degradation pathway. Both ISO, OECD and other standardising bodies have suggested appropriated methods. Much information in the pharmaceutical literature related to hydrolysis of VMPs may be found. Little knowledge on field scale level is available, as to the dominant process governing removal in the 'real world'. Again manure amendment can affects abiotic processes in water systems – pH, solid content, etcetera.

2.3.13 Aqueous photolysis

VMPs often contain multiple functional groups in the structure suggesting that aqueous photolysis may be an important factor and pH depended in the abiotic degradation pathway. Both ISO, OECD and other standardising bodies have suggested appropriated methods. Much information in the pharmaceutical literature related to direct photolysis of VMPs might be found. Little knowledge on field scale level is available, as to the dominant process governing removal in the 'real world'. Again manure amendment can affects abiotic processes in water systems – pH, solid content, etcetera. Information about indirect photolysis due to matrix constituents is hardly covered in the literature.

2.4 General research needs

Finalising the ERAVMIS project and synthesising all our data have lead us to draw the below conclusions about urgent research needs related to fate assessment of VMPs.

- Better baseline information on environmental concentrations especially in manure.

- Improve analytical methods especially for Tier B studies – i.e. very low concentrations in soil and manure.

- QSAR development for distribution predictions.

- Prediction of fate for homologues (tetracyclines, macrolides, sulphonamides). Will such a concept work?

- Refining modifications of Tier A studies to relate better to more advanced Tier B studies.

3. Effect testing strategies and effect assessment

As for other chemicals, ecotoxicology is the main contributor to the effect assessment of veterinary pharmaceuticals. The (eco)toxicological profile of any molecule can be defined, under pathological concepts, as the intimate association of the relationships between the chemical and the living organisms under a set of environmental conditions. This relationship is expected to vary significantly between:

- chemicals.
- living organism, including species within a community, individual within a population, and developmental/physiological stages within individuals.
- environmental conditions.

Each of these factors can, independently account for a variability covering several orders of magnitude, and differences up to four to nine orders of magnitude even for the same chemical are not unusual.

Toxicokinetic and toxicodynamic factors, and the influence of environmental conditions on bioavailability, potential for recovery, etc. are supposed to be responsible for these differences. However, we must recognise that nowadays no enough information on comparative physiology and ecological relevance of individual effects is available to fully explain this variability.

For comparative purposes, a set of standardised tests running under controlled conditions has been designed. The OECD is the main leader for tests used under EU regulations, but other sources, such as ISO, SETAC, USEPA, ASTM, etcetera, are also considered.

These tests are basically employed for hazard identification purposes (such as assessments of inherent toxicity) and lower tier risk assessment, while it is clearly recognised that standardisation for higher tier assays is much complicated and not necessarily useful.
This document summarises the ecotoxicological tools available for the effect assessment of veterinary drugs, a tiered approach for setting the effect profile on the basis of the available information, and present a set of specific recommendations including a proposal on the testing strategy.

3.1 The ecotoxicological toolbox for an assessment of effects of veterinary drugs

This section of the document describes the tests that are available to assess the effects of veterinary medicines on both aquatic and terrestrial organisms. The section begins with a description of the standard OECD studies (of the type that are currently used in risk assessment schemes for veterinary medicines) and then describes other standard tests that might provide additional useful information. A number of non-standardised tests, which could be of relevance to veterinary medicines are also listed. With the exception of a few studies, the section does not go into detail on the test methodologies. The user is directed to the individual test guidelines or the original scientific papers to obtain information on how exactly the studies are performed.

3.1.1 Standard OECD tests

Several national and international organisations are responsible for the validation and normalisation of experimental assays including ecotoxicity tests. Standard tests offer several advantages, from the existence of a well described and validated methodology to the reproducibility of the results. The standardisation of a test is a relatively long and expensive process, and therefore the number of standardised test is very low when compared to the total number of ecotoxicity tests available.
Obviously, if available standardised tests should chosen for the effect assessment. In particular, the OECD guidelines are used as reference in the EU and other parts of the world. Tests that have been standardised by the OECD are in this document treated as the "best choice" tests as these have been gained wide acknowledgement in different risk assessment frameworks. The OECD tests that can be relevant for the assessment of environmental effects of veterinary drugs are summarised below. For a detailed description, consult the source.

3.1.1.1 Aquatic compartment

Three taxonomic groups, fish, aquatic invertebrates and algae are covered by OECD tests. All these tests are recommended in the EMEA guidance document and included in Table 2.

Table 2. Recommended OECD test guidelines for aquatic organisms.

Tests	Effect parameters	Guideline	Adoption/Update
Fish acute lethality	Survival (LC50)	203	1981/1992
Fish prolonged toxicity	Growth (NOEC)	204	1984
Fish early life stage	Development (NOEC)	210	1992
Fish, short term toxicity test on embryo and sac-fry stages	Survival (LC50) Development (NOEC)	212	1998
Fish juvenile growth	Development (NOEC)	215	2000
Daphnia magna immobilisation	Mobility as survival measurement (EC50)	202 Part A	1981/2002 Draft
Daphnia magna reproduction	Reproduction (NOEC)	202 Part B 211	1981/1998
Sediment-water chironomid toxicity	Emergence (NOEC)	218 spiked sediment 219 spiked water	2003 Draft
Algae growth inhibition	Biomass reduction (EC50 and NOEC)	201	1981/2002 Draft
Lemna sp. growth inhibition	Growth inhibition (EC50 and NOEC)	221	2002 Draft

Lethality or related (mobility) endpoints based on dose/response curves are employed in the acute tests on animals to estimate the L(E)C50. Growth inhibition, also on a dose/response basis and EC50 estimation, are used for algae and aquatic plants.
NOECs (the use of ECx is under discussion) based on statistical analysis are used as endpoints for the chronic tests. Reproduction is considered in some cases as the only relevant endpoint for chronic tests, and therefore, the fish growth inhibition test should not be considered as a chronic test under these bases.

3.1.1.2 Terrestrial compartment

The terrestrial compartment is covered by tests on vertebrates (specific tests on birds and the test on mammals developed for the assessment of human health effects), plants, soil invertebrates and soil micro-organisms. These tests are included in the EMEA guidance.

Tests on terrestrial vertebrates (mostly mammals, birds in some cases) are included in the clinical and/or human risk assessment dossiers. It is strongly recommended to use this information as much as possible before considering additional testing. Therefore, tests on terrestrial vertebrates will not be considered here.

For the terrestrial compartment, an overview of existing tests has been given by the CSTEE (Table 3).

Table 3. Recommended OECD test guidelines for terrestrial organisms

Tests	Effect parameters	Guideline	Adoption/Update
Earthworm acute toxicity	Survival (LC50)	207	1984
Earthworm reproduction	Reproduction (NOEC)	222	2003 Draft
Enchytraeidae reproduction	Reproduction (NOEC)	220	2003 Draft
Honeybees acute oral toxicity	Survival (LD50)	213	1998
Honeybees acute contact toxicity	Survival (LC50)	214	1998
Terrestrial plants growth	Emergency inhibition (EC50) Growth, biomass (NOEC)	208	1984/2002 Draft
Avian dietary toxicity	Survival, body weight	205	1984
Avian reproduction test	Reproduction (NOEC)	206	1984
Avian acute oral toxicity	Survival (LC50)	223	2002 Draft
Avian reproduction toxicity	Reproduction (NOEC)	Not yet assigned	2000 Draft
Soil micro-organisms, nitrogen transformation	Nitrification	216	2000
Soil micro-organisms, carbon transformation	Respiration	217	2000
Mammalian tests	A large list (currently 36 guidelines)of acute, subchronic and chronic tests is available		

3.1.2 Other non-OECD standardised tests

In addition to the standard OECD studies described above, there are a large number of test guidelines available for other aquatic and terrestrial organisms standardised by other national and international organisation. The use of these studies might provide useful additional information for veterinary medicines. The availability of such approaches may also assist when focusing a testing regime on a particular group of interest (see testing strategy section) and when developing species sensitivity distributions. A compilation of aquatic tests that have been standardised by bodies other than the OECD is presented in Table 4 for the aquatic compartment and Table 5 for the terrestrial compartment.

Table 4 – Available test guidelines for aquatic species.

Group	Species	Authority Recommendation
Insect larvae	Plecopterans	
	Pteronarcys dorsata (stonefly)	APHA
	P. californica	APHA
	P. spp.	ASTM, FAO
	Hesperoperla lycorias	APHA
	H. pacifica	APHA
	Isogenus fontalis	APHA
	I. spp.	FAO
	Perlesta placida	APHA
	Paragnetina media	APHA
	P. spp.	FAO
	Phasgonophora capitata	APHA
	P. spp.	FAO
	Acroneuria californica	APHA
	A. spp.	FAO
	Ephemopterans (mayflies)	
	Hexagenia bilineata	APHA, ASTM, EPA
	H. limbata	APHA, ASTM, EPA
	H. rigida	APHA
	H. spp.	FAO
	Ephemerella subvaria	APHA
	E. cornuta	APHA
	E. grandis	APHA
	E. doddsi	APHA
	E. needhanii	APHA
	E. tuberculata	APHA
	E. spp.	ASTM, FAO, EPA
	Stenomena Ithaca	APHA
	S. spp.	FAO
	Baetis spp.	ASTM, EPA
	Trichopterans	
	Brachycentrus americanus	APHA
	B. occidentalis	APHA
	B. spp.	FAO
	Clistoronia magnifica	APHA
	Hydropsyche bettini	APHA
	H. bifida	APHA
	H. spp.	FAO
	Macromenum zebratum	APHA
	M. spp.	FAO
	Dipterans	
	Chironomus plmosus	APHA
	C. attenuatus	APHA
	C. tentans	APHA
	C. californicus	APHA
	C. spp.	ASTM, FAO, EPA, OECD
	Glyptochironomus labiferus	APHA
	Goeldichironomus labiferus	APHA
	G. holoprasinus	APHA
	Tanypus grodhausi	FAO
	T. spp.	APHA
	Tanytarsus dissimilis	APHA
	T. spp.	FAO
Protozoans	Ciliates	
	Tetrahymena pyriformis	APHA
Molluscs	Gastropods	
	Physa integra	ASTM
	Physa heterostropha	ASTM
	Amnicola limosa	ASTM
Vermes	Platyhelminths	
	Dugesia tirgrina	ASTM
	Annelids	
	Limnodrilus hoffmeisteri	APHA, FAO
	Tubifex tubifex	APHA, FAO
	Branchiura sowerbyi	APHA, FAO
	Stylodrilus heringianus	APHA
Fish	*Salvelinus fontinalis*	ASTM
	Oncorhynchus kisutch	ASTM
	O. tschawytscha	ASTM
	O. mykiss	ASTM
	Carassius auratus	ASTM
	Cyprinus carpio	ASTM
	Pimephales promelas	ASTM
	Catostomus commersoni	ASTM
	Ictalurus puntuatus	ASTM
	Lepomis macrochirus	ASTM
	L. cyanellus	ASTM
	Esox lucius	ASTM
	Gasterosteus aculeatus	ASTM
	Brachydanio rerio	ASTM
	Poecilia reticulata	ASTM
Crustaceans	Branchiopods	
	Daphnia magna	Many
	D. pulex	ASTM, USEPA, OECD
	D. pulicaria	ASTM
	Daphnia spp.	OECD
	Ceriodaphnia spp.	USEPA
	Rotifers	
	Brachionus calyciflorus	ASTM
	B. rubens	ASTM
	B. plicatilis	ASTM
	Amphipods	
	Gammarus lacustris	APHA, ASTM, FAO, EPA
	G. pseudolimnaeus	APHA, ASTM, EPA
	G. fasciatus	APHA, ASTM, FAO, EPA
	Hyallela azteca	APHA, FAO
	Pontoporeia affinis	APHA
	Hyallela spp.	EPA
	Mysids	
	Mysis relicta	APHA, FAO
	Decapods (crayfish)	
	Plaemonetes cummingi	APHA
	Plaemonetes kadakiensis	APHA
	Gambarus spp.	APHA, EPA, FAO, ASTM
	Orconectes rusticus	APHA
	Orconectes spp.	EPA, ASTM
	Procambarus spp.	ASTM
	Pacifastacus lenisculus	EPA
Macrophyte	*Lemna minor*	OECD
	Lemna gibba	EPA

Table 5 – Main standardised non-OECD effect tests for the terrrestrial medium (after Römbke and Moltmann [14] and CSTEE [15]).

Tests	Effect parameters	Guideline
Soil micro-organisms		
• Microflora test	Dehydrogenase activity, short-term	BBA VI, 1-1
Terrestrial plants		
• Plant growth test	Emergence, growth (EC50)	BBA (draft guideline)
• Seed germination/ root elongation test	Seed germination, root growth (EC10, EC50)	EPA CFR 40-1-R
• Early seedling growth toxicity test	Root, shoot and total plant growth	EPA CFR 40-1-R
• Root elongation test	Root growth (ECx)	ISO 11269/1
• Seed germination test	Emergence, growth (NOEC, LOEC)	ISO 11269/2
• Whole plant test	Total plant, root and shoot growth (EC50)	ASTM STP1115
Terrestrial invertebrates		
• Carabid test	Survival, feeding rate	BBA VI, 23-2.1.8
• Carabid test	Survival	IOBC
• *Collembola* test	Reproduction (NOEC)	ISO 11267
• *Staphylinid* generation test	Hatching, survival	BBA VI, 23-2.1.10
• Predatory mites test	Survival, reproduction	EPPO 151
• Parasitoid beetle test	Survival, reproduction	IOBC
• Parasitic waps test	Survival, parasitism, reproduction	EPPO 142/1& 142/2
• Spider test	Survival, food consumption, behaviour	BBA VI, 23-2.1.9
• Spider test	Survival, food consumption	IOBC

3.1.3 Other low-tier single species tests

In addition to the standardised test designs described in the previous section, there are numerous other test designs that have been developed for research purposes. Whilst these approaches should not be used as the primary basis of the effects assessment work (because they have not been extensively discussed, agreed or ring tested), they can provide useful additional information to the standardised studies.
This is a typical need for some effect assessment approaches such as the use of species sensitivity distributions.
Test conditions from standard tests can be just adjusted for testing additional species.
Methods assayed and published can also be found for a significant number of aquatic and terrestrial organisms, for example the CSTEE opinion [15] that summarised a large list of test candidates covering several taxonomic groups representing the terrestrial compartment.

3.1.4 Microbial community structure: PICT tests with biolog plates

3.1.4.1 Aim of test

The PICT test investigates changes in the structure of the soil microbial community upon exposure to a compound. It can be used to assess a possible reduction in microbial biodiversity brought about by a veterinary pharmaceutical.
The PICT test has been tested on veterinary pharmaceuticals in the course of the ERAVMIS project and has been shown to be well suitable for the assessment of their effects on microbial communities.

3.1.4.2 Advantages

PICT testing with Biolog plates [16-18]proved to be a test for microbial community structure and biodiversity, which is more sensitive than tests that investigate an overall function such as total bacterial counts. PICT can be tested under laboratory circumstances closely resembling field conditions and therefore has a high degree of representation. Especially the possible formation of breakdown products and sorption processes can be assumed to be taken into account in the PICT test in a realistic manner. PICT testing with Biolog plates is highly automated. PICT with Biolog plates can also give an indication of effects of antibiotics on certain soil functions. In field investigations with metals, PICT testing has been shown to be able to establish a clear cause-effect relationship than other test systems, as only the toxicant itself is thought to be able to change the tolerance of a community to that given toxicant [19].

3.1.4.3 Soil selection, handling, amendment and treatment

The test is based on natural agricultural soils in order to investigate a standing microbial community (highlighting the need for proper soil storage). After amendment of the soil with manure as organic substrate and the test compound, changes in the microbial community are brought about through physiological adaptations, disappearance of the most sensitive species, and preferential growth of (genetically) tolerant species.

3.1.4.4 PICT detection with Biolog plates

Microbial extracts are prepared after a soil exposure of 1-2 weeks and can be kept frozen until testing. Prefilled 96-well plates (ECO microplate≠ ; Biolog, Hayward, CA, USA) are used for determining the relative tolerance of the metabolic activities of the microbial inocula of each soil sample. ECO plates contain 31different organic substrates such as sugars or amino acids, as well as a tetrazolium redox dye and a dried mineral salts medium. The dye colours when the cellular redox state changes due to substrate metabolisation.
Testing is performed by inoculating the Biolog plates with dilutions of the microbial extracts and different amounts of the test compound. The inoculum density has to be defined in preparatory experiments in order to reach comparable values per sample. The well absorbance at 590 nm is then followed until day 12, reflecting the metabolic activity per substrate. Dose-response curves established from the absorbance data versus veterinary drug concentration per plate are compared. A change in community composition can be seen if bacterial inocula of treated soils show higher tolerance towards addition of the test compound

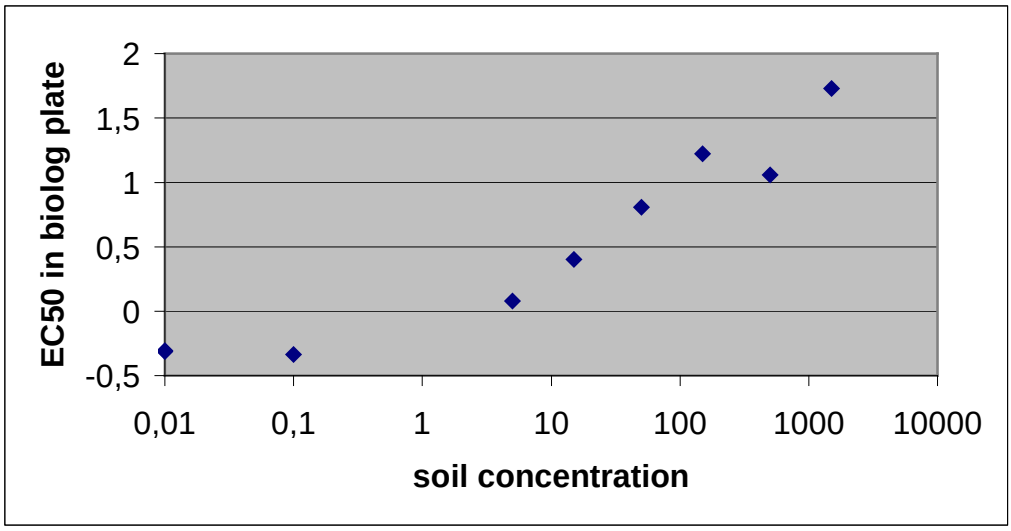


Figure 1 Effects of oxytetracycline on soil micro-organisms using the Biolog plate (from Schmitt, personal communication).

in Biolog plates (thus, increased EC50 values in the Biolog plates). Classical dose-response testing as well as sensitivity distributions can be used to establish NOEC concentrations. Effects of veterinary drugs over long time scales cannot be adequately tested, as the soils cannot be kept in acceptable conditions for more than several weeks. Testing is much facilitated upon existence of an automatic plate reader. Figure 1 shows an increase in EC50s (average of all EC50 values per soil) versus soil oxytetracycline concentration.

3.1.5 Modified exposure tests

Standard ecotoxicity bioassays are generally performed using one matrix (i.e. water, soil, sediment) and are performed over a fixed period of time (e.g. 48 h for daphnids, 96 h for fish, 72 h for algae). The method of entry and rate at which the compound dissipates in the environment may mean that the standard ecotoxicity studies are not particularly relevant. A number of approaches have therefore been proposed that incorporate the exposure characteristics for a particular substance into the ecotoxicity study. These have generally focused on aquatic organisms and have been applied to pesticides. However there is no reason why these approaches cannot be applied to veterinary medicines. The approaches that might be appropriate for the assessment of veterinary medicines are described below.

3.1.5.1 Inclusion of dissipation processes

Standard aquatic ecotoxicity studies usually require that the test concentration at the end of the study is 80% of the starting concentration. For compound which are volatile or which are degraded abiotically (i.e. through hydrolysis), this can mean that the standard test system is either covered or that a flow through test system is used or that the test solution is regularly replaced. This approach will not only account for the effect of the dissipation process but will also include the effects of major hydrolytic degradation products.

Box 1 Antimicrobial resistance / antimicrobial tolerance

In the medical literature, resistance is mainly regarded as the genetically encoded ability of micro-organisms to withstand higher concentrations of antibiotics than a control population. Resistance in this sense has to be differentiated from tolerance. Tolerance is defined here as an increased ability of the whole microbial community to withstand higher concentrations of an antibiotic. This can be caused by genetically resistant species, but also by physiological adaptations, or by the disappearance of specifically sensitive species.

The increased incidence of antibiotic resistant pathogens, especially in hospitals, has led to a debate about the linkage between the wide use of antibiotics and the resistance level. In this discussion, it has also been touched upon whether the use of veterinary drugs might also lead to a reduced efficiency of human antibiotics in the treatment of pathogens [20,21]. This has been motivated by the fact that human and veterinary antibiotics share the same mechanisms of action. This process of resistance development is triggered at the Minimum Effect Concentration (MEC) at which growth is reduced, which is tenfold below the Minimum Inhibitory Concentration (MIC), the endpoint used to characterise the efficacy of the strain [22]. This indicates that at and below the MIC level a selection pressure for resistance is present. The survival of adapted bacteria in absence of the compound that the bacteria have adapted to, is usually said to be limited, but the acquired functionality (e.g. resistance genes) remains present at low levels and the costs for resistance can be compensated [23-25]. Resistance genes may be favoured, and be transferred from manure to soil and groundwater [26-28].

The management of resistance development in water and sediment face comparable challenges [29]. Results from fish farms highlight that the proportion of resistant environmental bacteria might be elevated in sediments below the farm [30].

In this context, it has been addressed whether the release of both antibiotics and resistant intestinal bacteria with the spreading of manure might increase the pool of resistant micro-organism in soil. Plate counting reveal that an increase in the proportion of resistant bacteria can also be observed in field tests where manure of treated animals (fortified with antimicrobial agents) is spread onto agricultural land. The level of resistance observed in agricultural land is reduced back to levels close to the initial resistance levels over a time range of 150 days [28].

Further research is necessary to investigate the building up of a pool of resistance genes in both animal waste and agricultural land, also taking into account techniques focussing on genetic material (PCR).

3.1.5.1.1 Approach

The approach used is simple. For compounds that are known to be volatile or that are hydrolytically unstable (e.g. based on Table 6, the approach may be appropriate for use on ceftiofur), rather than using a flow-through system, covering the vessel or replacing the test solution, an open static system with no renewal of test solution is used. The approach may not be appropriate for longer-term exposures due to decline in the quality of the test medium

Table 6. Hydrolysis of veterinary medicines at pH 7

Veterinary medicine	DT50 (d)	Modified exposure stuffy?
Ceftiofur	8	possibly
Diazinon	78	x
Emamectin benzoate	>42	x

3.1.5.1.2 Experience with the approach

The approach has been applied frequently on selected pesticides, and could be extrapolated to pharmaceuticals.
When static toxicity studies are used, the dissipation in the test system should not be more rapid than that expected under natural conditions (e.g. comparing the Area Under the Curve for the test should be equal to or higher than the predicted exposure) for assuming that the data are valid for the risk assessment. The assessment of both exposure and effects must be done on the basis of the initial concentration.

3.1.5.1.3 Advantages of the approach

The approach is quick and easy to perform. It considers the impact of the abiotic dissipation processes along with associated metabolites.

3.1.5.1.4 Disadvantages

The approach may not be appropriate for certain test systems e.g. those studies that are performed over prolonged periods of time.

3.1.5.2 Variable-duration exposure studies

For compounds that dissipate rapidly, the duration of exposure to the veterinary medicine might be quite short. Studies can be performed with varying exposure duration, to determine the effects if any of such short exposures.

3.1.5.2.1 Approach used

Test organisms can be exposed to the test chemical for varying periods of time. After exposure, organisms are transferred to clean media and effects are then recorded at the standard test duration. For example, if a medicine, that is toxic to *Lemna* is predicted to dissipate in water within 1 d, *Lemna* would be exposed to a range of concentrations of test compound for 1 d and then removed and transferred to clean media for 6 d. This will then give a test duration of 7 d which corresponds to the standard *Lemna* study duration.

3.1.5.2.2 Previous experience with the approach

The approach has not been applied to veterinary medicines but has been used on pesticides. In a study by Maund et al. [31], *Gammarus pulex* were exposed to lambda-cyhalothrin for 1, 3, 6, 12 or 96 h and after exposure animals were transferred to clean test media and kept in this media until the total test duration of 96 h had been reached. There was a highly significant relationship between effects and duration of exposure with the effect concentration for 1 h exposure being 18 times higher (less toxic) than that for the 96 h exposure.

3.1.5.2.3 Advantages of the approach

The approach is simple to perform and will provide information on the likely impact of the actual exposure scenario.

3.1.5.2.4 Disadvantages of the approach

The approach may not be appropriate for certain test species and endpoints (e.g. reproduction) where the expected exposure duration is too short when compared to the species life span and significant differences could be expected depending on the developmental status of exposed organisms.

3.1.5.3 Pulsed exposure studies

Veterinary medicines may be released to surface water in pulsed (e.g. Figure 2). The continuous exposures used in standard tests may therefore not provide a true estimate of effects in the real environment.

Whilst data from standard toxicity studies can be used to provide an initial assessment of the effects of these types of exposures, it may be appropriate to perform 'custom-designed studies that mimic the exposure profile.

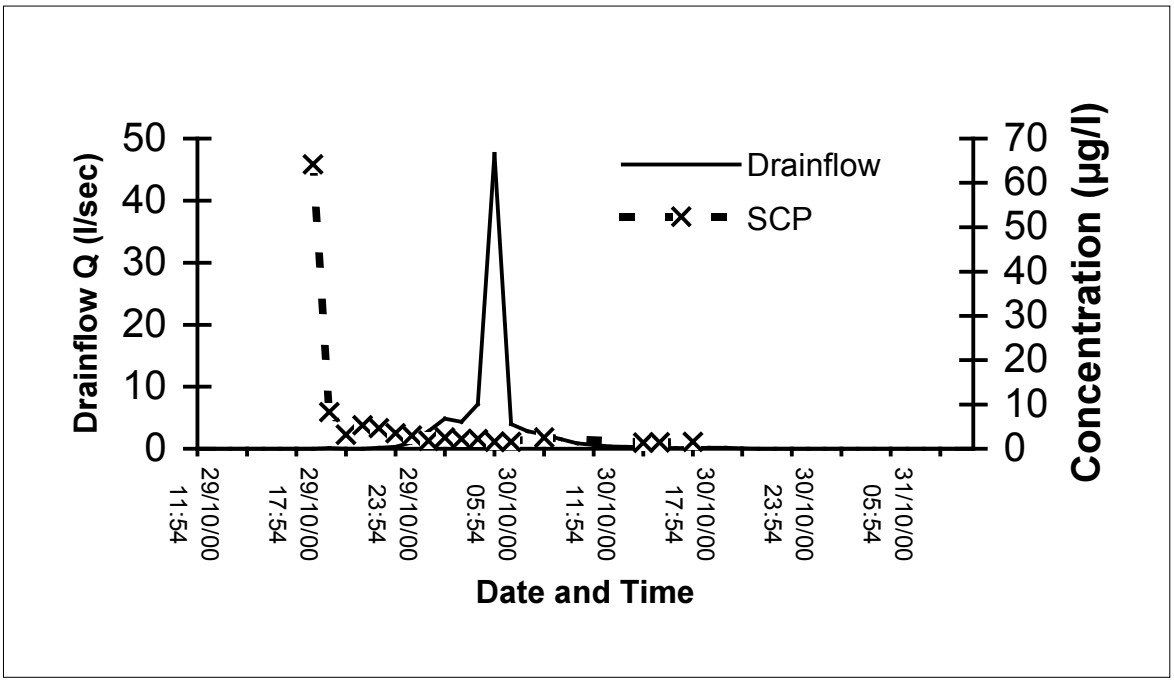


Figure 2. Measured concentrations of sulphachloropyridazine (SCP) in drainflow over time [32].

3.1.5.3.1 Approach used

Two general approaches can be used: static exposure studies or flow-through studies. The static exposure approach will generate square pulses whilst the flow-through systems allow pulsed exposure to be more realistically simulated with gradual changes in concentration.

3.1.5.3.2 Previous experience with the approach

The approach has not been used on veterinary medicines. However, it has been applied extensively to pesticides including fenoxycarb, fenitrothion, tebufenozide, chlorpyrifos and fenvalerate. In a study by Grade et al. (2000) [33], three substances were used in the simulation, namely the parent compound and two major metabolites.

3.1.5.3.3 Advantages

Provides assessment of impacts of real exposure scenarios.

3.1.5.3.4 Disadvantages

Approach may not be appropriate for certain test organisms (see variable exposure studies). Flow through approach resource intensive (e.g. ideally requires a lot of chemical analysis). The realism of the predicted exposure becomes critical and data extrapolation must be considered with care.

3.1.6 Organism recovery

For substances that dissipate rapidly in the environment and which are shown to be toxic, even when realistic exposure studies are used, it may be appropriate to perform an organism recovery study.

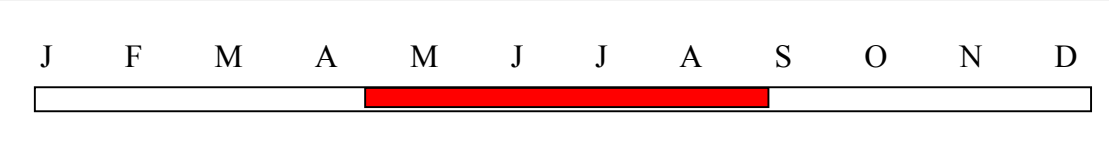
For aquatic organisms, such tests are often performed for *Lemna* and algae. A portion of exposed cells being transferred, once the test is complete, to clean media to determine whether cell division is irreversibly or reversibly retarded. The principle can also be extended to other studies (where mortality is not the endpoint) involving invertebrates and sub-lethal effects in fish. A few studies of this type have been reported for pesticides including organophosphates and carbamate pesticides and for organisms including daphnids, chironomids and blackflies (e.g. [34]). There is however considerable debate over the interpretation of the results of such studies and over what recovery timeframe is acceptable. Laboratory recovery studies can be used to estimate the expected effect on populations dynamics. However, it must be considered that extrapolation is limited to species with similar reproduction rates.
The reproduction cycles represent the time points where the new generation reach maturity and starts a reproduction. Obviously, these effects cannot be extrapolated to other invertebrates, even within the same taxonomic group, with other reproduction strategies.

Box 2 A simple model for assessing impacts on dung fauna.

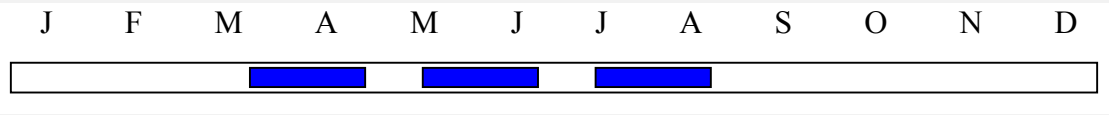
Parasiticides are used widely to treat a range of parasites in animals. These substances may be excreted in dung or washed off onto dung. Many of the substances are persistent and have been shown to be highly toxic to dung beetles and dung flies. Concern has also been raised over the knock-on impacts of these substances on rare predators such as selected birds and bat species which rely on flies and beetles as a food source

A number of models have been developed for assessing the impact of parasiticides on populations of dung fauna. Most of these models are detailed and hence require a large number of input data. As few data are available on the ecology of dung fauna and the usage and effects of parasiticides, a more simplistic screening level model might be useful that can provides a 'worst case' assessment of impacts based on a limited dataset. Such a model might assist in identifying chemicals or scenarios of concern that warrant further investigation or higher level modelling. A simple modelling approach has therefore developed and this is described below:

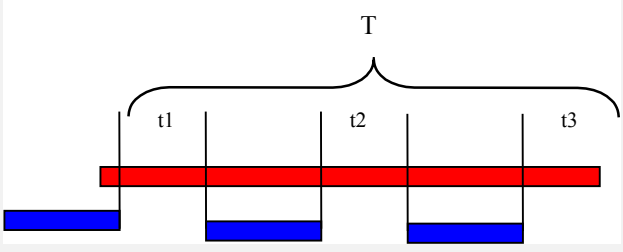
Firstly, determine broadly when the sensitive the stage(s) of the organism are likely to occur in dung



Next, identify the periods over which dung arising from animals treated with a parasticide is active (clearly this will be dependent on the rate at which the compound and its active metabolites are excreted, amongst other things).



Let *q* represent the proportion of time in which the sensitive stage(s) of the organism are theoretically capable of coming into contact with dung containing residues.



In this case for instance q = [T-($t1$+$t2$+$t3$)]/T

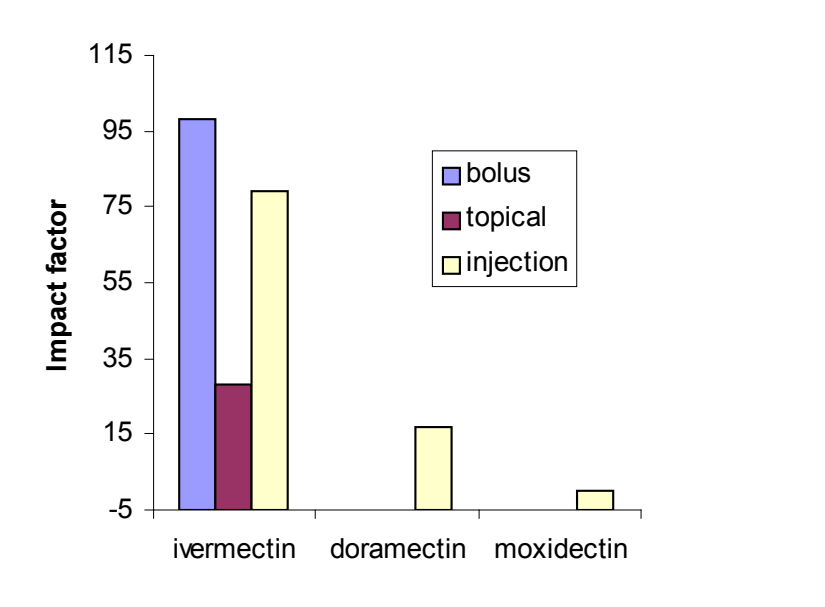
Similarly let *p* be the maximum proportion of *N* cattle treated at any one time, and let *v* be the maximum proportion of the life stage that are killed as a consequence of exposure to the highest field concentration of the anthelminthic in dung over the entire duration of this life stage.

If
i) toxicity does not change over the period of time dung it is attractive to the colonists
ii) the sensitive life stage does not move between pats (e.g. a larvae)
ii) the organism exhibits relatively quick development, so that dung colonised in periods t1, t2, t3 etc are eventually capable of supporting the life stage

then
a crude estimate of the impact of the anthelminthic (effectively the percentage of individuals killed as a consequence of its use) is:

impact = 100 . *p* . *q* . *v*

Calculated impact factors for a range of parasiticides and treatment types, obtained using data on the toxicity dung excreted by treated animals, are shown in Figure x. From the results it can be clearly seen that ivermectin administered either bolus or injection will have the highest impact on the populations whereas moxidectin administered by injection will have the lowest impact. Therefore it might be recommended that in sensitive areas (e.g. sites of special scientific interest), moxidectin is used rather than ivermectin or doramectin.



3.1.7 Multispecies soil systems (MS·3)

3.1.7.1 Description of the method

MS·3s consist of columns of natural sieved soil, designed as medium tier level assays, where soil dwelling macro-organisms are incorporated (Figure 3).

The soil-air interface, water transport and degradation/sorption kinetics are reproduced in a better way than in the standard soil bioassays, while the use of an homogeneous sieved soil and laboratory cultured macro-organisms facilitate the reproducibility of the results. The system also allows a realistic incorporation of the chemical, resembling the expected agricultural practices for the incorporation of manure. Fate properties, including leaching, can be investigated, and the combination of toxicity tests on leachates allows the assessment of the expected overall effects including those related to the parent and the metabolites produced within the time schedule for the test.

MS·3 can be used either for an initial screening assessment or as a tool for higher tier testing (Figure 4).

Figure 3. The arable soil MS·3 developed within the ERAVMIS project.

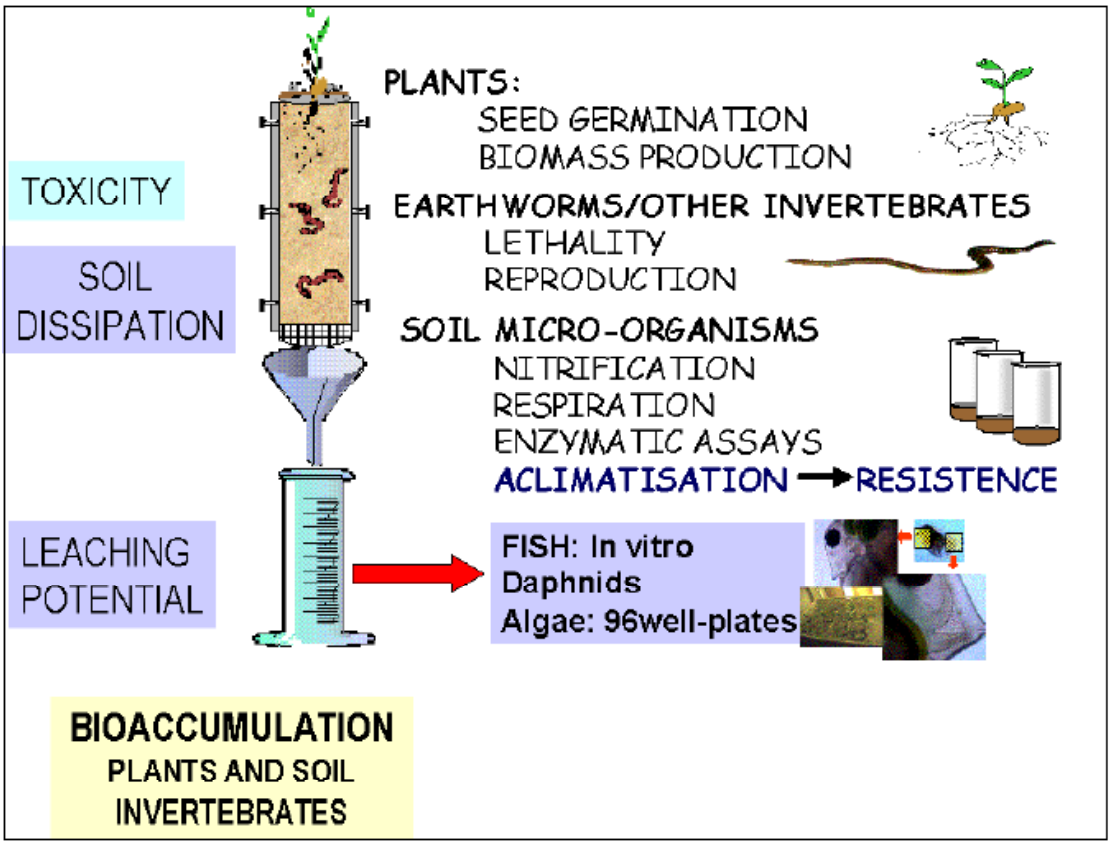


Figure 4 Summary of endpoints and information types addressed in the MS·3.

3.1.7.2 Soil characteristics

The assay is conducted on natural soils. Agricultural soils not treated with pesticides and not fertilised (either by manure, inorganic fertilisers, sludge, etc.) must be used. Soil physical-chemical and texture characterisation, and residue analysis must be presented. Soils are sieved (mesh size: 2mm) and homogenised.

3.1.7.3 Column design

Columns from an inert material, 15 to 25 cm diameter and 30-40 cm long, should be used. For simulating the arable soil, soil column depth should range between 20 and 25 cm. A system for collecting leachates should be placed below the soil column. Exposure of leachates to light must be avoided.

3.1.7.4 Test organisms

Natural soil microbial populations are used and therefore it is essential to handle and store the soil under conditions allowing soil micro-organisms development (Rules for soil degradation tests can be used guarantee these conditions).
Plants and soil invertebrates are added from laboratory cultures.
Certified seeds from at least three plant species (covering mono- and dicotyledonous) must be used.
Different soil invertebrates can be added. Earthworms, *Eisenia foetida*, have been used for the test design, while other soil and ground/soil invertebrates can also be used if required.
Plants and invertebrates must be added before applying the chemical for surface application or immediately after assembling the soil column when the chemical has been homogeneously mixed with the soil before filling the column.

3.1.7.5 Testing conditions
Columns are kept for 21 days, under 10/14 hours light/dark conditions (8000 lux), at about 20ºC, and irrigated, 3 times per week, with a suitable (spring, dechlorinated tap) water resembling 1000 mm annual rainfall. Temperature, light and irrigation conditions can be modified for specific assessment in order to resemble expected local conditions.

3.1.7.6 Leachate testing
Chemical analysis (parent and know metabolites) and toxicological analysis (fish, *Daphnia* and algae) of the leachates should be conducted. In vitro alternatives for fish testing using fish cell lines, and low volume testing alternatives for *Daphnia* and algae should be used.

3.1.7.7 Soil organisms end points
The final list of toxicity endpoints can be established depending on the introduced species and the testing needs. The suitability of the following endpoints has been already tested:
Soil micro-organisms: respiration, enzymatic activities. Effects must be assessed at least on two soil layers.
Plants: germination, biomass production, length, and accumulation of the tested chemical (metabolites).
Earthworms: lethality, accumulation of the tested chemical (metabolites).

3.1.7.8 Screening test
A screening test, mixing the chemical with the soil at 0 (control), 0.01, 1 and 100 mg/kg soil, in duplicate/triplicate columns (additional duplicates for microbial activities and chemical analysis) should cover the initial assessment. Effects should be tested after 7 and 21 days of exposure.

3.1.7.9 Higher tier testing
Higher tier testing conditions should require and specific protocol designed to fulfil the assessment needs. The protocol must be designed on the basis of the available information on the fate and effect profile, agricultural application conditions, etcetera, Some of the possible variables to be considered are presented below:
- species of soil macro-organims to be included and endpoints.
- toxicity endpoints for soil micro-organisms (including acclimatisation, biodiversity, …).
- local and climatic conditions (soil, temperature, soil, light, rainfall, …).
- soil column depth (arable only, arable plus additional soil layer).
- application of the tested chemical (on soil surface, homogeneous distribution on soil top layer or soil arable layer, resembling liquid manure application)
- co-application of manure (including application of spiked and aged manure).

3.1.7.10 Advantages/Limitations
The MS·3 offer a better simulation of the soil agricultural environment than the standardised ecotoxicity assays on soil organisms. It reproduces the air/soil interface, climatic factors and dissipation kinetics (degradation, mobility). The use of natural soil obviously limits the standardisation of the test.
Species are not assembled in food-chains; therefore, the inter-species interactions are limited to indirect effects (e.g. nutrient availability).
The screening test can be considered, basically, as a cost/effective alternative to conventional assessment, were several effect endpoints (mostly resembling those obtained from standard tests) and fate properties (are resembling lower tier fate test) are obtained from a single test,

under more realistic conditions and reducing cost (e.g. cost for handling and chemical analysis). In addition, metabolites formed during the test timeframe are also tested.

3.1.8 Microcosm / Mesocosms

There are several guidelines on the design of aquatic micro-and mesocosms. Still, the design has to be adapted to the test compound on a case-by-case basis.
The new guidance document for assessing the effects of pesticides on aquatic organisms offers an extensive revision of the current knowledge on microcosms and mesocosms studies, which can be applicable to veterinary medicines [35] .

An alternative to food-wed designed microcosms, focuses on simpler assemblages of several species from the same taxonomic group. These single taxa microcosms have been sporadically used for pesticides, mostly for herbicides. Some design have been developed for invertebrates and can be particularly useful for veterinary pharmaceuticals (e.g. [36]).

3.1.9 Terrestrial model ecosystems

TMEs consist of encased intact (non-homogenised) soil-cores that are extracted from the site of interest (e.g. from a natural grassland). By this method the disturbance of the natural vegetation, soil microflora and fauna, and the layering of the soil inside the cores is minimised. The size of a soil-core is 17.5 cm in diameter and 40 cm in depth. All soil-cores are placed in special containers and kept under temperature-, moisture-, and light-controlled conditions in a greenhouse/growth chamber. Each soil-core is watered via special rain-heads and leachates are sampled at the bottom of each core.
Comparisons among TMEs and field results have been carried out within an EU project co-ordinated by Dr. Thomas Knacker, from ECT. The following end-points were addressed:

Fate End-Points:
- vertical distribution of the test substance (carbendazim) in the soil-cores (HPLC)
- amount of test substance in leachates (HPLC)
- accumulation of test substance in vegetation

Effect End-Points:
- amount of nutrients (C, N, P, K, S) in soil (KCl extraction)
- amount of nutrients (C, N, P, K, S) in leachate
- soil microbial biomass (SIR)
- bacterial growth rate (Tritiated Thymidine Assay)
- enzyme activities in soil (Cellulase, Dehydrogenase)
- feeding activity of the soil biocenosis (Bait Lamina)
- abundance and diversity of earthworms (hand sorting)
- abundance and diversity of enchytraeids (wet extraction)
- abundance and diversity of *Collembola* and Gamasid mites (Tullgren extraction)
- abundance and trophic groups of nematodes (wet extraction)
- decomposition (litter bags)
- plant biomass (aboveground, fresh weight)

The project results were presented in a specific workshop and will be published as a special issue of the journal Ecotoxicology.

A critical aspect for using TMEs when assessing the effects of veterinary drugs in sludge is the application method. As TMEs are cores of undisturbed soil the application of chemicals must be limited to over-spray or application of liquid solutions.

3.1.10 Degradation tests (focus on litter bags)

The litter bag test is a functional test designed to address simultaneously the effects of chemicals on soil degradation processes where both soil micro and macro-organisms are involved.
The new guidance document on risk assessment of pesticides to terrestrial systems [15] requires this test for highly persistent molecules (DT90 > 365 days) and for persistent pesticides (DT90 between 100 and 365 days) showing a potential risk in studies on soil respiration/nitrification, or single species test on soil invertebrates (earthworms, ground arthropods, Collembola or mite).
As specific workshop on the Effects of Plant Protection Products on Functional Endpoints in Soil – EPFES, was held in Lisbon, Portugal on 2002 for discussing the methodology and a guideline on this test is under development.

3.1.11 Field tests

Field tests are considered the highest tier testing possibility in environmental risk assessment. Nevertheless, field-testing is not only expensive but also produce complex results that must be carefully analysed.
Field test are frequently used in the regulatory assessment for pesticides, however, for veterinary pharmaceuticals they have been mostly used at experimental level.
Field studies have been mostly conducted for addressing fate properties, however some examples of studies addressing also effects are available, such as those conducted in Denmark by Dr Halling-Sørensen or in Canada by Dr Solomon.
Field studies must be designed to fulfil the uncertainties and gaps remaining after an evaluation of the basic properties, exposure and effect profiles of the chemical. Therefore, field studies are not conducted in the first round of testing but only for risk refinement. It is a common practice to submit the protocol of field studies to the regulatory authorities for authorisation prior to conduct the test. This method may allow the application of field studies in the regulation of veterinary pharmaceuticals even for those cases where no methodological guidelines are available.

3.2 Integrating the ecotoxicological information in the effect assessment: quantifying expected ecosystem effects

Nowadays the methodological development of ecotoxicology covers a huge amount of possibilities, from in vitro testing to large mesocosms and field studies. These tests should provide the information required for the effect assessment of chemicals, including the hazard identification and the quantification of the dose/response relationships.
Following the suggestion from the EU Scientific Steering Committee [37,38], the ecotoxicity tests and the effect assessment conducted on their basis could be classified in 5 different levels or tiers, represented in the diagram below:

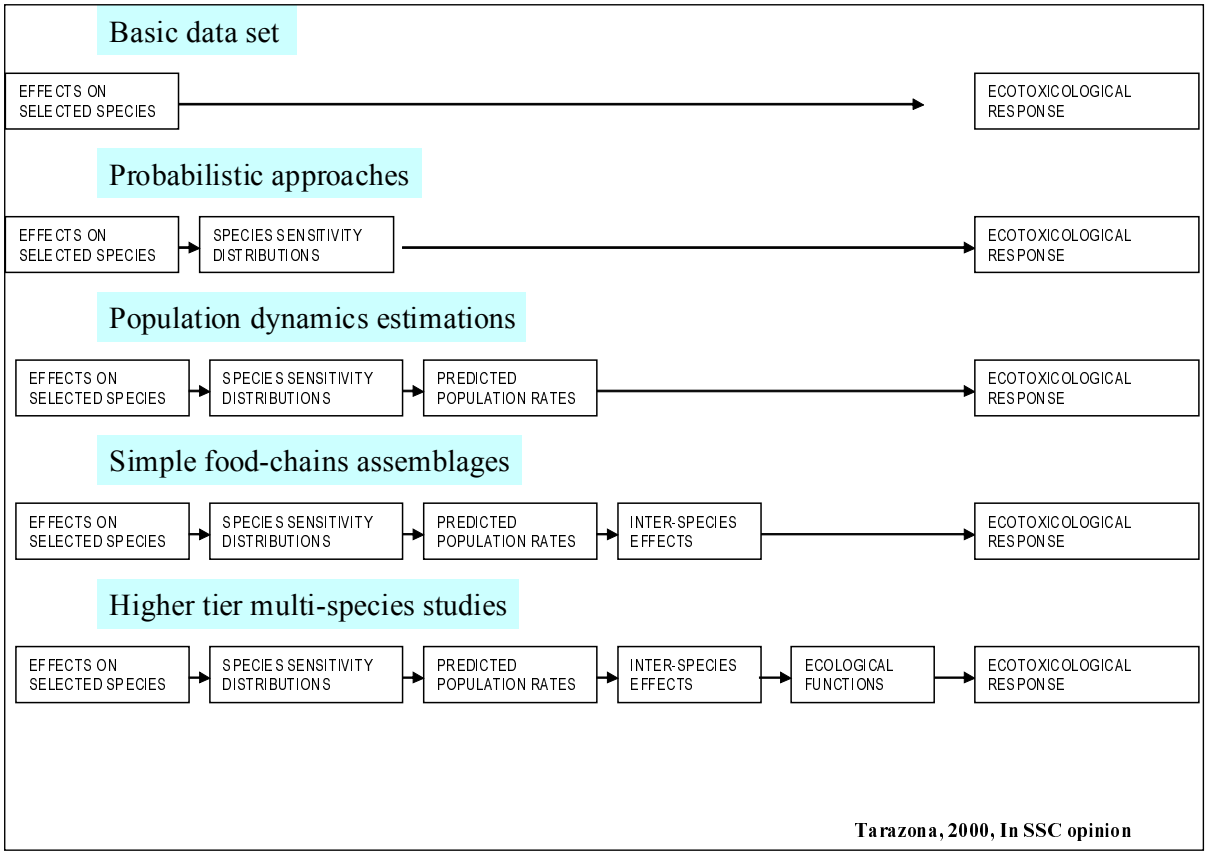


Figure 5 Tiered alternatives for the ecotoxicological effect assessment (from SSC, 2000).

The level of extrapolation from the measured endpoints to the ecotoxicological response of communities and ecosystems, as well as the realism of the estimations, increases dramatically when moving from the lower to the higher tier assessments. However, this situation does not necessarily imply that the testing strategy must follow strictly the sequence. Most risk assessment protocols starts with an initial, tier 1, screening assessment, but if potential risk is identified, the following alternatives, from tier 2 to tier 5, should be specifically considered and a decision must be taking considering the advantages and disadvantages of each possibility. As a general rule, the lower tier toxicity test can provide very useful information for the experimental design of higher tier testing, and therefore when the higher tier studies must be designed on a case-by-case basis, it is strongly recommended to get previously a basic set of lower tier toxicity tests.

The reduction in the level of extrapolation, required for interpreting test results on the basis of ecological relevance, obviously reduce the uncertainty in the assessment. Most ERA protocols present clear triggers for lower tier assessments but consider case-by-case decisions for analysis based on higher tier assays. If a higher tier test has been well designed and conducted, is fully validable, and represents realistic worst-case conditions, no additional uncertainty factors could be required when setting the ecotoxicological thresholds. The assessors must, however, conduct a specific assessment of the remaining uncertainty when considering the results of higher tier assays. The increase in the realism, can also limit the extrapolation capacity of these tests outside the particular conditions investigated in the specific study. Therefore, large uncertainty factors are required in same cases even for well-conducted higher tier studies, if the assessed conditions do not represent a realistic worst-case for the overall variability expected to be covered in the ERA.

3.2.1 Determination of the effect profile based on PNEC derivation

There are several possibilities for presenting the outcome of the environmental effect assessment of chemicals [38]. The most widely employed method is the determination of the Predicted No Effect Concentration (PNEC) currently used for the aquatic effect assessment of veterinary medicines [3] and the aquatic and terrestrial risk assessment of feed additives (Directive 2001/79/EC [39]) and industrial chemicals [40]. Predicted no effect concentrations are aimed at giving a concentration of the compound in a specific compartment at which no adverse effects should occur, taking into account the relevant endpoints and species.
The main benefits of this approach are described below:
- offers a community/ecosystem approach, covering all relevant taxonomic groups, and therefore allowing the inclusion of indirect effects
- it can be derived from all kind of tests, from lower to higher tier methodologies
- the risk and the associated uncertainty can be presented in a transparent way.

It is suggested to use the PNEC as the basis for the effect assessment both for the aquatic and the terrestrial environment.
PNEC derivation follows different approaches, depending on the amount and type of information, which is available. Following the SSC recommendation five tier levels are suggested, which have been adapted to veterinary medicines and are presented below [37,38].

3.2.2 Tier 1. Deterministic derivation of the PNEC

Methodological approach
The initial, lower tier, PNEC derivation is conducted on an initial data base of available single species toxicity tests. The information must cover all relevant taxonomic groups. The PNEC is derived by applying a factor to the most sensitive ecotoxicological endpoint. This application factor is selected on the basis of the amount and type (acute/chronic) of available information.

The application factors employed in EU regulations have not been harmonised. The PNEC approach is currently used for industrial chemicals and feed additives, and the EMEA guideline [3] recommends the use of the PNEC approach for aquatic organisms (but not for terrestrial ones).

The Scientific Steering Committee [38] has very recently published a comparison of the application factors employed by different EU regulations. The comparison does not only present the factors adopted for those guidelines based on PNECs but also the equivalent adjustment factors extrapolated after and in-depth assessment of other regulations. These tables are reproduced below.

Required methods
Single species (single taxonomic groups for micro-organisms) are employed. Both, acute and chronic tests, can be used, and it is strongly recommended to use standardised test whenever available. Currently, guidelines for aquatic and terrestrial organisms are available and can be used for veterinary medicines as expressed before.

Table 7. Comparison of the adjustment factors between laboratory toxicity results and expected exposure levels for assuming low risk (called margins of safety in the CSTEE opinion) (ratio between toxicity and the expected exposure level) for the protection of terrestrial organisms employed in the environmental risk assessment of feed additives, veterinary medicines, industrial chemicals and pesticides. From the European Commission-SSC, 2003 report.

Group	**Exposure route**	**Timing**	**Adjustment Factor (Margin of safety*)**			
			Feed Additives	**Veterinary Medicines**	**Industrial chemicals**	**Pesticides**
Vertebrates (birds and mammals)	Direct	Acute Chronic	Not considered	10 -	Not considered Not considered	10 5
	Secondary poisoning	Acute Chronic	Not considered	10 -	1000 100-**10**	10 5
Plants	Soil	Acute Chronic	100- >1000 -	10 -	1000 100-**10**	Not considered
Earthworms	Soil	Acute Chronic	100- >1000 -	10 or 100 depending on persistence in soil	1000 100-**10**	10 5
Bees	Oral Contact	Acute Acute	Not considered	Not considered	Not considered Not considered	5-17 5-1500
Other arthropods	Contact	Acute	Not considered	<1-1	Not considered	1-5
Soil micro-organisms	Soil	Acute Chronic	100- >1000 -	10 -	1000 100-**10**	1-5

Table 8. Comparison of the adjustment factors between laboratory toxicity results and expected exposure levels for assuming low risk (called margins of safety in the CSTEE opinion) (ratio between toxicity and the expected exposure level) for the protection of aquatic organisms employed in the environmental risk assessment of feed additives, veterinary medicines, industrial chemicals and pesticides. From the European Commission-SCC 2003 report.

Group	**Exposure route**	**Timing**	**Adjustment factor (Margin of safety*)**			
			Feed Additives	**Veterinary Medicines**	**Industrial chemicals**	**Pesticides**
Fish	Water column	**Acute** **Chronic**	100- >1000 -	100	1000 100-**10**	100 10
Invertebrates (Daphnia)	Water column	**Acute** **Chronic**	100- >1000 -	100 -	1000 100-**10**	100 10
Algae	Water column	**Acute** **Chronic**	100- >1000 -	100 -	1000 100-**10**	10 10
Aquatic plants	Water column	**Acute**	Not considered	Not considered	1000 100-**10**	10 10

Suitability of this approach for veterinary medicines

Veterinary medicines are biologically active chemicals and therefore, some ecological receptors and endpoints can be particularly sensitive. This sensitivity can be higher that the application factors (see examples in the recommendation chapter). Therefore it is critical to select the species and endpoints to be tested according to the mechanism or action and characteristics of the molecule.

3.2.3 Tier 2. Probabilistic derivation of the PNEC

3.2.3.1 Methodological approach

The available information on the toxicity of a chemical to different species is employed to establish the Species Sensitivity Distribution. The information can cover all relevant taxonomic groups, or just the most sensitive taxonomic group when there is enough information for establishing this group.

SSD can be established for the overall range of species within a compartment, selecting species covering all representative taxonomic groups, or for each particular taxonomic group within a compartment. The first option has been proposed for non-biologically active chemicals [40], while the independent assessment of each taxonomic group is proposed for chemicals with specific mechanisms of action, including pesticides and veterinary medicines.

There are several options for fitting the available data to the SSD, and the selection of the curve is essential for the further estimation. Some models, such as fitting to a triangular curve, estimate a concentration/dose which is expected to protect all species (0 percentile); while other fitting approaches (normal, lognormal, logistic) does not estimate this 0 percentile, and therefore the assessment must be based on the 1^{st}, 5^{th}, 10^{th}, …, percentile. The uncertainty in the fitting procedure can be quantified through statistical methods.
The PNEC is derived by applying a factor to the value or values extracted from the curve (i.e. to the 95 confidence interval of the selected percentile). This factor must cover the remaining uncertainty in the assessment. Factors between 1 and 5 are frequently considered but they must be selected on a case-by-case basis.

In general SSD should be based, whenever possible, on chronic NOEC values, however, if only acute LC(E)50s are available, and the information permit a sound determination of the acute to chronic ratios, acute data can also be employed. The environment fate of the molecule can also inform on the relevance of acute risk estimations.

Required information
Single species (single taxonomic groups for micro-organisms) are employed, and results on several species is required.
SSDs must be handled with caution when the data base is small (which can be expected for several veterinary drugs). In this case, care has to be taken to appropriately reflect uncertainty in the dataset. Further, SSDs should only be derived independently for each taxonomic group, because the special mechanisms of action of veterinary drugs do not allow extrapolation of toxicity between taxonomic groups. It has been recommended to use not less than 8 species (HARAP), apart from vertebrate tests, where a reduction in the number of animal testing should be aimed at (choice of 5 fish species). The choice of species should be adapted taking into account the mode of action of the compound.

For certain groups, no standardised protocols are available for the required number and diversity of species and therefore, non-standard methods should be employed.
The EU concerted action EUFRAM will produce information on the probabilistic risk assessment of pesticides. This information will be useful for the use of these methods for veterinary medicines.

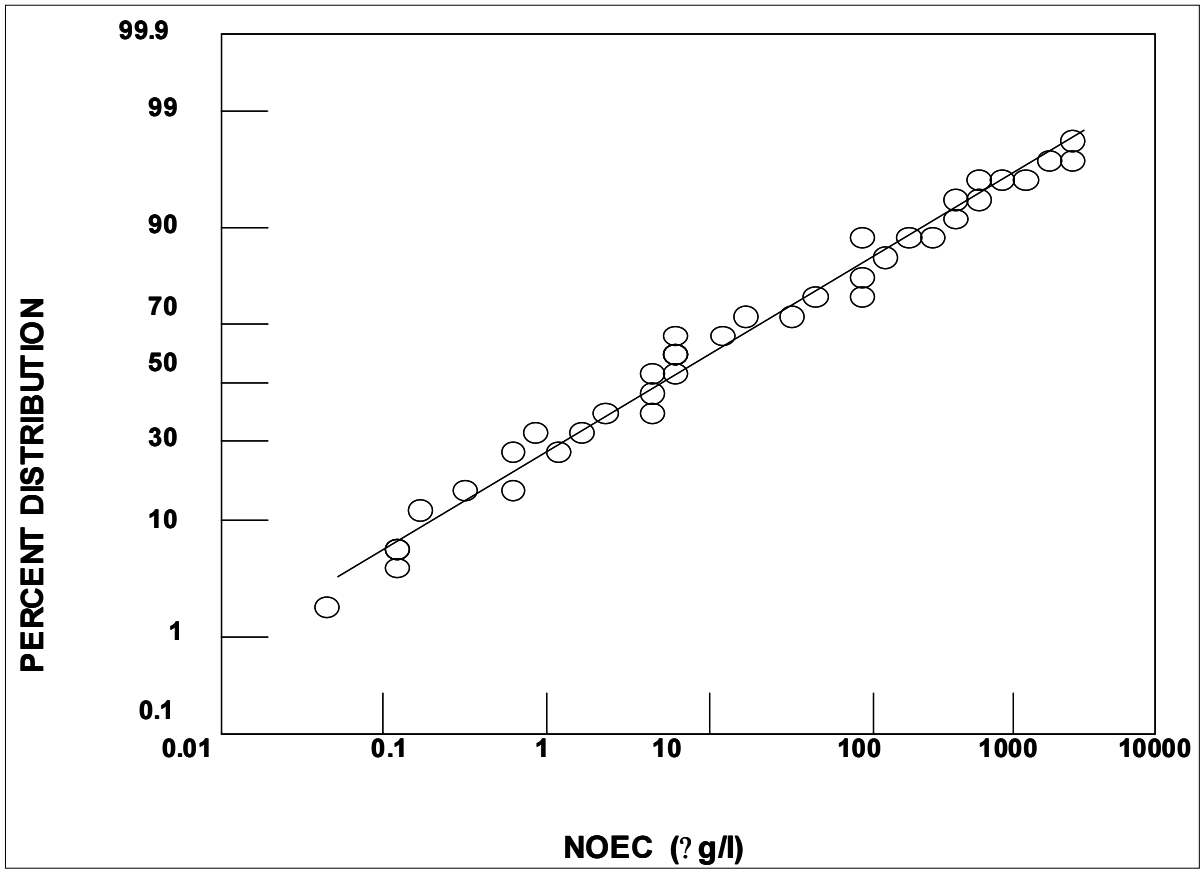


Figure 6. Example: chronic toxicity data obtained for an insecticide are fitted to a log-normal distribution. Fitting allows statistical estimations (including confidence intervals) for the selected percentile.

Advantages and limitations

Probabilistic estimations employ all available information, and the shape of the distribution curve allows a better assessment of the expected uncertainty. The use of statistical methods is also an advantage.

The interpretation of the outcome in the regulatory context requires a clear explanation. Probabilistic approaches are just an alternative methodology for a PNEC estimation and cannot be presented as a direct estimation of the protection goals. The distribution, and their confidence intervals, are referred to the laboratory studies and endpoints, and should not be interpreted as level of protections achieved for species in the natural ecosystems.
SSDs must be handled with caution when the data base is small (which can be expected for several veterinary drugs). In this case, care has to be taken to appropriately reflect uncertainty in the dataset.

Further, SSDs should only be derived for one taxonomic group, because the special mechanisms of action of veterinary drugs do not allow extrapolation of toxicity between taxonomic groups. It has been recommended to use not less than 8 species (HARAP), apart from vertebrate tests, where a reduction in the number of animal testing should be aimed at (choice of 5 fish species). The choice of species should be adapted taking into account the mode of action of the compound.

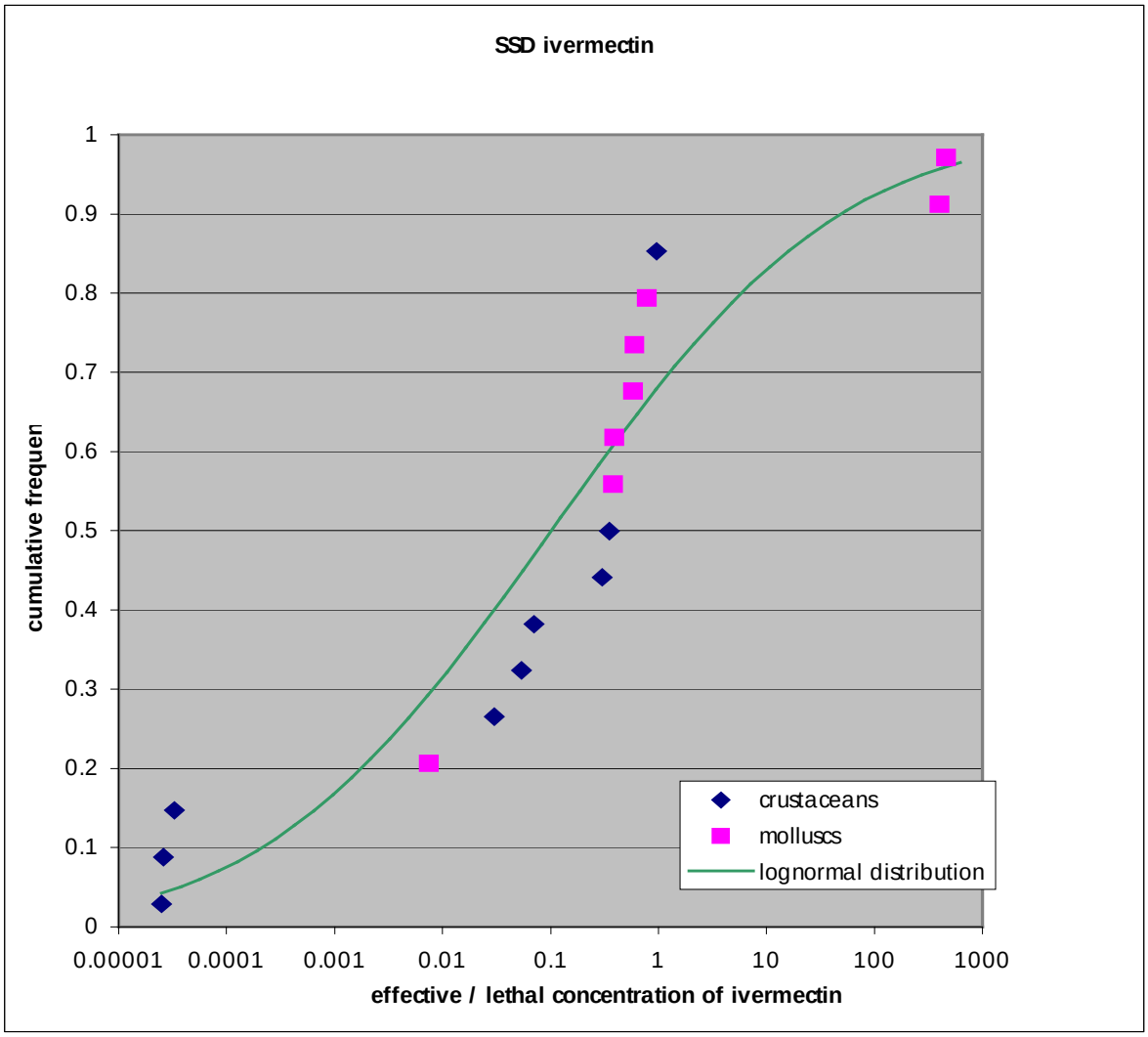


Figure 7 Example: SSD for ivermectin and different invertebrates, including molluscs and arthropods.

3.2.4 Tier 3. PNEC derivation based on expected population effects

Methodological approach

The standard acute and chronic endpoints are, in general, crude estimations of effects measured at the individual level assumed to be ecologically relevant.

Tier 3 methods present a refinement of the expected population effects, accounting for more realistic estimations of the exposure (e.g. including bioavailability), estimations of the expected effects on populations (e.g. using population dynamics models), potential for recovery, etc.

The PNEC is determined from the concentration/dose expected to produce no significant effects at the population level. The uncertainty in this determination is also considered when establishing the PNEC value.

Required information

Specific testing protocols, covering the conditions to be tested must be developed. Model for estimating the population dynamics are also required in same cases.

Box 3 Assessment of metabolite ecotoxicity

Following administration, veterinary medicines may be metabolised and these metabolites may be excreted along with the parent compound in the urine and the faeces. Once released to the environment, the parent compound and the metabolites may be further transformed by both biotic and abiotic degradation processes. As a consequence, terrestrial and aquatic organisms may be exposed to a mixture of parent compound, metabolites and soil/sediment and water transformation products.

In the absence of experimental data on the ecotoxicity of metabolites and transformation products (which are referred to from now on as metabolites), current regulatory assessment schemes generally use a total residue approach which assumes that the metabolites have equal toxicity and behave in a similar way to the parent compound. As many metabolites are likely to be significantly less toxic than the parent compound (e.g. Figure 8), this approach is likely to be precautionary.

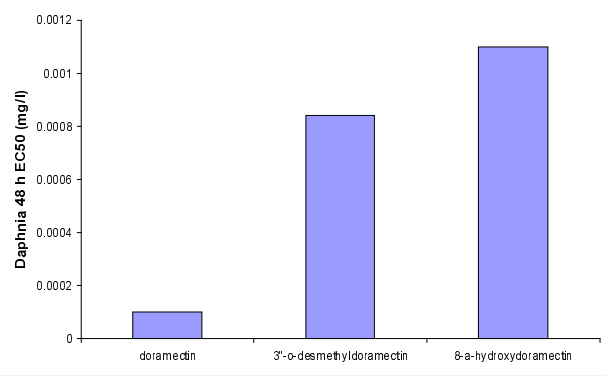


Figure 8 Ecotoxicity of doramectin and two of its major metabolites to daphnids [41].

However, in some instances it is possible that a metabolite is more toxic and due to differences in the behaviour of the parent compound and the metabolites (e.g. Figure 9), it may underestimate the exposure of a particular environmental compartment.

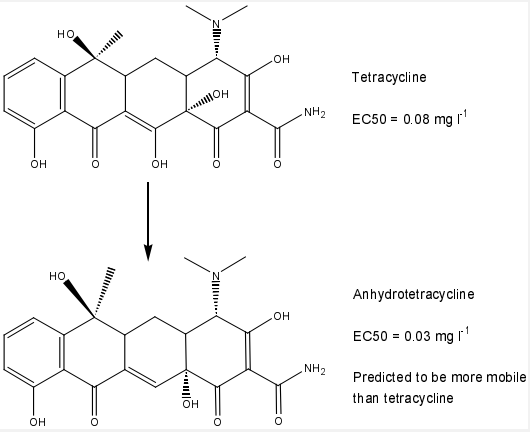


Figure 9 Differences in ecotoxicity and mobility of oxytetracycline and one of its degradation products, anhydrotetracycline

Standard ecotoxicity studies could be performed on each of the metabolites and transformation products to determine potential effects on aquatic and terrestrial organisms, such as was done for doramectin in the example given above. The problem when using the approach is identifying what metabolite (there can be many) to test and which endpoint to investigate. In the pesticide area, this area has been subject of much debate for many years and a number of proposals have been produced for identifying metabolites of potential concern, the approaches suggested for pesticides include an assessment of information on the:

- amount of the metabolite produced relative to the parent
- mode of action of the parent compound and the metabolite
- relative uptake of the metabolite compared to the parent
- the toxicity values for the parent

It is possible that a similar approach could be applicable to veterinary medicine although our current knowledge of the behaviour and effects of veterinary medicines may not be yet developed enough to propose guidance in this area. For those metabolites identified as of concern, they will need to be synthesised and extensive testing may be required – this is obviously costly and time consuming.

Metabolites of veterinary drugs of concern should be the ones that
- are formed in the animal in high proportions
- possess higher persistence
- possess higher mobility
- possess more likelihood to bioaccumulate / being taken up
- possess the toxic moiety
- possess reactive groups
- can free the parent compound

An alternative is to use test approaches that integrate an assessment of the effects of parent compounds and metabolites. For example, rather than applying the parent compound to the MS3 test system (which is described elsewhere in this report), manure from treated animals would be applied. As this manure would contain not only the parent but also the metabolites and as the MS3 column can incorporate important dissipation processes (i.e. degradation in manure, degradation in soil and leaching) and an assessment of toxicity of column leachate, the impacts, if any, of the mixture of parent compounds and metabolites on both terrestrial and aquatic organisms could be readily established. In the context of the ERAVMIS project, a set of experiments have been conducted on doxycycline, applying the chemical to pig manure, ageing the manure and studying the effects of aged spiked manure in the MS·3. Soil microbial activities were inhibited by doxycycline, but showing different patterns between the aged manure and the pure chemical.
This approach has already been used for coccidiostats (Tarazona, personal communication) and these results have been submitted to regulatory bodies and have been found to be acceptable as long as the parent compound and, if possible, metabolites have been analysed for in the manure. In some instances, the approach could also be applied to earthworm studies and soil microbial inhibition studies, where manure is part of the test matrix. Mesocosms and field trials could also be used.

Advantages/Limitations
This methods offers a more realistic and ecologically relevant approach for estimating the expected consequences of the chemical exposure. They require much more information than the previous approach and must cover the overall exposure timeframe.
A limitation for population dynamics modelling and recovery tests is the extrapolation between species. For obvious reasons, typical laboratory test species have high reproduction rates, therefore, the extrapolation of expected population effects (based on models or recovery experiments) to other species with different reproduction rates must be done with care.

3.2.5 Tier 4. PNEC derivation from lower tier multispecies assays

Methodological approach
Tests are conducted on system assemblages, which include the co-exposure of several species. The typical aquatic assemblages are based on food-chain relationships were indirect effects on prey/predator relationships (from primary producers to predatory vertebrates) are studied.
For the terrestrial systems these food-chain assemblages although possible, are not commonly employed due to the difficulties for including terrestrial vertebrates. The alternatives have focussed on the use of vertebrate-free systems, covering soil micro-organisms, soil and ground macro-invertebrates and plants.
The PNEC is derived from the concentration producing no relevant effects on the endpoints, which can be either structural or functional, plus the additional consideration of the uncertainty.

Required information
Two basic testing alternatives can be employed. Artificial assemblages, such as the MS·3 or undisturbed soil columns, such as the Terrestrial Model Ecosystems.

Advantages/Limitations
These systems reproduce more realistic exposure conditions and can include more relevant effects, particularly indirect effects. Reproducibility and standardisation possibilities depends on each test design but in general are better than for the higher tier multispecies assays.

3.2.6 Tier 5. PNEC derivation based on mesocosms and field studies

Methodology
The effects of controlled exposures are directly assessed on the field or on artificial systems reflecting the complexity of field situations (large outdoors mesocosms) including ecological parameters trying to measure directly structural and functional effects on populations and communities.
For each study, a concentration/dose, which does not produce ecologically relevant effects, is settled. This concentration must be settled on biological, not purely statistical, basis.
The PNEC is derived from the selected concentration accounting for the remaining uncertainty for extrapolating the assayed conditions to a generic scenario.

Information required
Mesocosms or field studies.

Advantages/Limitations
The interpretation of the test design requires an in-depth analysis of the results. These studies must be designed and interpreted case-by-case, and requires a preliminary evaluation of the effect profile of the chemical.
The representativeness of the selected conditions is essential for allowing the extrapolation of the results outside the studied area/conditions.

3.3 Recommendations on the testing strategy for assessing the effects of veterinary pharmaceuticals

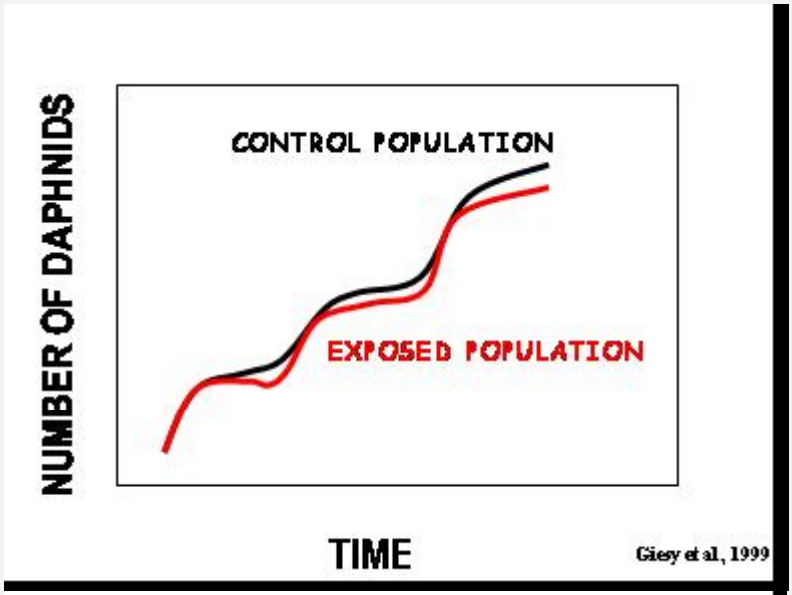
Risk management decisions are a compromise of several aspects related not only to the scientific evaluation of available information but also to the socio-economic implications of the decisions to be taken.
In the regulatory arena, the decision on the testing strategy is considered a key element, trying to balance the assessment needs and the costs (economic, time, animal use, etcetera) required for getting the required information.
Obviously, enlarging the testing requirements reduce the uncertainty of the assessment but increase the costs. A typical solution for solving this dilemma is the use of tiered testing strategies.
All EU environmental risk assessment protocols have been constructed under tiered approach, although the basic information requirements are not harmonised. The larger amount of initial ecotoxicological information is required for pesticides, where a set of acute and chronic studies on aquatic and terrestrial organisms are basic elements of the initial dossier. Veterinary medicines and feed additives are just the opposite. Ecotoxicity tests are currently requested only for Phase II, therefore, most products are commercialised on the assumption of low environmental risk based exclusively on the postulation of low exposure levels, following the Phase I estimations.
The effect assessment tools developed and/or used in ERAVMIS constitute an updated state of the art of effect assessment for veterinary medicines.
On the basis of these results and the review of new information a tiered testing strategy is proposed.

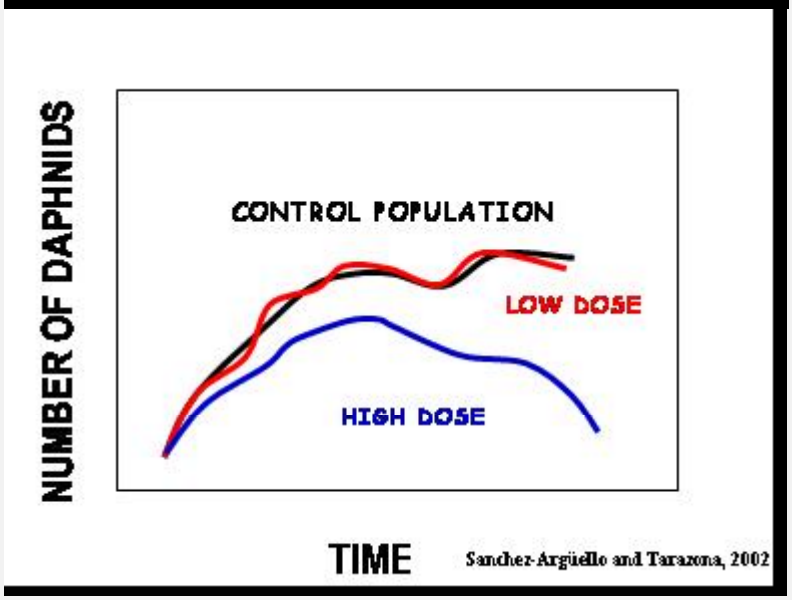
3.3.1 Generic testing strategy for veterinary medicines

The proposed testing strategy is described in four different levels. Levels 1 and 2 should be required for all veterinary medicines while levels 3 and 4 are part of the effect assessment refinement, and therefore, are only required if the risk characterisation conducted on the basis of the information generated in levels 1 and 2 cannot exclude that the proposed use represent a low environmental risk.

Box 4 Assessing effects on population dynamics

Two basic approaches for assessing the effects on population dynamics from laboratory experiments can be used, modelling and testing. Modelling approaches determine through experimental assays the parameters regulating population dynamics (e.g. survival and reproduction rates) and fit the data into the model. The extrapolation is based on single generation assays.





Experimental approaches include the design of multigeneration assays and the direct measurement of the number of individuals under the tested conditions. The effect of long term exposures, including potential effect on the second, third and further generations, resulting in delaying population declines are only covered in this methodological approaches.

3.3.2 Testing strategy: Level 1

Level 1 tries to summarise the basic profile of the molecule. It comprise two types of information, information on a set of toxicity tests and a summary of additional information extracted from the efficacy and safety dossier.

The toxicity test covers two main groups:

MAMMALIAN TOXICITY
No additional studies on mammals are initially required for the ecotoxicological assessment, however, the studies conducted for assessing the safety of the veterinary medicine (mostly the acute, subchronic and chronic oral studies) must be submitted and re-evaluated by experienced ecotoxicologists. This aspect is particularly critical for the subchronic and chronic studies as the NOAELs selected for animal and/or human protection may be based on endpoints with are considered of no ecological relevance.
For pesticides and other chemicals the endpoints assumed to be ecologically relevant are those related to survival, growth and reproduction. The same approach can be used for pharmaceutical products.

AQUATIC TOXICITY TESTS
It is proposed to request the standard 'aquatic base set', comprising acute toxicity studies on fish, daphnia, and algae following the OECD guidelines.
This base set has a relatively low cost, offers very useful information, and is also required for general chemical regulations, such as directives on classification and labelling and those based on initial hazard identification such as the Seveso II Directive or the IPPC Directive. Therefore this information will not only be required for the risk assessment of the product used by the farmers, but also to the other phases of the product life cycle, such as production and formulation (e.g. for assessing the risk of the industrial effluents), transport and storage (e.g. through classification and labelling and related 'downstream' regulations), and disposal (e.g. assessment of residues and wastes).
The requirement of this base set is in agreement with the current EMEA proposal.

ADDITIONAL INFORMATION
The dossiers for pharmaceutical products also include a significant amount of very relevant information that is not always considered in the environmental risk assessment.

All this information should be addressed in the level 1, for getting a better profile of the molecule. This information includes a.o.:
- mechanism of action
- chemical structure
- QSARs
- expected effects.

3.3.3 Testing strategy: Level 2. Introduction and proposed decision tool

The level 2 offers an innovative proposal, based on a new conceptual model for covering non-homogeneous risk assessments.
This conceptual model was initially designed for covering terrestrial ecosystems [42], which has been already implemented for supporting regulatory risk assessments [43]. The conceptual model can be expanded to other non-homogeneous assessments, considering, for example, the inhomogeneous responses among organisms expected for biologically active molecules.
The model is proposed in level 2 as a decision tool for identifying the additional testing required for a particular veterinary medicine on the basis of the available results.
Figure 10 present a schematic representation of the model proposed by the ERAVMIS partners.
Basically, the decision tool covers three main issues. First the use patterns regulates the expected environmental emissions/releases; second, the basic physical-chemical properties, the studies on fate and behaviour (degradation, adsorption/desorption, leaching, etc.), and the toxicokinetic studies will identify which environmental compartments are expected to receive the highest level of exposure; and, third, the toxicity studies and information analysed in level 2 will determine the most relevant ecological receptors. This information will identify the critical exposure-to-receptor routes on which level 2 studies should be required.

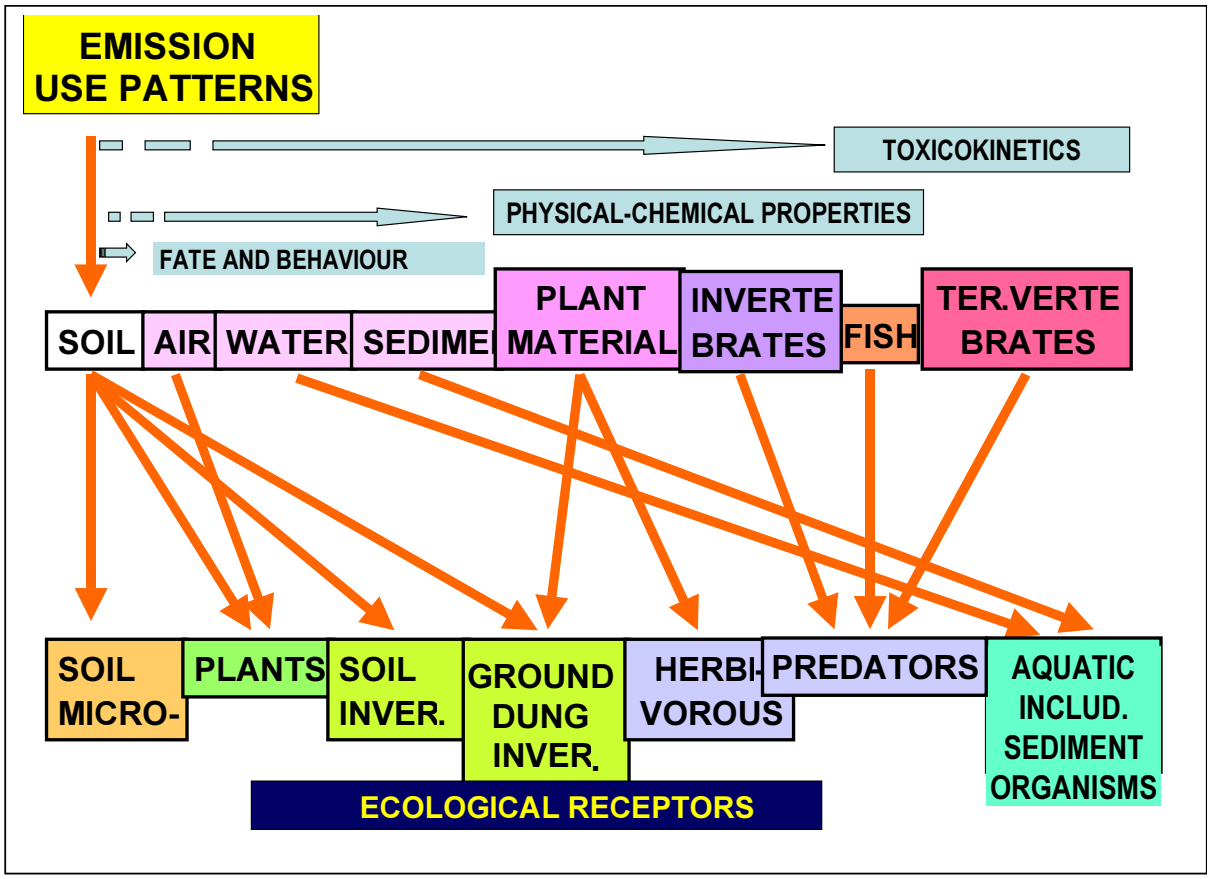


Figure 10 Schematic representation of the decision-making tool for level 2.

Testing could then focus on the ecological receptor, which is thought to be most at risk. To this end, an endpoint and finally a test should be chosen from the range of available standard and non-standard tests. If existing, results from tests already undertaken can be extrapolated to judge environmental effects for this particular ecological receptor. For example, data on mammalian toxicity, which has been prepared in the development of the drug, can be used for the estimation of effects on predators.

To give an indication about how knowledge on the modes of action and structural features of veterinary drugs could be used to identify ecological receptors, the following table lists modes of action and possible sensitive organisms for a range of veterinary medicines. Level 2 studies cover the required studies on soil organisms, and possible additional studies on aquatic organisms.

Justifications for this innovative approach and some examples are presented below.

3.3.4 Testing strategy: Level 2. Selection of specific lower tier tests for the aquatic and terrestrial compartment

3.3.4.1 Aquatic environment – base set versus other endpoints

The proposed Level 1 request the base set of fish, daphnia, and alga toxicity in the first tier for the aquatic compartment. However, taking into account the specific mechanisms of action of veterinary drugs, it might not always be protective to rely on this base set. A comparison of the effect concentrations of a range of veterinary drugs assessed using base set tests with results of other test endpoints (that are considered to be ecologically relevant) reveals that in about 30% of the cases, the use of the base set with an assessment factor of 100 is not protective for other aquatic species (such as *M. aeruginosa*). Data has been taken as summarised in [44] (Figure 11).

Effects concentrations for around 20% of the veterinary compounds were more than three orders of magnitude lower than the lowest standard test endpoint and a further 10% were between one to three orders of magnitude lower.

The TGD assumes that each of the uncertainties arising from the extrapolation from laboratory tests to the ecosystem, make a significant and equal contribution to the overall uncertainty. The role and magnitude of these uncertainties are however not well validated [45]. Notably the relation between acute and chronic effects with the same test species will depend on the qualities of the chemical under consideration, in contrast to intra- and interspecies relations. Based on acute and chronic data averaged over all species, the ratio is found to be between 1 and 100 [46]. In an analysis of acute and chronic endpoints within single species, acute-to-chronic ratios (ACR) were highly variable, ranging from 0.79 to 5000 [47]. At least 30% of the variation in ACR could be explained by chemical class. Furthermore, there was a 100% probability that the extrapolation factor derived from the combination of ACR within species and interspecies variability based on acute data, is greater that 1000 (instead of the 100 as expected). Conceptually, based on the specific mode of actions of pharmaceuticals, i.e. modulation of receptors at sub-toxic levels, a large discrepancy between chronic effects on reproduction or individual growth, and acute effects, i.e. based on mortality or population growth rate, are to be expected. It is therefore hypothesised that an AF of 1000 on acute data will not be protective for chronic exposure.

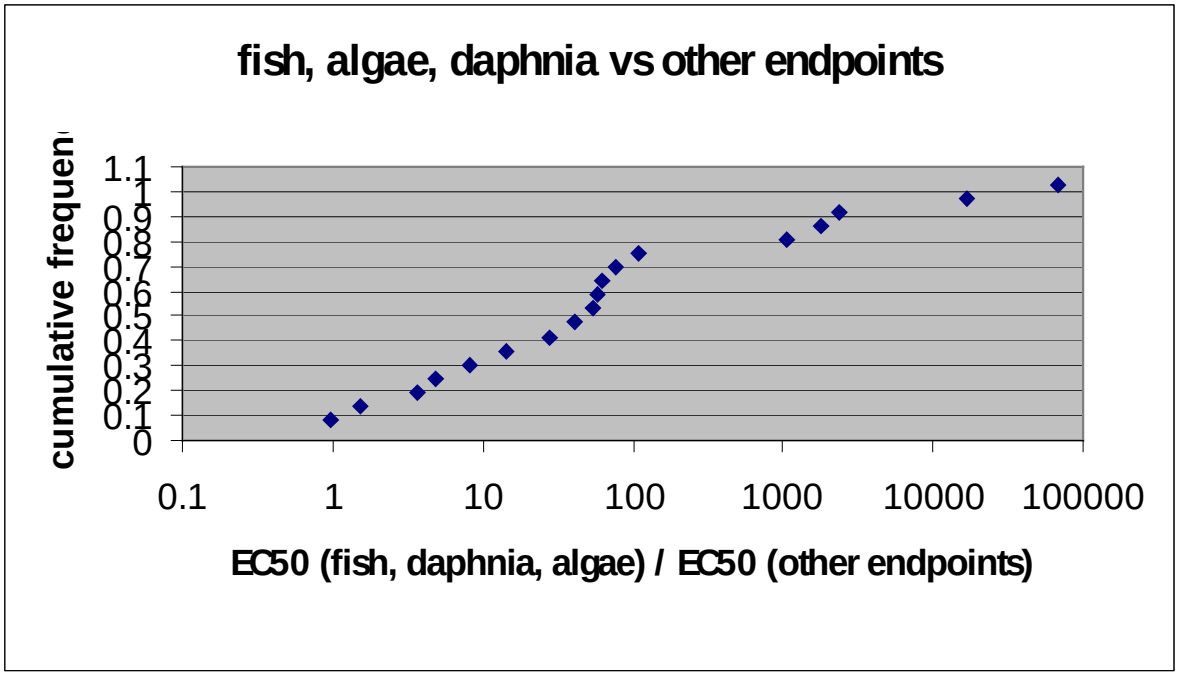


Figure 11 Cumulative frequencies of EC50-ratios between standard aquatic species and other species.

The hypothesis is tested against the limited information on ACR available. ACR determined for diclofenac, clofibric acid and carbamazepine for the same batch of the crustacean *Cerodaphnia dubia* were 23, 213 and 5100 [48]. Invertebrates (*C. dubia, Daphnia magna* and *Hyalella azteca*) were exposed to atenolol, metoprolol, nadolol and propranolol and average invertebrate 48h LC50 ranged from 0.85-29.8 mg/L. Reproduction of *H. azteca* after a 27 days exposure was impacted at sublethal levels of propranolol with a NOEC of 0.001 and a LOEC of 0.1 mg/L. *C. dubia* reproduction NOEC and LOEC were 0.125 and 0.250 mg/L [49]. The ACR for these compounds is hence at least 850. ACR were calculated for seven compounds by Webb (2001). Iopromide, a radiopaque medium, has an ACR of 1, as there were neither acute nor chronic effects at 1000 mg/L. The other ratios were: clofibrate 1428 in *D. magna*, etidronic acid 44 in *D. magna*; nicotine 43 in *D. pulex*, and the metabolite salicylic acid 6 in *D. magna*. The ACR for endocrine disrupting agents may be in orders of magnitude: for diethylstilbestrol and ethyniloestradiol in *D. magna* 17.6 and 570, but in the fish *Oncorhynchus mykiss* the ACR for ethyniloestradiol is 800,000 [50]. These limited data substantiate rather than refute the hypothesis that an AF of 1000 on acute data will not be protective for pharmaceuticals.

Increasing the general application factor for the PNEC derivation in order to cover those sensitive endpoints would produce an excessive enhancement of requirements for higher tier testing. Instead, it is proposed to use the additional available information to establish if the basic data set is expected to cover the specific characteristics of the veterinary drug. Some recommendations are presented below:

3.3.4.2 Recommendation for an assessment of effects of veterinary drugs in the aquatic compartment

- The base set of fish, *Daphnia*, and algae can be used to get a overview of the range of ecotoxicological effects.

- Taking into account the mode of action and the exposure regime that is likely to be found in the environment, either acute or chronic tests should be chosen. For compounds that are assumed to have endocrine or antibacterial properties, chronic tests should be used.
- Additional aquatic tests can be identified taking into account information from the base set (fish, *Daphnia*, and algae), the mode of action, and additional information.
- The chemical structure and the mechanisms of action can trigger specific studies and/or the selection of specific species and endpoints.

3.3.4.3 Terrestrial compartment – standardised versus non-standardised tests

As it became clear during the ERAVMIS project that non-standardised tests and standardised tests sometimes deviate significantly in their outcome regarding the ecotoxicity of a given veterinary drug, we have investigated if the exclusive use of standardised tests hinders the identification of possible environmental effects. To this end, we have determined the ratio of the effective or lethal concentrations of standardised and non-standardised tests that had been undertaken in the ERAVMIS project (Figure 12).

In the given dataset, non-standardised terrestrial tests are generally more sensitive than standardised test. This holds true for plant tests and especially for microbial tests, where soil respiration has been compared with enzymatic tests and community structure tests. The sensitivity increase can be as high as a factor of 100 000 when using the latter in order to identify effects on the microbial biodiversity.

Based on the above findings, it is recommended to choose appropriate terrestrial tests not only from the existing standardised tests, but to also take into account non-standardised tests. A rationale for a choice of non-standardised tests for both the aquatic and terrestrial compartment is described below.

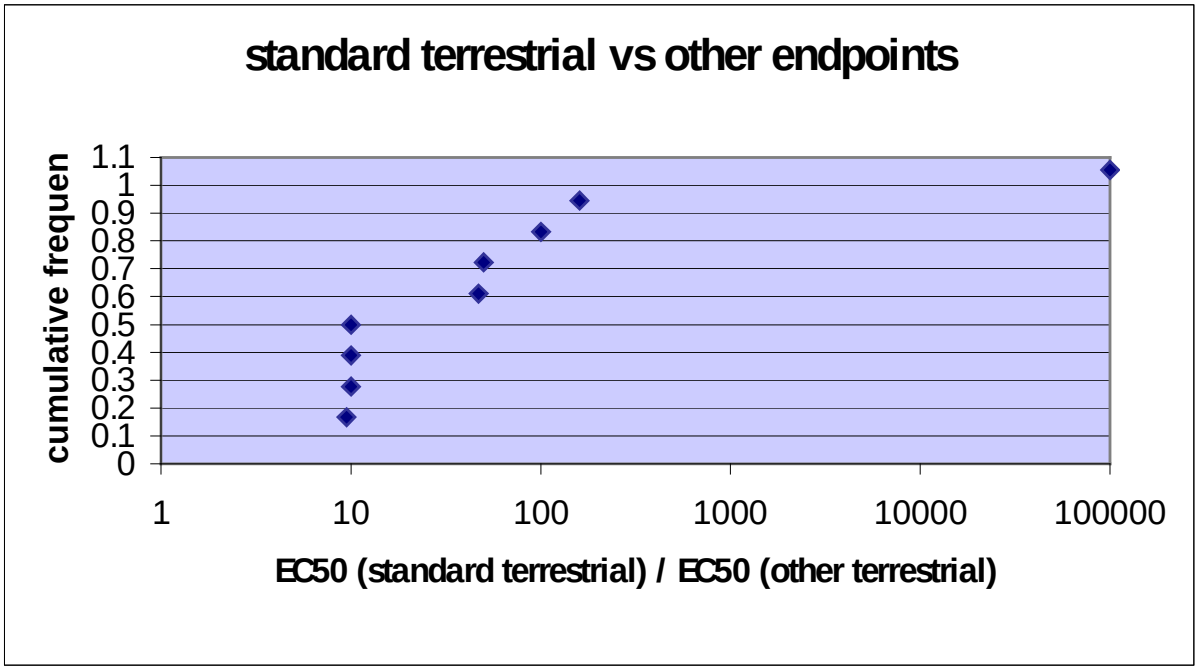


Figure 12 Cumulative frequencies of EC50-ratios between standard terrestrial species and other species.

3.3.4.4 Rationale for the choice of tests on terrestrial organisms

The level of standardisation of test on soil organisms is not as well developed as for the terrestrial environment. An harmonised basic data set is not available at the EU level and therefore, it is proposed to choice of tests for the terrestrial environment on the basis of the available information using the decision tool.
Considerations on the biological activity of the drugs are also essential.
In the recent debate on biodiversity, several concepts have been formulated regarding the importance of a diverse range of species for maintaining ecosystem functions. The 'insurance' concept has gained wide acceptance, stating that biodiversity ensures that a community is able to cope with a wide range of changes in its environment.
This not only applies to above-ground biodiversity: For micro-organisms, it has currently been acknowledged by the OECD working group on genetically modified organisms that maintenance of microbial biodiversity is a protection goal in its own.
Experimentally, it has been shown that changes in the microbial community directly affect community functions, as the ability to degrade specific organic compounds declines with increasing metal tolerance, reflecting a reduced diversity.

From information on the fate of the compound, the compartments that are most at risk can be identified. For most veterinary drugs, exposure of soil can be expected. Based on the compartments, the ecological receptors that might be most important can be identified, taking into account knowledge on the mechanism of action, structural similarity with other reactive compounds and the open literature.

- The ecological receptors that should be taken into account are: soil micro-organisms, plants, soil invertebrates, ground and dung invertebrates, herbivorous organisms and predators.
- If information exists that suggests insensitivity of a certain ecological receptor, testing of this receptor should not be a priority.
- If no standard tests cover the endpoints to be tested, the choice of other tests is recommended.
- For microbial tests, a range of non-standardised tests such as structural tests (PICT) show a greatly enhanced sensitivity for identifying hazards for diversity.
- MS-3 offers the possibility for testing all relevant taxonomic group, in addition to some fate properties, in a single test. Additional tests/endpoints, e.g. PICT can be incorporated in the MS·3 design.

3.3.5 Testing strategy: Level 2. Some examples on the use of the proposed decision tool

The revision of available information suggest that is currently possible to identify some testing requirements on the basis of available information. Some examples are included in Table 9.

To illustrate the use of the proposed tool, the example of sulphonamide antibiotics can be taken. The mode of action of sulphonamides suggests that soil micro-organisms could be affected. In addition, structural similarity with sulphonylurea herbicides suggests that they could also affect terrestrial plants. Existing data on sulphonamide effects on invertebrates shows that toxicity to earthworms and *Daphnia* is low. Therefore, terrestrial testing should focus on soil microbes and plants.

Being a selective antimicrobial the effects on soil microbial biodiversity should be included. The PICT method is proposed as an alternative. In addition, toxicity studies for aquatic vascular plants should be conducted.

ERAVMIS results show that indeed, micro-organism and plant toxicity is high – with NOEC values of 1 mg/kg for the endpoints of plant growth and microbial biodiversity. In addition, the aquatic plant *Lemna* was observed to be several orders of magnitude more sensitive than algae.

3.3.6 Testing strategy: Level 3

Level 3 will a refinement of exposure conditions for getting a more realistic exposure of laboratory organisms to the expected fate and exposure under field conditions.
Two specific aspects are particularly relevant for veterinary drugs, first the drug will be applied with manure, and this application can be resembled in some tests such as the MS·3; second the degradation of the molecule occurs at different levels (within the organisms; during storage of manure; in soil, water and sediment after environmental release), the risk associated to metabolites should be covered by testing the metabolites or with direct toxicity testing of aged degraded samples.
For chemicals with rapid dissipation the tool box included several possibilities already tested for pesticides.

3.3.7 Testing strategy: Level 4

Level 4 include the higher tier testing based on multi-species testing (micro and mesocosms) and field studies.
For soil microcosms the potential use of TMEs is restricted in some cases by the limitations on the possibilities for adding the chemical. The MS·3 can be designed to cover several species for each taxonomic groups, representing a realistic alternative for arable soil.

Table 9. Possible environmentally-relevant modes of action for selected groups of medicines and potential sensitive species

Major Group	Class	Mode of action	Possible sensitive organisms
Antimicrobials	tetracycline	block bacterial translation by binding to the 30S subunit of ribosomes	soil and sediment bacteria
	sulphonamides	analogues of p-aminobenzoic acid and competitively inhibit formation of dihydropteroic acid structurally similar to sulphonylureas which have herbicidal activity	soil and sediment bacteria possible effects on macrophytes
	macrolides	inhibit translocation	gram-positive bacteria in soil and sediment
	β-lactams	inhibits transpeptidase enzyme, critical in the production of bacterial cell walls	gram-positive bacteria in soil and sediment
	quinolones	bind to A sub-unit of DNA gyrase and inhibit DNA synthesis	gram-positive bacteria in soil and sediment
Fungicides	imidazoles	inhibit action of fungal cytochrome P450	soil fungi and aquatic hyphomycetes
Sex hormones	estrogens	mimic estrogen, the female sex hormone	fish birds
Parasiticides	macrolide endectins	stimulation of γ-amino butyric acid, an inhibitory neurotransmitter	dung beetles and flies other arthropods
	benzimidazoles	interfere with energy metabolism by inhibition of polymerisation of microtubules. Also effect fungi	dung beetles and flies soil fungi and aquatic hyphomycetes
	Tetrahydropyrimidines	cholinergic inhibitors	dung beetles and flies and other arthropods
	Organophosphates	inhibitors of cholinesterase	arthropods, fish, birds
Cytotoxics		cytotoxicity	fish, invertebrates
Antiprotozoa	xxx	xxx	protozoa

4. Scenario definition and calculation of PECs

The exposure assessment is based on scenarios and models. A model is just the set of formulas describing the relations between parameters. The scenario is the package of parameter values that represent the conditions for which the model calculations hold true. This may be a worst-case scenario, or a scenario for a specific area of interest. Defining scenarios is one way of coping with the variability in the real world. Addressing the uncertainty and variability associated to the scenarios and models is not easy. The European environmental conditions cover several ecoregions, from the Northern latitudes to the temperate Mediterranean area; therefore, a large variability for most parameters should be expected.

In this chapter general considerations on the nature of models and scenarios are shared, indicating the limitations to the results of the modelling. It indicates the relative importance of the model algorithms (multiplications, order of processes), model parameters (substance characteristics, compartment characteristics) and boundary conditions (dosage, conditions that determine the processes at the virtual border of a compartment).

First some considerations on modelling, model types and scenarios are shared. Then the available model approaches are presented in a formalised way as to address their characteristics, possibilities and limitations. Both screening level and advanced level models are discussed, for every compartment.

4.1 Model considerations

A model is an abstraction of reality: either a physical object or and a mathematical representation or description of this abstraction. Physical exposure models are further addressed as field simulations or field studies. Both laboratory toxicity tests and field effects tests are physical effect models. Models that link substance properties to effects are theoretical effect models. Effect testing and effect assessment are further addressed in Chapter 3. The term model is reserved for exposure models.

An exposure model may describe a multitude of processes through several environmental compartments, or just one process. A model may consist of several sub-models. A model is to be seen as the concepts and the algorithms, isolated from the values selected for the different boundary conditions and process parameters.

The use of models enables predictions to be used for decision making. The additional use of field measurements is an option in all frameworks. The design of a representative monitoring strategy and the final selection of representative data is imperative in order to validate or falsify the model predictions with the field measurements and reach decisions. Only in case of the assessment of an ongoing activity, real-time measurements can replace model results for the assessment of that activity. In general, field studies can provide only additional information to laboratory model results.

A critical component of any modelling procedure is the identification of relevant scenarios to characterise the environmental conditions determining model input parameters. A scenario comprises a unique combination of agronomic and environmental conditions that realistically represent areas in which a substance is to be applied. Relevant means in this context that the selected parameters should be realistic and that the scale of the modelling is adequate.

In Table 1 the tiered approach in the risk assessment is presented, defining a screening stage and an advanced stage. For generic assessments, standard scenarios based on a set of assumptions are usually proposed. Screening scenarios usually represent worst-case conditions, while higher tier assessments present trends to include more information in the evaluations. A similar situation is observed for models. The regulatory goals of model predictions are divers and three groups of models are discerned based on their applicability: screening, primary and secondary models; or on their scale: local, regional or continental. Models are also classified according to their algorithmic design. Mathematical modelling approaches for estimating mass transfer and concentration in environmental compartments can be either deterministic or stochastic, and are either mechanistic (rate models) or functional/empirical (capacity models). Some characteristics of the models of interest are presented below:

- screening models are used to provide rapid prediction of the potential environmental fate of a compound. Primary models provide a standardised approach to characterise substance behaviour and should permit rapid review of modelling submissions by regulators and help to ensure consistent regulatory decision making. Secondary models are appropriate for chemical and site-specific predictions. Secondary models might be applicable for higher tier assessments and will require calibration.
- a deterministic model uses a single set of assumed conditions taken from the range of conditions that can be present in reality. The practical use of the predictions depends on the nature and extent of the variability within the actual system (see [51,52]). For example, several parameter values in pesticide exposure models are selected from stochastic distributions, e.g. pesticide residues on feed and drift values for repeated applications of pesticides.
- all models operate within certain dimensions (time units, distances and areas) on which the parameter values depend, and the type of model is not necessarily related to the spatial scale of the simulation. Local, regional and continental scales are complementary to each other, as different processes can be modelled. However, within several frameworks only one scale of modelling is applied and comparative situations may be handled differently (e.g. exposure of agricultural soil by fertilisers, veterinary medicines, feed additives, biocides, pesticides and sewage sludge).

The parameter values in a model are considered representative for the range of rates for the modelled process within the selected area and interval in space and time. In general the variability of input parameters increases with the size of the area or duration for which the prediction is made.

4.2 Scenario considerations

For registration of veterinary medicines a realistic worst case scenario is needed in conjunction with a model to identify acceptable uses and measures that mitigate the risk when

the product is used. Using the scenarios it will be possible to facilitate decentral and central registration procedures and European harmonisation of risk assessment methodology.
All exposure models operate within certain dimensions: time units, distances and areas, on which the parameter values depend, and the type of model is not necessarily related to the spatial scale of the simulation. The parameter value is considered representative for the range of values, or rates for the modelled process, encountered in the field, for both the selected area and interval in space and time. In general the accuracy of input values decrease with the size of the area or duration for which the prediction is made, because the variation in the parameter increases. There are two ways of increasing the applicability of the modelling result together with decreasing variability. Models can be applied to grid cells of a topographic GIS-chart, each cell with its own characteristics (parameter values). By downsizing the cell dimensions and time steps, the variability per cell decreases. Evidently, the efforts to parameterise and calibrate all cells will be huge, and one has to consider how to interpret the detailed outcomes. One may find the definition of representative vulnerable conditions (scenario) for a single calculation a suitable option. This has certain advantages: the outcome is fairly simple; this point simulation allows for validation of the model and the characteristics (scenario) can be assigned by means of spatial analysis of all information.

The exposure modelling can be split in three major submodules: on for the animal husbandry phase, one for the slurry handling and one for the environmental phase. The scenario defines parameters (distributions) in the exposure models, all of which are related:
1. emission
2. storage
3. immission into soil
4. environmental conditions.

Pathology and dosage are parameters, which are not variable, because the modelling is performed using a given prescribed dosage: dose rate (mg/kg body weight) and duration of treatment. Several animals have more production cycles in one year, which may all need treatment. Excretion of residues is also an input-parameter in the model. Excretion patterns and cumulative excretion may differ depending on species, race, mode of application and dosage.

The input, storage and outflow of contaminated and uncontaminated slurry determine the loads that will reach the soil. Slurry storage, production and removal should be defined in scenarios.

The most realistic period that degradation can take place in slurry and in soil cannot be determined with a rule of thumb. Storage time is a function of output (spreading intervals) and input (time of application or excretion). However, as these functions largely depend on the (unknown and individual) substance handling, either a complete subroutine (model) with detailed inputs are needed, or a scenario for every livestock category of interest is to be defined. In this way dosing, excretion and manure handling are made part of the scenario rather than of the algorithms.

Climate and soils are important factors in the determination of chemical concentrations in the environment. In 1993, the European Commission and the European Crop Protection Association jointly established FOCUS (Forum for the Co-ordination of pesticide fate models and their USe) who, as one of its tasks, established standard leaching and surface water

exposure scenarios for pesticide registration in Europe. These scenarios are probably also appropriate for use in the environmental risk assessment of veterinary pharmaceuticals.

4.2.1 Animal husbandry and manure

Agricultural practices will play a very important role in determining the concentrations of veterinary drugs in the environment. Livestock manure is the second most important source of nutrient inputs to agricultural land [53]. The nutrient content of manure varies from country to country and from one region to another within a country. The relative contribution of the animal types to the manure-N input per country is depicted in Figure 13.

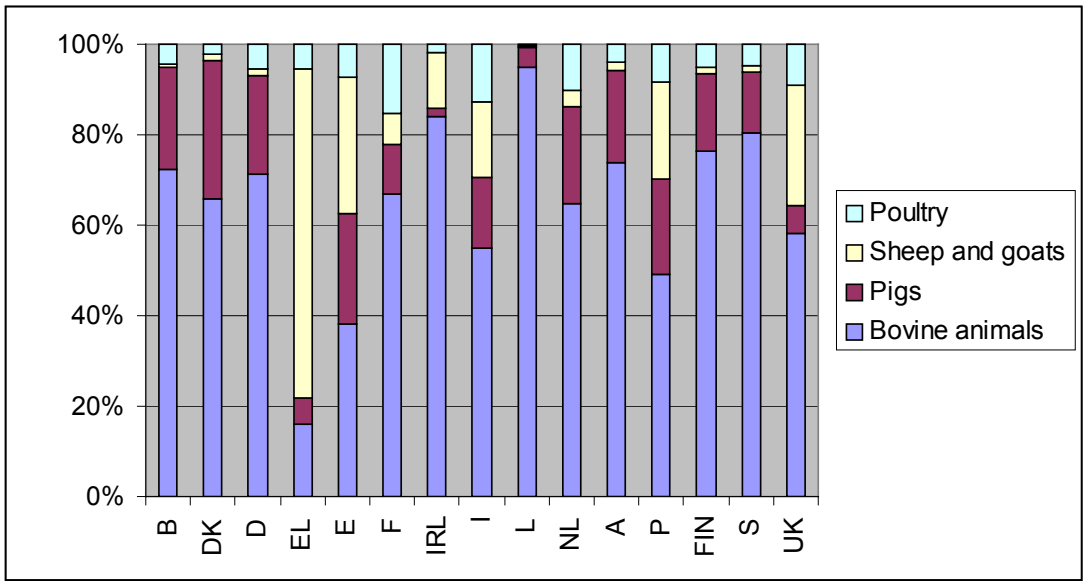


Figure 13 Nitrogen input from manure in EU member states in 1995.

These data indicate that bovine and pig manure is the main slurry types used to fertilise land (if organic fertilisers are used). However, sheep and chicken manure are regionally and locally important. The pattern of agriculture and manure use can vary widely from one region to another.

Manure quality depends on species, race, age, sex, and feeding. We propose to use the lower nitrogen production standards presented to the European Resource Management of the European Commission DG XI [54] (Table 10). Body weights at treatment are proposed based on adult weights for parent animals and the mean of slaughter weight and starting weight for production animals.

4.2.2 Manure and slurry management

The excreta obtained indoors, referred to as manure, are collected and stored for some time. Slurry is the mixture of manure and materials from the housing of animals (e.g. spilled feed, straw, litter, sand, and water, down, carcasses). Different animal types may contribute to the same slurry storage system and the treated animals do not exclusively determine final concentrations.

Table 10 Standardised nitrogen production standards, rounds, and treatment body weights, for different livestock categories.

Type of Livestock	Nitrogen production standard [kg N/place/year]	Rounds per year	Proposed body weight at treatment
Cattle			
Dairy cows	60		425 adult, 25 kg per calf (0.6 calves per place per year)
Other cows	44		425 adult, 25 kg per calf (0.6 calves per place per year)
Veal[#]	10	1.8	140
0-1 year	18		200
1-2 year	31		400
>2 year	35		450
Pigs			
Sows with piglets till 25 kg	32		240 adult; 9 kg per piglet (20 piglets per place)
Slaughter pigs 25-105 kg	7.5	3	65
Poultry			
Laying Hens	0.35		1.6
Broilers, 1.8 kg	0.23	9	1
Ducks, 3.3 kg	0.41	7	1.6
Turkeys, 13 kg	0.90	2.7	6.5
Sheep			
Ewes with lambs till 40 kg	13		75 adult; 20 kg per lamb (1.6 lambs per place)
Goat			
Females with kids till 7 kg	13		65 adult; 3.5 kg per kid (1.8 kids per place)
Rabbit			
Females with kittens	3.9	6.75 births	2 kg adult; 1 kg per kitten (50 kittens per place)
Horses			
Horse – pony - mule	35		400

[#] veal data based on [55] and [56]

In general, both dosing and excretion are model processes that last a certain time span, depending on method of administration and substance. Representative minimal time spans, based on the larger part of the dosage excreted, are in the range of 3-21 days. Residue concentrations in the manure might be modelled provided the driving factors are quantified and standardised testing conditions are operational. Manure models that describe manure loading, quality change and fate of constituents (i.e. CO_2, NH_3) exist and could be adapted, but also have to be improved, a/o. on insufficiently developed manure production submodels, and the restriction to liquid manure facilities [57-59]. Some manure models operate at regional levels because the fate of omnipresent nutrients or oocytes is modelled [60,61]. For

the modelling of incidental contamination with medicinal products these models are not suitable.

Depending on the manure structure (solid or liquid), the manure is kept in bedding, piled, or stored in tanks or lagoons. Storage can be above ground and underground. Depending on structure and handling, the manure can be aerobic or anaerobic. Different manure types and storage systems will influence storage conditions and manure composition in different ways [62]. Conditions like oxygen levels, manure age, microbial activity and temperature will determine the fate of organic contaminants to a large extent, but are highly diverse within and between storage systems.

In the period June to September, the pit temperature for cattle in the Netherlands is considered to be 15°C; in the remaining months this is 10°C. The temperature in liquid pig and poultry manure is considered to be 15°C year-round. This is based on measurements and theoretical consideration that the temperature will be between the soil temperature and stable temperature. In out-door silos the temperature is defined at an average soil temperature 10°C [63]. The actual temperature in the slurry pit of a Dutch pig finishing facility with 1000 places ranged from 15 to 19 °C during the year, mean 16.8, sd 1.4 °C [64], cited in [65]. Pig manure temperatures under rearing facilities in Canada were reported to range from 16°C-23°C over the year [66]. Typical values of air temperature (15-35 °C), air velocity (0.1-0.5 m/s), and liquid manure temperature (15-35 °C) found in under-floor swine manure storage pits were recorded in Illinois, USA [67]. Temperatures in manure/bedding packs used in hoop structures for finishing pigs ranged from –1°C to 47°C, 15-45 cm below the surface during February (Iowa, USA) [68]. Manure collected from Wisconsin USA cattle stables (floor) in February ranged from 6-8°C in a free-stall and 14.5-14.9 °C in a tie-stall. In all samples the pH was around 8 [69]. In a calf manure pile erected outdoors in winter (New York, USA) the temperature rose from an initial 10°C to 29 °C in the first five days, fell to 15 °C after 30 days, was at its lowest, 4°C, after 80 days and then steadily increased until termination of the study [70]. In biogas production cells in Texas, during summer and winter, the temperature in the beef cattle manure pile was initially about 25 °C, but temperature dropped rapidly during the first month as the manure became anaerobic. Temperature began rising during summer months, a result of warmer ambient temperatures, and peaked around the first of August at 22°C. The temperature dropped below 15°C in the middle of October and has remained there until May 2000. The pH at the start was 7 [71]. In poultry manure row piles stored in a high-rise poultry house (25°C air temperature) the temperature at 15 cm below the surface was found to be in the range 34-43°C. In the piles amended with cardboard, hay or saw dust, temperatures were higher: 52-63°C [72]. The following data refer to Dutch good agricultural for poultry. Poultry manure is used in arable land and as compost for mushrooms [73].

Average soil temperatures for Europe are summarised in the FOCUS scenario document on groundwater leaching [74] and range from 4 to 18°C.

In conclusion, depending on climate, season, storage systems and manure structure, temperatures can range from ambient (freezing) to 65°C (composting [75]). For underground slurry storage systems this range is narrowed down to 4-18°C. For storage of solid manure (piles, containers) temperatures can be quite higher and varying due to composting processes (until anaerobicity is reached). Manure can also be treated during storage in order to optimise composting and reduce odour and methane emissions.

Table 11 Manure types, storage and treatment in poultry housing in the Netherlands.

Animal category	Manure	Storage/ treatment	Dry matter content (%)		Temperature (°C)	
			Stable	Storage/ treatment	Stable	Storage/ treatment
Hen, caged	Wet	Pit or silo		±15		20-25
	Dry	Removal in container	40-60		20-25	?
		Shed	40-60	40-80	20-25	20-60
		Drying by aeration	40-60	60-80	20-25	20-25
		Drying by composting	40-60	60-80	20-25	50-70
Broilers	Dry	-	50-70		25-40	

Storage systems differ between countries and animal types. The frequency at which slurry is taken out of the storage facilities can have a different timing. For cattle and pig slurry, seven respectively three moments are considered by [63], which gives the following picture for storage in pits or in silos (Figure 14). During the grazing season, 60% of the cattle manure is excreted in the stable. At emptying the storage tanks, 10% is left behind.

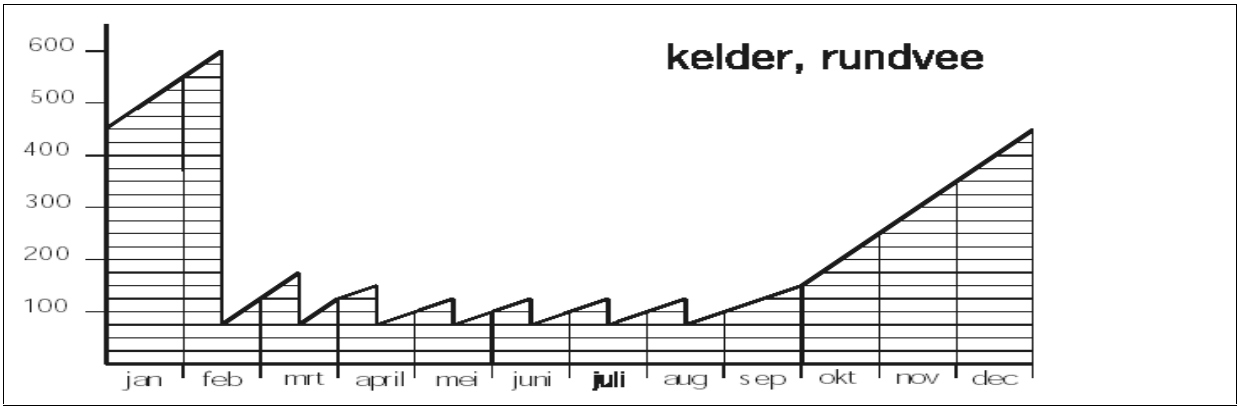


Figure 14 Utilisation of slurry storage capacity in farms with exclusively pits or silos, based on a capacity of 600 m^3, and a production of 100 m^3 per month. Rundvee = cattle. Kelder = pit.

Spreading events are monitored at regional levels [55,76-83]. In the Netherlands and in Belgium, over 95% of the agricultural (arable) land is manured one to three times per year. In the Netherlands, chicken and pig manure is usually spread on arable land in one event, and cattle manure on grassland in up to four events. In the Belgium province of Limburg, 20-60% of the farmers manured once per year, and 30-45% twice, and 2-30% three times [55,84]. Another source reports the following data on slurry storage and application rates (Table 12). It is not specified to what animals the data apply [85].

Table 12 Amounts of slurry stored and applied, time of application and length of storage (WRc-NSF, 2000).

	% applied	Quantity stored (gallon)	storage time (months)	Application rate (kg/ha/y)	plough depth (cm)	waste content (kg N/m3)
slurry						
Average	100	1042571	9.1	21212.88	16.49	2.69
min	100	0	0	80	0	0.99
max	100	5000000	50	100000	28	7
manure		**(tonne)**				**(tonne)**
average	98.44	2358.5	8.06	12636.89	16.53	2.11
min	25	0	0	3	0	2.11
max	100	110000	48	55000	28	2.11

The amount of manure that is spread containing the residue of the treatment is delimited by the storage capacity of the system and the opportunities to take the slurry out. Manure storage times ranging from zero weeks to 4 years have been established in the UK, and from 2 to 12 months in the Netherlands. Most UK farms only have the capacity to store slurry for less than one month. In Figure 14 a minimum storage time of 1 month for cattle is proposed for the Netherlands.

The depth of incorporation depends on the method of application. In the field, no incorporation has been registered as general agricultural practice.

Realistic worst case conditions are hence proposed in a simple scenario assuming:
- single treatment per animal place,
- standard European nitrogen production values (Table 10),
- an application rate of 170 kg N/ha/year in one event,
- a manure production volume of 1 month (30 days) containing the full residue [P_N],
- no dissipation during storage, and no after-treatment of slurry,
- Incorporation into 5 cm soil with a bulk density of 1500 $kg.m^{-3}$.

The PECsoil can be used in conjunction with screening level models that predict mass transfer to groundwater and surface water. Screening level and mechanistic models provided by FOCUS are considered as suitable for veterinary drugs as for pesticides and can be run using European scenarios for soil and weather, with and without incorporation of the substance in the topsoil.

4.2.3 Climate and soils

Climate and soils are important factors in the determination of chemical concentrations in the environment.

At the screening level worst case conditions are usually selected based on theoretical considerations or empirical data. The scenarios usually define a soil texture in terms of bulk density and organic matter, clay, and moisture content, and the profile in terms of depth. Weather is usually not defined, but captured in empirically derived transfer factors for soil moisture and substance load.

In 1993, the European Commission and the European Crop Protection Association jointly established FOCUS (Forum for the Co-ordination of pesticide fate models and their Use) who, as one of its tasks, established standard leaching and surface water exposure scenarios for pesticide registration in Europe. These scenarios are also appropriate for use in the environmental risk assessment of veterinary medicines spread with manure, as this activity targets the same agricultural fields. The FOCUS report on surface water scenarios was not finalised at the time this guidance was conceived. Therefore we base the further description on the FOCUS report on groundwater scenarios.

Locations were selected by an iterative procedure with the objective that they should:
- represent major agricultural regions (as much as possible).
- span the range of temperature and rainfall occurring in EU arable agriculture.
- be distributed across the EU with no more than one scenario per Member State.

Below the scenario characteristics for soil and climate in groundwater leaching models are presented in Figure 15 and Table 13.

Table 13 Overview of the nine FOCUS groundwater scenarios.

Location	Mean annual temp. (ºC)	Annual rainfall (mm)	Soil texture	Organic matter (%)
Châteaudun	11.4	648 + I	Silty clay loam	2.4
Hamburg	9.2	786	Sandy loam	2.6
Jokioinen	4.3	638	Loamy sand	7.0
Kremsmünster	8.8	900	Loam/silt loam	3.6
Okehampton	10.4	1038	Loam	3.8
Piacenza	13.3	857 + I	Loam	1.7
Porto	14.8	1150	Loam	6.6
Sevilla	18.1	493 + I	Silt loam	1.6
Thiva	16.2	500 + I	Loam	1.3

Screening level and mechanistic models for distribution of residues from soil provided by FOCUS are considered as suitable for veterinary drugs as for pesticides and can be run using European scenarios for soil and weather, with and without incorporation of the substance in the topsoil [74,86-88].

The simple fact that this methodology encompasses the same agricultural fields that are relevant for manure application, predestined these scenarios to be applicable to residues spread by manure as well. Application timers in the models should be set to relevant regional conditions for manure application. These may depend on spreading restrictions and where these do not apply, by worst case conditions (e.g. autumn vs. spring conditions).

Several medicinal substances display a sorption behaviour that does not correlate with the organic matter content of the medium [12,13,89]. The sorption parameter is used at different levels with different percentages of organic carbon in the model. Therefore, the possibility to use a Kd value instead of Koc values prevails over deriving pseudo-Koc values for the top soil layer. Distinctions between leaching, run-off en drainage can be made. The FOCUS drainage and run-off models have not been assessed.

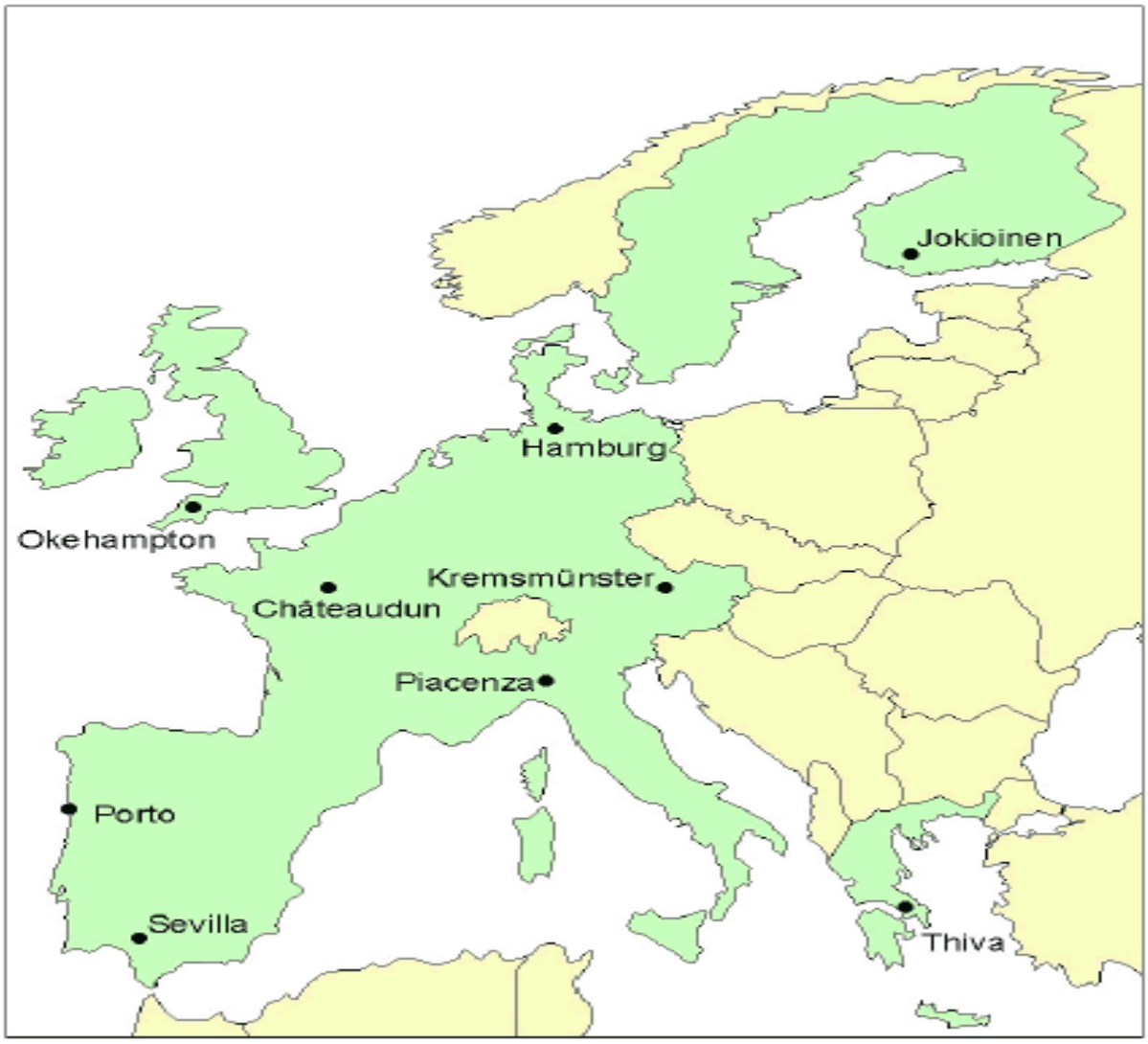


Figure 15 Location of the FOCUS groundwater scenarios

For run-off and erosion in Mediterranean regions a river-catchment model is proposed. The transfer of the veterinary pharmaceutical from soil to surface water is obviously associated to likelihood for rainfall evens. The heterogeneity of rainfall distribution within the year is also a relevant characteristic of the Mediterranean region. Two basic factors, the magnitude of the rain even and the concentration of chemical remaining in the soil/manure when the rain even occurs, will regulate the expected concentration in water. Obviously, the second factor depends on the soil dissipation characteristics of each particular chemical. Persistent chemicals will have a much higher probability for reaching surface water than those chemicals which are rapidly degraded in soil.

Soil erosion distributions for the Ebro river basin are presented in Figure 16. Soil losses ranging from 12 to 100 Tm per year are frequently observed for agricultural soil in the Ebro river basin. These values represent a loss of 0.4 to 3.3 % of the arable soil layer per year, and of the chemicals associated to this soil.

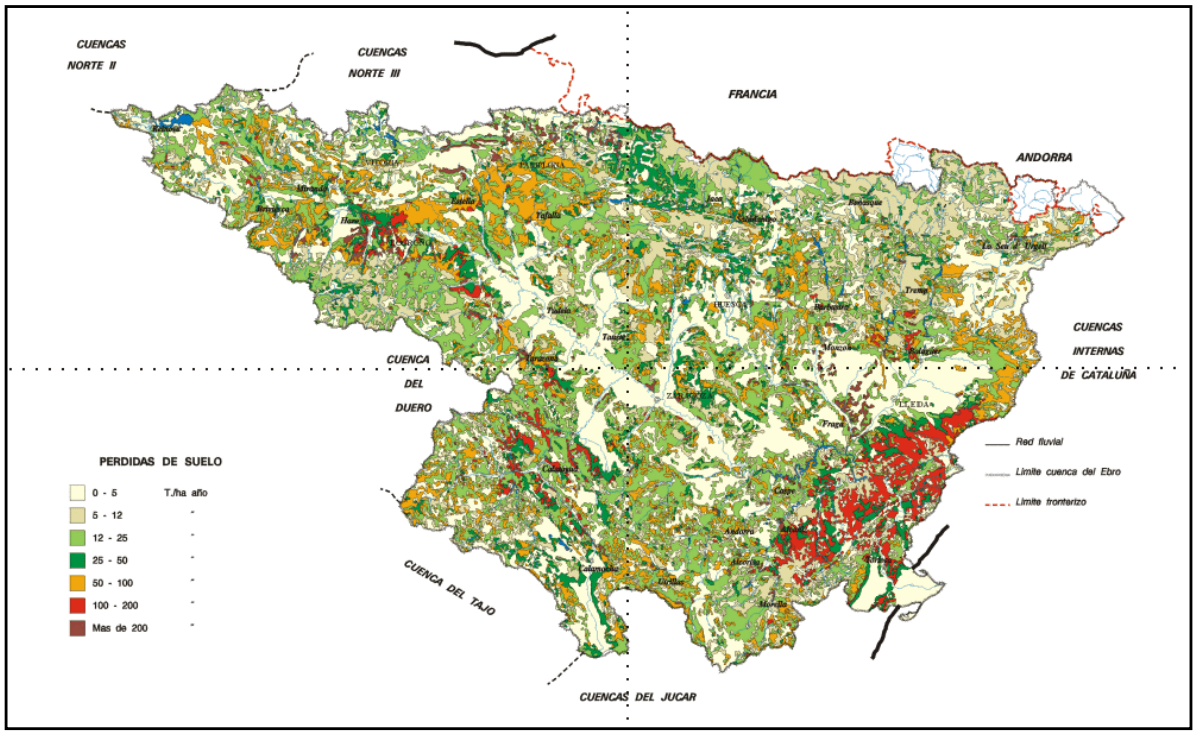


Figure 16. Soil losses in the Ebro river basin. Taken from the CHE website, 2003.

Monte Carlo analysis, based river flow changes for small rivers within the Ebro river basin, have been employed for refining the likelihood for surface water contamination. The introduction of the soil degradation rates produces large changes in the outcome of this probabilistic assessment, as presented in Figure 17. For the Ebro river tributary, the edge of field deterministic estimation corresponds to the 90th percentile of the PECsw distribution for a rapidly degraded chemical, while much higher surface water concentrations are expected for more persistent chemicals.

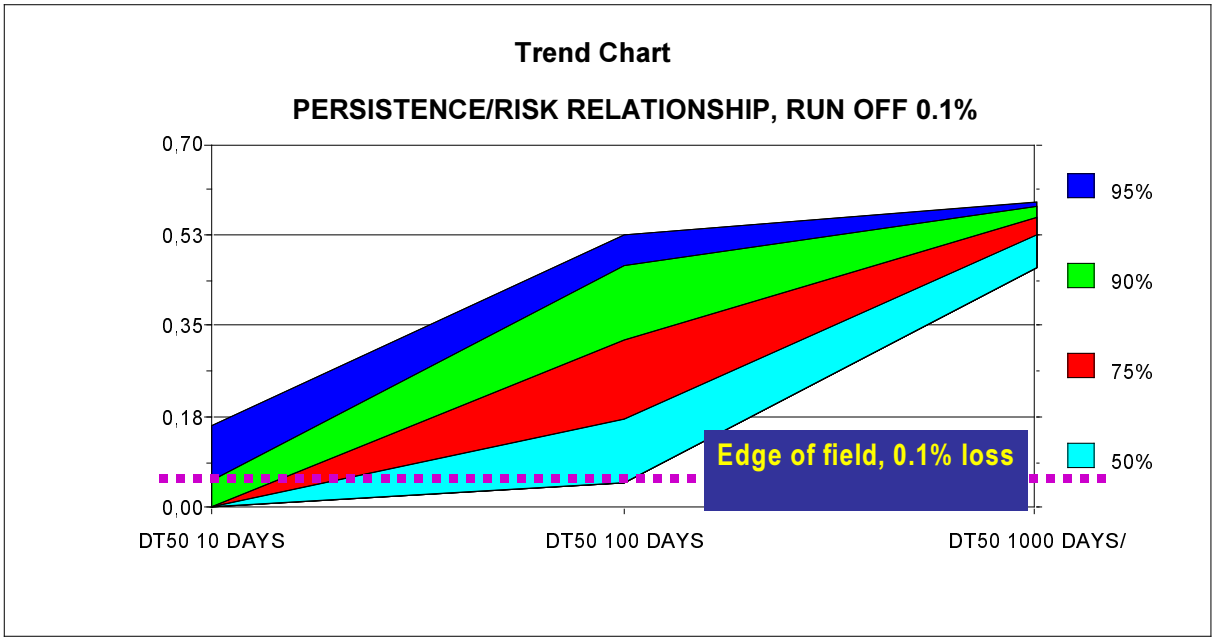


Figure 17. Probabilistic Monte Carlo analysis of the PECsurface water associated to soil losses of 0.1% of the remaining amount for chemicals with DT50 values of 10, 100 and 1000 days.

5. Risk Management

5.1 Risk Characterisation

In the risk characterisation stage the risk of the use of the product for the environment is addressed. Exposure to all relevant compartments and the complementary effect assessments are combined as to give information on the risk of the use of the product. The EMEA guidance is considered to target the central Marketing Authorisation Decision at the community level for the specific use of the product. The risk is characterised under conditions of exposure and for levels of effects that are relevant for this community level.

The risk characteristics based on the risk model are compatible to the decision making criteria, as these will have been harmonised in the process. Risk characterisation should provide information that enables decision making: be it on the direction of further assessment (pre- or post registration) or on risk management: e.g. risk mitigation measures, or risk elimination measures.

Scale and timeframe of exposure modelling including the type of endpoint should be compatible with the effect assessment endpoint of concern. Both exposure and effect are to be considered at the appropriate integration level for the stage in the risk assessment: at the screening level less information on variability and uncertainty in outcomes is required than in higher tier level.

5.2 Risk Mitigation

Risk reduction can be achieved by instructing the user through the SPC to use the product in such a way that emission of the product to the environment is further limited. It should be motivated in what way the instruction affects the emission to the environment and alters the conclusions of the assessment.
All risk management options that target the modus operandi at the farm are outside the scope of the registration and belong to the arena of environmental policy making.

The EMEA guidance is considered to target the central Marketing Authorisation Decision at the community level for the specific use of the product. Risk mitigation options are therefore to be considered within this framework of registration. The environmental risk assessment is performed taking codes of conduct according to Good Agricultural Practice into account. This means that the Summary of Product Characteristics (SPC), that contains instructions for use of the product is followed by the users and that the users operate under agricultural conditions that are considered representative for the branch. This allows for the use of standardised or generalised data on manure production, storage, handling and spreading. An important assumption in the assessment has been that a certain treatment is given only once to every cycle or once every year, whichever comes first.

A second important assumption is that spreading of manure is a given fact. Whether or not a certain amount of contaminated manure is legally allowed to be spread on land depends on the legislation governing soil protection, water quality protection, and animal fertiliser

management. The competent authorities for the enforcement of these acts are not the competent authorities for registration of the products. The environmental risk assessment at registration is performed under the assumption that the contamination by the veterinary medicinal products do not restrict the spreading of the slurry, as this is the practice in most European member states. Restrictions to the use of manure on land are set by the Nitrate Directive for vulnerable areas, advisory standards for crop fertilisation and tolerance of crop for excessive manuring.

The Summary of Product Characteristics (SPC), including the product label, cannot be used to control the fate of the excreted residues, i.e. the manipulation of the slurry (see e.g. [90]) [3]. This is because the legal person addressed by the SPC may be the veterinarian practitioner or the farmer, who cannot, respectively, account for the manure management carried out by the farmer, or by a contracted consumer, i.c. a third party. Even if the farmer is managing the manure, he cannot foresee at the moment of administration what the exact fate of the manure will be, thus he cannot decide on these grounds if he can administer the product.

Drug substances may degrade during storage in the manure tank. If this is the case, selection of sufficiently long storage times before spreading the manure provide an option to reduce the concentration of the drug substance in the manure to a level that constitutes an acceptable risk to the environment. Given the variability in manure management a straightforward approach for the manure model was selected here. The efficacy of risk reduction measures related to manure management and manure treatment cannot be assessed using the proposed methodology.

There is a risk that veterinary medicines applied to soil enter surface waters by runoff following storm events. This risk can be reduced by appropriate tillage practices.

[3] The Safety Working Party of the Commission for Proprietary Medicinal Products (CPMP) decided in the June 2003 meeting to delete similar risk management options from the guidance document on the environmental risk assessment for human pharmaceuticals: " The decision to delete these was based on formal/legal reasons, as monitoring and restricted landspreading are measures that cannot appear in the SPC and would be up to local authorities rather than the CPMP".

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