RIVM report 601450016/2003

Environmental risk assessment for veterinary medicinal products
Part 3. Validation of environmental exposure models

M.H.M.M. Montforts, A.J. Verschoor

This investigation has been performed by order and for the account of account of the EU Energy, Environment and Sustainable Development programme of the Fifth framework Programme, within the project Environmental Risk Assessment of Veterinary Medicines in Sludge, ERAVMIS, contract number EVK1-CT-1999-00003, and of the Netherlands Ministry of VROM, within the project M/601450 Development of Risk Assessment Methodology.

RIVM, P.O. Box 1, 3720 BA Bilthoven, telephone: 31 - 30 - 274 91 11; telefax: 31 - 30 - 274 29 71

Abstract

This report investigates the validity of exposure and distribution models for soil, groundwater and surface water for the environmental risk assessment (ERA) of veterinary medicinal products (VMPs) at registration. The functional validations with (oxy)tetracycline and sulphonamides indicate that it is impossible to analyse the contribution of every single model parameter to the variability in the model predictions using random field samples. It can be concluded that the available field data do not allow for validation of the parameter selection in the models investigated.

A lysimeter study with sulphachloropyridazine was used for a functional validition of the groundwater model PEARL. A simulation error of 0.02 was established, which means that computed values underestimated the measured values by a factor 50. In this study two major factors for uncertainty in the simulation are discerned. First, the untimely ending of the lysimeter hampered the full expression of downward transport. Second, the uncertainty in the sorption process and parameter is of major importance for a reliable simulation. Despite the shortcomings of the case study the potential of pesticide leaching models in general and of PEARL in particular is demonstrated.

Contents

Samenvatting

In dit rapport wordt de validatie van blootstellings- en verspreidingsmodellen voor bodem, grondwater en oppervlaktewater ten behoeve van de milieurisicobeoordeling bij de registratie van diergeneesmiddelen onderzocht.
De algemene uitgangspunten voor blootstelling van de bodem, die in de toelatingsmodellen worden gehanteerd, worden door onafhankelijke onderzoekers als valide aangemerkt. Vastgesteld kan worden dat Montforts [1] en VetPec [2] op een juiste wijze het modelconcept vertalen naar rekenregels. De functionele validatie met (oxy)tetracycline en sulfonamiden geven een indicatie dat het onmogelijk is de bijdrage van elke afzonderlijke modelparameter aan de variabiliteit in de modelvoorspellingen te bepalen op basis van willekeurige veldbemonstering. Niet alleen variatie in dosering, uitscheiding, verdunning, afbraak, mestafzet, en bodemtypen, maar ook factoren als een representatieve monstername in mest en bodem, en de veldhistorie, compliceren de validatie van dit deel van het model. Er zijn aanwijzingen dat het bodem-model van Spaepen onder-beschermend is bij vergelijking met genormaliseerde resultaten van de Duitse en Engelse veldexperimenten. Het Montforts model had meer succes in het voorspellen van maximale (nominale) waarden. Geconcludeerd moet worden dat de beschikbare veldgegevens niet voldoende zijn om de parameter selectie in de modellen te valideren of te verwerpen.
Een lysimeter studie met sulfachloropyridazine is gebruikt om de functionele validatie van het grondwatermodel PEARL uit te voeren. Een simulatiefout van 0,02 werd bepaald, hetgeen betekent dat de berekende waarden een factor 50 verschillen van de gemeten waarden. In deze studie worden twee factoren voor onzekerheid in de simulatie onderscheiden. Ten eerste, het voortijdig beëindigen van de studie belemmert de volledige expressie van het neerwaartse transport. Ten tweede, de onzekerheid in de adsorptieprocessen en –parameter is van groot belang ivoor een betrouwbare simulatie. De simulatie toonde aan dat het adsorptiemechanisme bekend moet zijn en dat het model in staat moet zijn dat proces goed te beschrijven. Bovendien moet het experiment aan zekere eisen voldoen. Gemeten concentraties dienen ver boven de detectielimiet te liggen en de uitspoeling moet doorgaan totdat tenminste concentratiepieken van zowel de tracer als de stof waargenomen zijn. Ondanks de tekortkomingen van de casus is de potentiële bruikbaarheid van uitspoelingsmodellen voor gewasbeschermingsmiddelen in het algemeen en van PEARL in het bijzonder aangetoond.

Summary

This report investigates the validity of exposure and distribution models for soil, groundwater and surface water for the environmental risk assessment (ERA) of veterinary medicinal products (VMPs) at registration. The general concepts of soil exposure, used in the regulatory models, have been considered valid by independent researchers. It is concluded that Montforts (1999) [1] and VetPec [2] adequately translate the model concept into algorithms. The functional validations with (oxy)tetracycline and sulphonamides indicate that it is impossible to analyse the contribution of every single model parameter to the variability in the model predictions using random field samples. Not only variation in doses and excretion factors, dilution, degradation, slurry application rates, and soil variability, but also factors such as representative sampling in slurry and soil, and field residue history, complicate the validation of this part of the model. There are indications that the soil concentration model by Spaepen is under-protective when compared to the normalised results of the German and UK field experiments. The Montforts model was more successful in predicting maximum (nominal) values. It can be concluded that the available field data do not allow for validation of the parameter selection in the models

A lysimeter study with sulphachloropyridazine was used for a functional validition of the groundwater model PEARL. A simulation error of 0.02 was established, which means that computed values underestimated the measured values by a factor 50. In this study two major factors for uncertainty in the simulation are discerned. First, the untimely ending of the lysimeter hampered the full expression of downward transport. Second, the uncertainty in the sorption process and parameter is of major importance for a reliable simulation. The simulation made clear that the sorption mechanism must be determined and the simulation model must be able to describe the appropriate sorption mechanism. Moreover, the lysimeter experiment should fulfil certain criteria. Measured concentrations must be well above the detection limit and leaching must continue at last until peak concentrations of the tracer and the compound under investigation have been observed. Despite the shortcomings of the case study the potential of pesticide leaching models in general and of PEARL in particular is demonstrated.

1. Introduction

1.1. Scope of the report

This report investigates the validity of exposure and distribution models for soil, groundwater and surface water for the environmental risk assessment (ERA) of veterinary medicinal products (VMPs) at registration.

The report defines the value and possibilities of model validation, makes a comparison of user needs and the available models (concepts, output), and compares empirical data on a selection of veterinary medicines (field study) with model predictions.

The first section introduces the context of the regulatory authorities in Europe that have to perform an environmental risk assessment at registration. The second chapter focuses on the purpose of the modelling. To do so, the chapter explains what models are, what validation entails, what the objectives of the regulator are and how these should be reflected in the models. It contains an inventory of models and modelling-related problems encountered by the regulators. The nature of the available models and the way they are implemented will be discussed in the following chapters. The possibility to use pesticide fate models for veterinary drugs risk assessment and the extent to which these models were capable of predicting field observations is investigated.

1.2. Risk assessment and veterinary medicines

The fate and behaviour of pharmaceuticals in the environment has been studied since several decades [3-5], and the presence and effects of residues in the environment is a concern that has been identified not long after that [6-10]. More recently several reviews on use, emission, fate, occurrence and effects of pharmaceuticals have been published and at national and international regulatory levels the environmental risks of pharmaceuticals are on the agenda [11-16].
Currently, the environmental risk of the use of medicinal products is assessed at registration. The methodology has not been finalised yet [17-19] and suggestions for risk assessment methodology are given in [11,20-24]. The proposed risk assessment procedure at registration of human medicines and veterinary medicines is discussed in [25-27] and [28]. Considerations on the assessment of pharmaceutical feed additives are given in [29].

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, European policy and case law, as well as global (trade) agreements, like the precautionary principle. Understanding of the way the assessment of environmental risk is considered in this framework is a starting point for validating the models applied in the assessment.

As a general observation it is stated here that the primary goal of any environmental assessment should be risk mitigation and risk management. The decision-making process and the risk models used should optimise (reduce) the costs to society in terms of environmental damage (due to registration of harmful products) and economic damage (due to refusal of

harmless products). Also the assessment process itself should not hamper product development or timely action [30]. Ideally, the processes of risk assessment and risk management are influencing each other when it comes to deciding on endpoints, accuracy and uncertainty factors [31]. 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] has published guidance on the environmental risk assessment (ERA) of VMPs, and this assessment was implemented in 1997 [17]. 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 [32]. The guidance document on Phase I was completed and finalised (15 June 2000) for implementation by July 2001 in the European Union and United States [19] and replaces the EMEA 1997 guidance on Phase I. This guidance document is at this moment leading for the registration procedure (see Figure 1).

Within the VICH guidance document a limited assessment is foreseen for substances with a generally accepted low hazard (vitamins, electrolytes), and with a presumed negligible emission and exposure level. The exposure level that is considered irrelevant for the total environment is quantified both for water (effluent) and soil for some groups of compounds and several routes of emission: 1 µg/L and 100 µg/kg, respectively (Phase I). These triggers are derived from a dataset of toxicity values of several antibiotics, although the determination of a safe level is criticised from an ecotoxicological point of view [25]. Not only these exposure trigger values define the desired level of quality for soil and effluent. Should these triggers be exceeded, a risk assessment based on the PEC/PNEC approach is warranted for soil, surface water, sediment and groundwater (Phase 2), according to the EMEA guidance (1997).
At the screening level methodology has been defined for soil exposure, in contrast to the advanced level where the assessment can be refined but no further restrictions are given. There is however no Phase I trigger for groundwater or sediment, and persistency and accumulation are addressed separately. Also the method to derive PNEC values is not harmonised with those for the setting of MPCs.

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

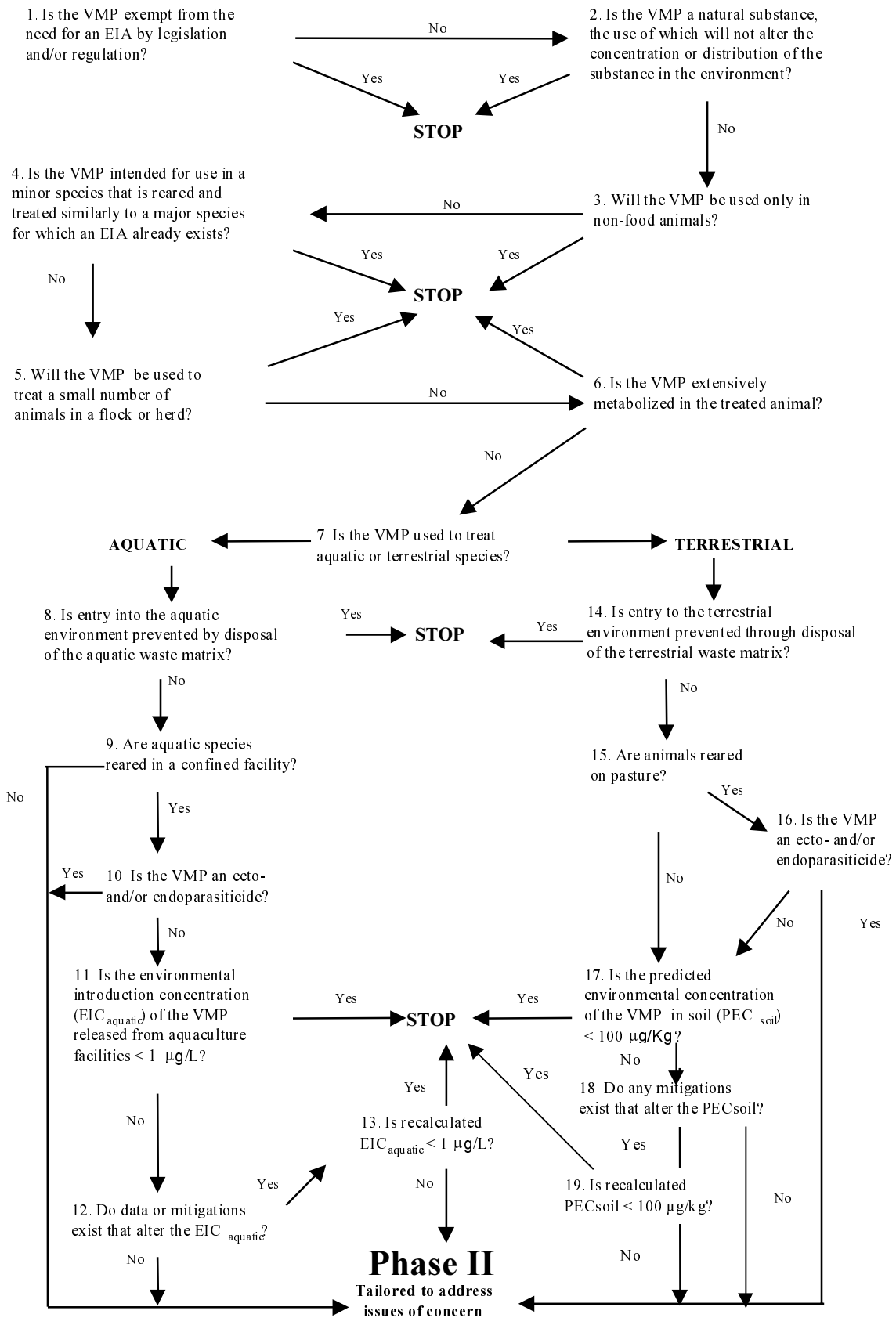


Figure 1 VICH Phase I decision tree

According to the EU directive 2001/82/EC on veterinary medicinal products an assessment of ecotoxicity shall be compulsory for any application for marketing authorisation for a veterinary medicinal product other than applications submitted in accordance with Articles 12(3)(j) and 13(1). This assessment shall normally be conducted in two phases.
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 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.

These further investigations shall be carried out in accordance with the test protocols laid down in Annex V of Council Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances, or where an end point is not adequately covered by these protocols, in accordance with other internationally recognised protocols on the veterinary medicinal product and/or the active substance(s) and/or the excreted metabolites as appropriate. The number and types of tests and the criteria for their evaluation shall depend upon the state of scientific knowledge at the time the application is submitted.

It is very well possible that environmental directives on the quality of water already contain standards for substances used in medicines and feed additives, even though the product groups ‘medicines’ and ‘feed additives’ are not named in the environmental directives [28]. The use of the terms ‘pesticide’ and ‘biocide’ in these directives do not refer to the product categories, but to the nature of the substances reaching environmental compartments after production, use or disposal of products. If an active substance in a medicine or feed additive should be denoted ‘pesticidal’ or ‘biocidal’ because of its properties, the standards in these directives do apply.
The use of the terms ‘pesticide’ and ‘biocide’ in these directives does not refer to the product categories, but to the nature of the substances reaching environmental compartments after production, use or disposal of products. Once in the environment, the competent authority is not concerned with the intended use of the compound, but with the unintended environmental effects of the compound itself. Medicines could be qualified as ‘biocidal’, because they are biologically active. Several compounds are actually registered as pesticide and as medicine, for example streptomycin, oxytetracycline, warfarin, and cypermethrin.
The Dutch government published in 1989 a document on quality criteria for substances in soil and groundwater [33]. It was considered necessary to specify the criteria for pesticides and biocides, “that constitute a special group of environmental hazardous substances: they are developed to repel organisms, modify the growth and development of organisms, or kill organisms, and are by definition biologically active. Also by their use they distinguish

themselves, because these substances –especially the agricultural applications- are brought into the environment directly and cannot be regained". These criteria (repel, modify, kill, biologically active, (direct) introduction, not regain) apply to many medicinal products as well. Moreover, the Netherlands Health Council advised the Ministers to treat medicines in a way comparable to pesticides and biocides because they are pharmacologically active, are spread continuously, and little is known on their effect [34].

The quality of drinking water is protected under the Directive 98/83/EC. This directive aims at protecting public health by setting quality objectives to drinking water. Within the Netherlands' environmental policy it has been the rule since 1989 that with respect to xenobiotics also groundwater should comply with the standards for drinking water, as it often concerns soluble compounds that cannot, or insufficiently, be removed using common purification techniques [33]. This point of view is reflected in the directives on pesticides and biocides [35] and [36] where the allowable concentration in groundwater (irrespective of a use as drinking water) is 0.1 µg/L.

Based on this reasoning, competent authorities have to set water quality standards to medicinal substances and feed additives that can be assigned to the List I and II of the water directives. Also, they have to develop action plans to control the pollution; medicines are acknowledged as a specific group of substances in the Netherlands' 4th Water Action Program [37]. Furthermore, to all substances that qualify as pesticidal, a standard is already available for drinking water, and at least in the Netherlands also for groundwater (0.1 µg/L).

2. Regulatory objectives of modelling

As stated in the previous chapter, the risk assessment targets a desired level of quality. This level of quality can be seen as a translation of the natural ecosystem: it defines how the environment should be. The assessment methodology translates the natural ecosystem in quantities: for example concentrations, dosages, and risks.
The environment is at risk when a product reaches the environment. Transport (mass transfer), concentration and impact of substances are influenced both by the environment and the substance. Environmental factors such as soil, climate, and receptors (populations) are subject to a considerable spatial and temporal variation. Because it is impossible to measure environmental concentrations and effects of all combinations of substances and environments, there is a well-established need to predict fate and exposure concentrations and risks. In order to do so, generic models of the environment and values for the quantities (parameters) described by the models are needed by regulators. Models are tools to help in the production of the environmental safety assessment. Data and other information in the environmental assessment should provide or support the input parameters used.
Ultimately, the quality of the model output will depend upon the adequacy of available data as well as a suitable choice of model and modelling parameters [38,39].
It is important to note that the model capabilities should have been reflected in the decision making process, e.g. in applying a worst-case scenario or in the use of safety factors [40-43]. In regulatory frameworks, known modelling limitations, the applicable effect assessment approach, and acceptability standards should have been harmonised in the process [44].

From the regulatory point of view important aspects of modelling are:
- the goal of the modelling versus the type of model;
- the relation between the model and the use of the product (both in time and space);
- the relation between model outcome and acceptability standard (quality level)

Before we go deeper into the modelling and methodology, we have to define several terms.

Risk assessment

There are many definitions of *risk assessment*. Two are given here, the first relating to a unified risk approach, the second focusing on the risk assessment of chemicals:
Risk assessment is the process of estimating the probability of occurrence of an event and the probable magnitude of adverse effects over a specified time period [45]. *Risk assessment* is a process that entails some or all of the following elements: hazard identification, effect assessment, exposure assessment and risk characterisation [31].

Exposure assessment

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 human populations or environmental compartments are or may be exposed [31].

Model

A *model* is an abstraction of reality and a mathematical representation or description of this abstraction. A model may describe a multitude of processes through several environmental compartments, or just one process. Emissions, concentrations, effects and risks can all be modelled. A model may consist of several sub-models. A model can be seen as the concepts and the algorithms, isolated from the values selected for the different boundary conditions and process parameters, as well as the total set of rules and values.

Scenario

A *scenario* is a representative combination of values selected for the different boundary conditions and process parameters (e.g. animal husbandry, agronomic, soil, and climate parameters and values to be used in *modelling*); representative means in this context that the selected parameters should be realistic and that the scale of the modelling is adequate.

Validation

Validation of a model is the process of formulating and substantiating explicit claims about the applicability and accuracy of computational results, with reference to the intended purposes of the model as well as to the reality (natural system) it represents [46].

In the following sections different model types are introduced, the theory of model validation is explained and the regulatory objectives of modelling are formulated.

2.1. The purpose of the modelling: the desired level of quality in the environment

Neither the EU directives on veterinary medicines and feed additives, nor the guidance documents explicitly state what the protection goals of the environmental assessment should be [17,19,47,48]. However, none of the other chemical substance frameworks described or defined the desired quality of the environment. Due to this lack of reference it would be not possible to validate the concept of the risk assessment methodology. Nevertheless, the directives give some considerations on the scope of the assessment, and the guidance documents contain several decision trees on risk for several endpoints. These are discussed below. The pesticide and biocide directives have codified the criteria and standards (uniform principles (91/414/EC and 98/8/EC)). Communautarian environmental protection goals also have been identified in literature [28] and are addressed below.

Important sources of information on protection goals are the legislation's and policy documents concerning environmental quality [49]. The European Commission (EC) has issued several directives on the protection of the environment. The EC, national authorities and multi-lateral commissions (e.g. International Commission for the protection of the Rhine) are the competent authorities that ought to enforce a program in order to reduce existing pollution and set specific quality and emission standards in law, according to the 76/464 [50], 80/68/EEC[51], 2000/60/EC[52], and 98/83/EEC[53] directives on water, groundwater and drinking water, respectively [54,55]. Specific substances of concern have been identified and listed in two lists (List I and II). Within the framework directive on water 2000/60 the goal is to protect aquatic ecosystems for deterioration or to support recovery. A good chemical condition and ecological condition are discerned; for groundwater a good chemical condition and quantitative condition. In the near future, the framework directive will replace the 76/464 and 80/68 directives.

There is no European legislation on soil quality; however, because sediment and river banks are considered part of the water system and soil contains groundwater, quality of sediment and soil can also be considered an objective of environmental policy. Many member states have national legislation on soil. Member states should at least implement the EU Directives, by setting criteria for emission of substances, for environmental quality, product quality (drinking water), waste, and transport of chemicals.

The registration process of products should primarily be concerned with the level of no effect and the risk that this level will be exceeded. This protection goal is generally pursued by determining a reasonably worst case situation, thus assuming that either the chance on a negative impact is reasonably small and/or that the affected fraction of the area (nation, water

catchment, crop area) and the impact itself are acceptably small. Both the model and the validation exercise should target the regulatory benchmarks: levels of no concern. Modelling at such low levels requires a rigorous understanding of all relevant transport and fate processes, or requires sufficient safety factors. Evidently, there should be good agreement between the protection goal and the methodology used to assess the impact, in the sense that it should be clear what situations the methodology represents, and what level of certainty the predictions have.

2.2. Member states' use of exposure models

In preparation to a European workshop for regulators of veterinary medicines, a survey was held. In the survey the question was asked: "What kinds of models are used? Could you indicate the method how your country calculates the PEC in soil and water?" The following conclusions can be drawn from the workshop results [56].

- Veterinary products are not assessed uniformly within some countries.
- Veterinary products are, despite the EU guidance, not assessed uniformly between countries. There are different approaches to the exposure calculations.
- Exposure assessment is not always based on model calculations.
- The guidance on the PEC calculations in soil, surface water and groundwater provided by the EMEA is used [17], [19]. The older documents contain descriptions of the models, algorithms, and parameter values (defaults). The most recent document does not contain algorithms and parameter values but refers to the so-called FEDESA model [23] for model calculations. These documents, and the models described by Montforts [57] and VetPec [38] are the starting point of the validation process.
- The EMEA and Montforts models are one-dimensional capacity type models using transport coefficients.
- Within VetPec the partitioning between air/soil/water is calculated using a cellular fugacity model at steady state conditions (partitioning). The concentration of residue in groundwater and surface water is then calculated for a range of predefined aquifers and catchments using computer simulation models PESTAQ and PESTCAT. PESTAQ is a function model, partly empirical and partly mechanistic, using confined cells with different scenarios based on river catchments in the UK. PESTCAT is an empirical capacity model based on river catchments in the UK and data on one pesticide.
- Assessors were aware of FOCUS models used in pesticide registration on drainage, run-off and groundwater leaching, but these were not used.
- There was a general agreement that models for pesticides are relevant for medicines, based on their use, properties and mode of action [34,58].
- Most assessors were not familiar with interpreting environmental fate studies or manipulating environmental fate data. It has been demonstrated that the subjectivity and skill of the modeller or validator leads to difference in the results of studies and modelling [59-61]. Protocols for study evaluation are needed and available [57,62,63].
- In the workshop it was agreed that a technical guidance on the calculation of PEC in soil was needed. Models for drainage, run-off and groundwater leaching were considered of later concern.

The Netherlands

In The Netherlands the calculations for Phase I are performed according the method described by Montforts (1999). The models are based on the EMEA guidance [17,23], the Technical Guidance Documents in support of the Commission Directive 93/67/EEC on risk assessment

for new and notified substances and Commission Regulation (EC) 1488/94 on risk assessment for existing substances (TGD), and the Uniform System for the Evaluation of Substances (USES) [64]. The description of parameters used by Montforts (1999) is in accordance with the one for biocides [65].

The United Kingdom

In the United Kingdom for the PEC_{soil} calculation in Phase I the EMEA method is used. It is assumed that the total residue (100% of the dose) is evenly distributed to 5 cm depth, regardless of whether ploughing might take place. This is because there was a previous meeting of an *ad hoc* group of EU ecotoxicity assessors to discuss the VICH proposal for a PEC_{soil} trigger value of 100 µg/kg. This higher trigger (cf. CVMP) value was agreed with the *proviso* that a 5 cm depth would be assumed and the calculation would be based on total residue. For Phase II assessment the VETPEC model would be used [38]. VetPec is a software simulation tool developed by WRc-NSF with funds provided by the Ministry of Agriculture Fisheries and Food. PEC_{water} calculations are also according to EMEA.

Finland

Finland normally uses PECs calculated by the applicant. If PECs are calculated using Central-European default values (number of animals etc.) they may be considered as worst case scenarios regarding the Finnish agricultural conditions. The FEDESA-model is available, but the agency has no experience in using it. Finland plans to use the VETPEC from now on. In the case Finland calculates PECs they use following schemes: for the PEC_{soil} calculation 100% of the a.i. is excreted as such (if no other evidence available), a typical size of stock in Finland is used, the manure is spread to fields at maximum rate allowed by the EU agro-environmental support scheme (almost all farms in Finland receive the support), no ploughing is assumed, i.e. PEC is calculated in top 5 cm of soil, and degradation in soil is not considered in the calculations but taken into account qualitatively. $PEC_{surface\ water}$ calculations are according to EMEA. It is assumed that 1% of the a.i. spread to the field ends up in the water. PEC is calculated in river 100 m long, 1 m deep and 1 m wide. Degradation in water is not considered in the calculations but taken into account qualitatively. $PEC_{groundwater}$ calculations: no models used so far, yet a qualitative assessment based on water solubility and K_{oc}.

Germany

The German environmental agency calculates PEC estimations on the basis of worst case scenarios. The worst case assessment considers the toxicokinetic data of medicines, metabolism in the target organism, fate in excreta and fate during, respectively, after spreading of manure on land. If the provided data is incomplete or not plausible UBA calculates PEC-values on the basis of worst case assumptions (e.g. 100% of the active ingredient is excreted unchanged; no degradation occurs in dung/manure/soil). If a medicine contains besides the medicinal active ingredient a carrier substance, then we consider an assessment for all substances, which are known to be a risk to the environment. The models are according to the EMEA guidance. Models for surface water are according to the German pesticide act and the EMEA guidance. The $PEC_{surface\ water}$ is calculated in a river 100 m long, 0.3 m deep and 1 m wide, with a 5 cm sediment layer.

France

France uses the FEDESA model for PEC_{soil} and the TGD method as reported in [57] for $PEC_{groundwater}$ calculations.

Denmark

In Denmark the applicant submits the calculations and therefore the method for the calculation of PECs varies.

2.3. Advanced models with potential for veterinary medicines risk assessment

Advanced models are used in pesticide risk assessment for many years. More recently a set of European consensus models including 9 European standard scenarios has become available through the Internet. These models are officially accepted for pesticide risk assessment aiming at registration on the European market. These models are:
PEARL: **P**esticide **E**mission **A**ssessment at **R**egional and **L**ocal scales [66]
PELMO: **PE**sticide **L**eaching **Mo**del [67]
PRZM: **P**esticide **R**oot **Z**one **M**odel [68,69]
MACRO: [70,71]

The model versions that are distributed for free through the internet (FOCUS website) are referred to as FOCUS_PEARL, FOCUS_PELMO, FOCUS_MACRO, and FOCUS_PRZM [72]. A summary of the main characteristics of the models PEARL, PRZM and PELMO is given in Annex II.
The models go together with a set of nine scenarios [73,74]. A scenario is a combination of weather, soil and cropping data, which collectively represent agriculture in the EU for the purposes of a Tier 1 EU-level assessment of leaching potential. The scenarios have been implemented as a set of input files for the four simulation models. Distribution of input files and model software, version control, documentation and user support is realised via the website [75]. The model concepts are generally considered valid. The functional validity has been subject of several studies. This however does not mean that their outputs are exactly the same when a particular case is computed. There are differences in the algorithms and/or processes included and different parameterisation that cause the differences. Moreover the models have a different suitability for different cases, as is summarised in Table 1.

Besides the official FOCUS version of the models, beta releases, with improved features, could be used. In this chapter the official FOCUS versions of the models are compared and commented on. When we were informed of other versions of the same models, information on other versions is included also, because it is expected that beta releases will replace the current versions on the Internet.

Table 1 Set of decision criteria to assist in the selection of a specific model.

Decision criteria	Model(s) suggested
Accounting for pesticide losses by volatilisation	PEARL, PELMO, PRZM
Evidence or strong suspicion of a significant influence of preferential flow on water hydrology or pesticide loss	MACRO
Simulation of complex degradation schemes	PELMO, PEARL
Simulation of the fate of compounds susceptible to ionisation	PEARL, PELMO, (PRZM), (MACRO)
Simulation of the interaction between the unsaturated zone and the upper groundwater	PEARL
Need for an accurate description of soil hydrology	PEARL, MACRO
Simulation of lysimeter experiments	PEARL, MACRO, PELMO, PRZM
Increase in sorption with time	PEARL, PRZM, PELMO, (MACRO)

Source: [76]

2.4. Model types

This section deals only with exposure models on distribution and concentration of substances in soil, surface water and groundwater. Effect models and risk models are not discussed. 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.

The regulatory goals of model predictions are diverse and three groups of models are discerned based on their applicability: screening, primary and secondary models [77]. Models are also classified according to their algorithmic design: models are either deterministic or stochastic, and are either mechanistic (rate models) or functional/empirical (capacity models) [78]. A brief introduction into model types is necessary to understand the possibilities and restrictions of model validation.

Screening models
Screening models should be used to provide rapid prediction of the potential environmental fate of a compound. Screening models are needed to assess the Tier I exposure triggers (see figure 1).

Primary models
Primary models should 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. For Phase II, primary models are required.

Secondary models
Secondary models are appropriate for chemical and site-specific predictions. Secondary models might be applicable for Phase II tier C assessments and will require calibration.

Deterministic versus stochastic
Stochastic models take the complete distribution of parameter values into account. With this probabilistic approach the full range of the resulting outcomes and the main sources of uncertainty are available for decision-makers. It is claimed that this approach may be useful for management purposes as it shows the information needed to refine the (deterministic) risk assessment [78,79]. In the Netherlands, a probabilistic decision making criterion on toxicity is operational in the setting of environmental quality criteria and in deriving effect data for the registration of pesticides [80-82]. Probabilistic approaches to the entire risk model are currently under development [83,84]. These models are however not fully integrated in the regulatory risk approach for products: the acceptability of risk for pesticides, biocides, chemicals, medicines and feed additives is expressed in risk thresholds where safety factors account for uncertainty. The acceptability of risk is not (yet) expressed in the probability of exceeding a certain threshold, in which case the uncertainty would be placed in the distribution [35,36,47,48,85-87].
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. Several parameter values in risk models are selection from the stochastic distributions, e.g. pesticide residues on feed [63], drift values for repeated applications of pesticides [88], climate parameters [89], or toxicity thresholds.

Mechanistic versus functional

Mechanistic or rate models describe processes and are time driven; capacity models define changes in amounts and consider time indirectly by using, for example, daily amounts of rain. Capacity models avoid spatial variability problems associated with rate inputs, but due to their empirical nature, they are of limited value for determining seasonal loads or evaluating water pollution control measures. With respect to the mechanistic or functional aspects, models can further be divided in the coefficients and functions.

Export coefficients express the average mass transfer per time unit to or from a given area. They can be determined on theoretical considerations or on actual measurements. The coefficients are capacity models. For example; the fraction of a dosage reaching the surface water due to drift is described by the export coefficient F_{drift} [88], the fraction reaching the soil by F_{soil} [90].

Loading functions are mathematical codes that describe transport processes using both mechanistic and empirical data. The actual mechanistic modelling is restricted to water and/or sediment movement (capacity models) and chemical behaviour of compounds is either ignored or described by simple empirical relationships. The loading function models have modest input data requirements, obtainable independently of the test data, which makes them useful for management purposes [78,91,92].

Simulation models like PEARL, PRZM or MACRO are mechanistic descriptions of availability, transport, degradation and retention (rate models) [84,93,94]. These models provide the most comprehensive descriptions of mass fluxes, but they are very data intensive and laborious to validate [95]. The distinction between loading functions and mechanistic models is not very clear in practice. All mechanistic models are clusters of sub-models describing separate processes (degradation, hydrology, heath transport, solute transport, particle transport, sorption, gas diffusion, etc), and some are empirical to a large extent. The groundwater models CMLS and CALF are categorised as simple functional models in [95], but perhaps for the end-user they are just as difficult to use as the mechanistic models are. A sophisticated mechanistic model of groundwater leaching consists of sub-models for several processes and several horizontal layers to account for changing environmental conditions, and the model actually consists of mixing cells with both rate and capacity codes [96].

The outcome of more empirical models may be biased under certain circumstances, but mechanistic models are no guarantee for success. Large validation exercises involving several models, field data sets and pesticides demonstrated that not the model design or hydrological component, but the assumed substance behaviour and the substance data generation are the most sensitive and determining steps in the modelling [59,60,77,95,97]. Mechanistic models might be most suitable for local modelling, and empirical models for regional modelling [98].

Scale of the model

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. First of all, most distribution models are one-dimensional: mass transfer is described in one direction only. The parameter value is considered representative for the range of rates for the modelled process encountered in the field, for 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. Alternatively these model approaches can be applied to grid cells of a topographic GIS-chart, each cell with its own characteristics (parameter values, see e.g. [99]). Evidently, the efforts to parameterise and calibrate all cells will be huge and one may find the definition of representative vulnerable conditions for one cell a suitable option: a point simulation allows for validation and the characteristics (scenario) can be assigned by means of spatial analysis [100].

2.5. Model validation

2.5.1. Definitions

Strictly speaking, a model cannot be validated in the sense that the validation proves the model is true. All that can be done is to show how small the probability is that the model has been rejected by a statistical comparison with measurements [98]. This implicates that eventually the policy maker must decide how much difference between reality and model results is acceptable. This level of acceptability depends on the purpose of the model [78]. Several authors have discussed statistical criteria for test performance and validation status, but it appears that other issues, such as model documentation and user friendliness, are more important in selecting appropriate models for the regulatory framework. The following elements of model validation are discerned [39,41,46,97,101]:

1. Documentation considerations: Availability of source code, version control, support and training, user manual (language, model description -concept, -mathematics, -parameterisation);
2. Modeller skills: problems in using models in the regulatory process include: lack of clearly defined objectives or standard procedures when using models, misuse of models by untrained staff, input data handling, output data handling, and inaccurate interpretation of modelling results.
3. System features: hardware requirements, reliability, clarity of error messages, the accuracy of models, graphical user interfaces (GUI).
4. Model science (concept, algorithm, and software):
   - Conceptual validation: to make explicit the consequences of the choices on what variables and relationships in the natural system are formalised in the model; assess the motivation for the applied definitions and choices; assess the applicability, usefulness and accuracy of the conceptual model; assess the demands to which the model input must comply.
   - Algorithmic validation: establish the extent to which the procedures for computation (codes, boundary conditions and parameter values) represent the conceptual model.
   - Software validation includes all activities, which build confidence in the quality of the software implementation, particularly its correctness with respect to the algorithmic implementation, but also in the data handling.
     Some examples on the validation of algorithms and software:
     - The relationship between water budget and run-off in the GLEAMS model on soil surface run-off is computed in a way it will not represent small daily fluctuations [102].
     - The model RZWQM was not able to cope with groundwater in the root zone due to instabilities in the numerical schemes used [103].
     - The TOXSWA 1.2 program [104] did not always update changes in the specified parameters, and data were not correctly saved in the parameter file, a problem also encountered in MACRO [105].
5. Most validation studies do not refer to the way a model is put together, but regard it as a black box: an input-output function, which might represent the system of interest. Some studies simply compared results with field observations [102]. This approach is denoted *functional* validation. For example, the Danish Environmental Protection Agency studied several groundwater and surface water leaching models with the objective to find the model that is most suitable for Danish conditions [103,105-107]. In fact, most validation studies are of the functional type, but hardly ever result in rejection of the models [108-111].

With respect to the regulatory objectives, validation contributes to a better understanding of the information generated in the risk assessment and thus to the transparency of the decision making process.

Parameterisation
Parameterisation and calibration are other terms related to model validation. Parameterisation is the process that leads to specific values for designated parameters in the model, and should be separated from the validation experiment: data that were used for parameterisation are evidently not suitable for validation [110]. Calibration is the process of changing parameter values in order to obtain a better match between predicted and measured values.

Sensitivity and uncertainty analysis
Sensitivity and uncertainty analyses may be part of a validation exercise to help identify the shortcomings of a model. Sensitivity analysis is the exercise of determining to what extent parameters determine the outcome of the model by varying the values (see e.g. [111]). It accounts for the variation in the input values by e.g. incorporating their probability distributions (= uncertainty), which leads to a probability distribution of the outcome. It is also possible to determine crucial parameter values using (reliable) data or (crude) estimations as investigated in [112]. Uncertainty represents a lack of knowledge about specific factors or parameters that characterise the physical system that is being modelled and includes parameter uncertainty (e.g. measurement errors and variation), model uncertainty (e.g. uncertainty due to necessary simplifications) and scenario uncertainty (e.g. descriptive errors). Uncertainty can lead to inaccurate or biased estimates and can be reduced through further measurements with for instance a larger sample size, an unbiased sample design, or with more appropriate target species (e.g. soil selection). The use of more sophisticated modelling and analysis tools can also reduce uncertainty.

Variability
The above-mentioned uncertainty arises due to a lack of knowledge of model-parameter values. In fact, these parameter values are fixed, but the actual value can not be determined accurately. Variability is another type of uncertainty and refers to observed differences due to heterogeneity or diversity. In other words, variability is the effect of chance and is a function of the system. Unlike uncertainty, variability is usually not reducible by further measurements or study. With increasing area size the variability of parameters increases, and instead of expanding the value–code combinations (defining more cells; e.g. [99]), rates may eventually be more efficiently replaced by lumped parameters: capacity. Total uncertainty is the combination of uncertainty and variability.

The variability between soil types (e.g. bulk density) and within a field will influence the range of concentrations encountered. Field variability might also be responsible if the measured concentration some time after application is higher than the measured concentration at t=0. To level off field variability a number of mixed samples should be prepared. From the comparison of the nominal amount of active ingredient with the measured concentration at t=0 (directly after application) it may be possible to estimate the influence of the field variability on the results.
Climate, soil texture and topography determine the transport of substances within and through compartments. Variability between and within waterbodies will influence the range of concentrations of parent compounds and metabolites encountered [58].

The scale of the model (one-dimensional, regional, catchment area, site specific, GIS-based) is not fastened down. The same applies for the time-window. Various scales might serve the regulatory objective, given the scenario and decision making criterion.

2.5.2. Conceptual validation

Conceptual validation consist of the following considerations:
- to make explicit the consequences of the choices on what variables and relationships in the natural system are formalised in the model;
- assess the motivation for the applied definitions and choices;
- assess the applicability, usefulness and accuracy of the conceptual model;
- assess the demands to which the model input must comply.

Manure and slurry are applied as organic fertilisers of agricultural soil and residues of medicines that are excreted by treated animals or are spilled will reach the soil [113,114]. The amount of residues that will reach the soil (immission) depends on:

- Manure parameters
  - manure spreading events (number, interval, amounts), dependent on crop and nutrient legislation
  - manure nutrient quality
  - nutrient immission standards
  - storage conditions.
- Soil and climate parameters
  - crop
  - soil type
  - mixing depth
  - season/climate
  - slope
  - hydrology.
- Animal husbandry:
  - animal body weight
  - manure production
  - storage conditions
  - pathology.
- Substance loading:
  - dosage,
  - excretion;
- The relative area of soil affected depends on the
  - soil use
  - fraction of slurry contaminated
  - slurry production surplus and
  - slurry allocation.

This approach underlies all models used at registration [19,23,57]. This approach has been formulated before [10,115-117,117,118], and is also applied for other contaminants such as heavy metals, pathogens and biocides [119-122]. Some existing models focusing on contaminants, apply one-dimensional models, also referred to as ‘local scale’ models. The models developed in the organic fertiliser and landfill pollution frameworks are two- or three-dimensional (topographic models, GIS based) [64,91,113,120,123,124].

2.5.3. Functional validation

None of the existing models reported in Chapter 2.2 have been functionally validated for veterinary medicines yet. Functional validation should target different variables in the exposure models, all of which are related:

1) Emission
   a) pathology and remediation: occurrence of infections and diseases throughout the year(s)
   b) Dose administered
   c) Duration of treatment
   d) excretion of residues by animals
2) Storage
   a) Slurry production
   b) storage time
   c) storage conditions
   d) slurry removal
   e) slurry quality
3) Substance behaviour in slurry
   a) degradation in slurry
   b) concentrations in slurry
4) Immission into soil
   a) Dosage applied
   b) repetitions
   c) soil management
5) Substance behaviour in soil and water
   a) degradation in soil
   b) concentrations in soil

Given the large number of variables, it is virtually impossible to control all variables and examine one parameter at the time. The nature of the available models and the way they are implemented will be discussed in the following chapters. The extent to which the models used for veterinary medicines were capable of predicting field observations is investigated. A field data base stretching 2 years (2000-2001) on soil leaching, drainage and run-off for three veterinary medicines applied in slurry is available. This functional validation is approached as recommended by FOCUS and DEPA, taking their experiences into account [77,89,93,106,125].

Advanced surface water and groundwater leaching models have been explored and validated against several field data sets in the last 15 years [60,77,93,95,97,102,103,105-107,111,125-129].

A thorough comparison of model validations for several groundwater leaching models and surface water models with respect to regulatory purposes (i.e. registration of pesticides) has been accomplished by the EU COST Action 66 and Forum for the Co-ordination of pesticide fate models and their Use (FOCUS) of DG Sanco [77,89,93,97].
These mechanistic models generally describe one-dimensional flow, therefore spatial heterogeneity has to be compromised in the parameterisation or in the input scenario, especially of the most sensitive parameters. The concentration in ground water depends on the load to the soil, the depth of the groundwater table, and the transport of the compound to the groundwater. Transport by water movement and retention in the soil matrix are the driving processes. Flow is generally considered to be in one direction in the unsaturated zone, and in three directions in the saturated zone. The models generally assume stratification in at least an unsaturated and saturated zone. Depending on the water balance, preferential flow through cracks may occur in some models. Substance immission and distribution in the soil is mostly modelled through deposition or mixing, without alteration of other topsoil parameter values. Substance transport processes are described in the rate models as functions of hydrology, heat transfer, plant uptake and evapotranspiration and solute transport, or as mass transfer

coefficients in capacity models. Substance behaviour is ruled by reversible instantaneous sorption and first-order degradation kinetics.
Groen (1997) studied the leaching of pesticides in cracked clay and sandy loam polder soils in the field and by modelling. The modelling was based on the SWACRO and PESTLA models, describing water and solute flow and pesticide behaviour in the (un)saturated zone, respectively. Calibration was performed in three steps: first calibration of soil water fluxes, then calibration of the concentration of conservative solutes in drainage water and the concentration profiles of conservative solutes, and finally calibration of the concentration of pesticides in drainage water and transformation of pesticides. The model was calibrated in three experimental fields. In general, scenario analysis using the calibrated model showed that:
- pesticide characteristics
- lateral boundary conditions
- time of application of the pesticide
- soil characteristics and
- weather conditions

define the concentration of the pesticide in the drain pipes and thereby the fraction of the dosage leached. Generally, the fraction of the dosage leached is higher for cracked clay soils than for loamy sand. Sensitivity for leaching is low to moderate for areas without preferential flow and a high organic matter content and high to very high for areas with preferential flow and a low organic matter content. For cracked clay soils it was calculated that leaching is mainly caused by preferential flow through cracks. The scenario studies showed that several measures can be taken in order to reduce leaching:
- reduction of leaching by allowing pesticide application only during a certain period
- introduction of new drainage criteria with increasing drain depth
- decreasing preferential flow by increasing the ploughing depth.

Bergström and Jarvis discuss the validation of several groundwater-leaching models. The models were: CALF, CMLS, GLEAMS, MACRO, PELMO, PESTLA, PLM and PRZM [95].
All models failed to predict leaching of dichlorprop if laboratory data on degradation were used as model input. Laboratory conditions do not describe field conditions; and degradation may be better described with more than one rate parameter. Temperature and moisture-dependent degradation rates should be modelled (perhaps related to depth). Gustafson and Holden suggest a spatially variable first-order constant [100], an approach that can be applied to both field and laboratory studies. The relative variability found for the rate constant is similar for both field and laboratory studies, suggesting that the length scale of the spatial variability is very small, possibly on the order of pore-size dimensions.
All models failed to predict leaching of dichlorprop if preferential flow was not modelled. The independent determination of the soil parameters that describe these pore level processes is a major challenge to users of those models. Such models will certainly need careful calibration for a variety of benchmark soil types and climates before they can be used with confidence as management tools. Outputs from the macropore flow models are sensitive to parameters related to the macropore region, but this information is generally lacking in datasets [89].
Leaching models are sensitive to degradation rate and sorption coefficients, both parameters with a large variability. This requires large safety factors, and in this respect it is questionable whether we are adding any useful information by using simulation models as a decision-support system, or if it is sufficient to simply consider physicochemical properties of chemicals. Stochastic models might be valuable as the decision can be based on the complete distribution. Soil properties affecting degradation and adsorption showed larger spatial variability than those controlling soil water retention and flow paths [89]. Understanding the model in terms of sensitivity can be a valuable tool [112,130], although a too limited analysis

may lead to the wrong interpretation of differences between model and field observations [111].
The hypotheses were that the simple models would perform better with scarce data, and the mechanistic models better with comprehensive data. The results were not conclusive on this issue. Some models performed well without calibration, but it was not revealed if this was due to a large similarity between model and field, or a matter of coincidence. How this modelling success should be rated remains unclear.
Substance properties and evapotranspiration had often to be changed after the hydrology was fitted. In PESTLA evapotranspiration had to be increased, but in PLM it was decreased. Leaching models are sensitive to substance properties, and the evaluation of these properties is not standardised, so that the differences in model concept may be obscured by difference in estimations of substance properties. Apparently most models simulated the lysimeter condition rather well, after adjustment of a very sensitive parameter. This does however not prove that the adjusted properties reflect field conditions, and not compensate for other faults, such as no description of preferential flow.

Furthermore, if one desires to use the model at a larger scale than used for parameterisation, not only parameter variance is increasing with scale, but in addition the variability of the soil parameters (land use) change. Measuring mechanistic parameters at a large scale is not feasible, but fitting them on measurements in the field is hampered by the scale problem as well. In this respect the mechanistic model is –just like the export coefficient- limited to the area is supposed to represent. However, a major advantage of mechanistic models over box-models is that the effect of control measures on components described in the model can be simulated [110,131].

Vanclooster et al. discusses the validation of several groundwater-leaching models. The models were: GLEAMS, MACRO, PELMO, PESTLA, PLM and PRZM2, PESTRAS, CRACKP, LEACHP, SIMULAT, CALF/VARLEACH and WAVE [97].
The validation exercise should target the regulatory benchmark of 0.1 µg/L for pesticides, or to leaching levels of 0.1% of the dose. Modelling at such low levels requires a rigorous understanding of all relevant pesticide transport and fate processes in soil.
The modelling protocol consisted of two phases. In the first phase, the model's fitting capacity is analysed. In the second phase, the ability of the model to extrapolate or to predict, is analysed. Calibration in this context is considered as a parameter estimation technique (first phase). This process is not performed at registration of a product, as it requires several field experiments for the same pesticide/soil combination that are usually not available.
A high quality data set is needed to validate the comprehensive models. The dataset should be gathered from fields that are fully characterised in soil, climate, land use and history. Before sampling, an a priori model simulation with generic modelling data may address the expected variations of the state variables of soil moisture and substance concentration in space and time, and so allow optimisation of the sampling program. Boundary conditions should be known: daily climatic data for matrix based flow models, but high-resolution data for macropore low models are needed. An early collection of data (some months before the experiment) is needed for some state variables to attain equilibrium. Correct application rates of substance and tracer are needed, and information on spatial variability may be valuable. Surface run-off fluxes during storm events are an important boundary problem. Soil moisture and temperature should be determined in all soil layers and frequent during the year. Information on soil hydrology, soil heat and tracer behaviour is essential to validate a leaching model. For the purpose of validation the raw data as well as the interpretation of the data should be provided. All individual measurements should be tagged with a label indicating the quality or the reliability of the individual measurement. Mass balance of the experiments

should further be checked to see if the recovery allows sound conclusions to be drawn from the results.
The hydrology component of the model should be reliable. Yet, there is no agreement about the level of detail needed in modelling the soil hydrology for assessing substance-leaching risk in a regulatory context. The fact that all the models based on a certain data set were able to target significant mobility differences between bentazone and ethoprofos, regardless of the hydrological description used. This may confirm an earlier observation that the sensitivity of the substance leaching fluxes is an order of magnitude lower than the sensitivity to the sorption and transformation parameters.
There is no agreement on the level of detail needed to model solute transport in a leaching model. The tracer data available from field experiments did not allow for elucidating what modelling concept is driving solute transport in soil (convective motion, hydrodynamic dispersion, and non-equilibrium chemical diffusion).
Heat transport processes were not considered as a bottleneck in leaching modelling.
Substance fluxes should be assessed in combination with substance concentration. Given the variability occurring within the field., current predictions of the fluxes should be considered as uncertain. Current models are successful in screening the potential mobility of pesticides. If a model is technically sound and if it gives sufficient support to the user during the parameterisation process, then it will often predict the observed data within the range of variability.

In the Brimstone dataset, the drain flow measurements integrated the spatial variability over the whole plot. MACRO predicted five times too large concentrations in the drainflow, although the timing of the breakthrough was remarkably well predicted [97].
Further scientific developments in leaching modelling must seek to reduce the uncertainty in estimated fluxes. One way of dealing with uncertainty in management applications is to combine model predictions with available monitoring and experimental data, using Bayesian statistics to assign confidence levels to both (as suggested by [110]). Another method is to directly account for variability in input parameters in deterministic models: either a full stochastic treatment, or running worst-average-best case simulations [40].
Lysimeter data do not represent the complete variability occurring within a field.
Models use mostly instantaneous linear or Freundlich isotherms, assuming fully reversible sorption, and no hysteresis by desorption characteristics is considered. Only two models allow for increased sorption in time, and three models simulate two-site sorption with a kinetic sorption process according to a first-order rate constant. Although these features are needed, the error generated using standardised laboratory data is understood. Estimating rates of transformation in sub-soils is poorly understood, but much needed. The influence of plant uptake and volatilisation has been paid little attention in the past, but is needed to increase confidence in model predictions.
Although a range of data was available for the present exercise, it was concluded that the subjectivity on the process of parameter estimation and input selection is critical [59]. The variation encompassed in substance properties determined in environmental matrices (sorption, degradation) and the subjectivity in the data evaluation process results in a wider range of results for one model than the different modelling concepts do using one dataset. Various modellers however changed substance parameters in order to fit measurement [95], but it is stressed that changing of the initial laboratory data to match observed field data should not be carried out unless a sound basis for such a change is given [97].
The required expertise to run a comprehensive leaching model might not be represented at the regulatory authority [89]. It has been demonstrated that the subjectivity of the modeller or validator leads to difference in the results [59,60].

In several reports it is stressed that criteria for acceptance should be defined before validation is started, and several statistical methods are mentioned. In the formal model validation exercises of FOCUS [77] the criteria for acceptance were however of a qualitative nature, not quantitative. The design of the model (relevant processes and compartments included; boundary conditions correct) was considered, and not the performance of the model in terms of statistical agreement with measurements. This was mainly due to the lack of relevant measurements in the field [77,106].

2.5.4. Validation of scenarios

In the current pesticide registration procedure, models are applied to a limited number of point locations (standard scenarios). These point locations are assumed to represent realistic worst case conditions. The FOCUS scenarios are derived to represent realistic worst case situations for major agricultural areas in Europe. In the framework of FOCUS realistic worst case is defined as the 90^{th} percentile vulnerable situation, with vulnerability attributed equally to soil and climatic conditions. The 90^{th} percentile vulnerability (FOCUS target concentration) is achieved by selection of an 80^{th} percentile vulnerable soil and the 80^{th} percentile of a 20-years weather sequence.

In the Dutch registration setting for pesticides, the model assessment intends to filter out those compounds for which model calculations clearly indicate no risk. Two (spring and autumn) realistic worst-case scenarios are used to evaluate all applications. The actual groundwater level is not considered: all evaluations are based on simulations down to 1 metre deep. If a great risk is predicted, there is reason to believe that also in a practical situation these is a risk present. For intermediate situations, additional experiments under climatic conditions outdoors have to be conducted.

A probabilistic decision making criterion on groundwater exposure in The Netherlands is being developed: the area under consideration (The Netherlands) is divided in 6000 plots with different (deterministic) model parameters. The outcome of these calculations is represented in a distribution considering space (plots) and time: for 95% of the plots at least 50% of the time the result should be below 0.1 μg/L. The model runs themselves are not (yet) stochastic: they do not consider a distribution of key parameters [132]. Models should not substitute, but complement experimental data. All models investigated are research models, which normally emphasise a comparison of the relative effect of different scenarios rather than the provision of a single value suitable for regulatory conditions. Currently it is proposed to use the simulation error for cumulative leaching in lysimeters to correct predictions made by the worst-case standard scenario [133].

An alternative is to use a spatially distributed model. Such a model provides policy makers with a wealth of additional information, particularly high and low risk areas. In an ongoing study, the EuroPEARL model was used to establish the pesticide leaching risk in the European Union. This model is one of the products that has been delivered within the framework of the APECOP project, which is a European project supporting the harmonised registration of pesticides in Europe. Simulations were performed for 1062 unique combinations of Soil Mapping Unit, Climate Zone and Country. Soil properties, including soil horizon designations, were obtained from the Soil Profile Analytical Database of Europe. Daily weather data were obtained from the MARS database. Other data like irrigation data, crop data and pesticide properties have been compiled from various sources, such as inventories, field-studies and the literature. The 1062 unique combinations together represent 75% of the total agricultural area of the European Union. Austria, Sweden and Finland could not be included in the simulations, because there was insufficient soil profile information for these countries. Results are presented with a resolution of 10x10 km^2, which is the highest justifiable resolution based on the EU soil map 1:1,000,000. Results indicate that the leaching concentration generally increases with precipitation and irrigation and decreases with increasing organic matter content. Because of the strong sensitivity of the leaching

concentration to soil properties, there is a strong variability of the calculated leaching concentration at relatively short distances. Results further indicate that due to large irrigation amounts combined with large temporal variation of rainfall in the Southern European countries, the trend in the calculated leaching risks from North to South was less extreme then expected. This implies that areas of high leaching risk (‘hotspots’) as assessed by means of the EuroPEARL model occur in all countries of the European Union, including the Southern European countries. [134]

3. Environmental data

3.1. Use and emission of veterinary medicines

Use and emission of veterinary drugs are not investigated in the ERAVMIS project and are recommended for further research. The existing observations are summarised in Annex I-1, in order to demonstrate realistic values for major input parameter in exposure models.

3.2. Slurry storage, production and removal

Amount of slurry produced, storage time and conditions, slurry removal and slurry quality are major input parameters required to calculate the amount of veterinary medicines applied to the soil. Calculations in the slurry compartment are considered as a pre-calculation for surface water and groundwater models. More information is given in report D9 Exposure scenarios and in Annex I-2.
The total load to the field soil depends on the dosage applied with every spreading event (governed by the factors addressed above), and the number of spreading events within the period of concern.
The soil management after and during spreading determines the distribution of the slurry and the concentration of the residue in soil.

3.3. Substance behaviour in slurry

Because there are different housing systems with various manure collection systems, and different slurry qualities, the storage conditions of the slurry are not uniform within a region or country. Residues may be subject to some extent of (bio)degradation depending on these circumstances [135,135,136,136]. Relevant investigations are addressed in Annex I-3.

It must be reminded that the nominal concentration of active ingredient applied to the top soil of the field is hardly ever equal to the actual concentration [137]. Patchy distributions of slurry will contribute strongly to variability in concentrations [138,139].

3.4. Fate of veterinary medicines in slurry, water, and soil

In the following paragraphs a set of experiments is described, performed by different research groups making use of the same soils and the same model compounds. The model compounds are oxytetracycline, sulphachloropyridazine and tylosin. The model soils are a clay soil from and a sandy soil from the UK. With these soils and compounds laboratory experiments to degradation and adsorption have been performed as well as field and lysimeter soils. The results of these studies will be combined for validation of exposure models.

Dissipation processes such as (bio)transformation [4,140-143,143-150], leaching, [9,151,152] run-off and drainage [153,154], plant-uptake [151,155,156], and volatilisation alter the concentrations and spatial distributions of the residues in soil and water. Depending on the

substance properties and the slurry spreading regime the residues in soil may reach an elevated equilibrium level after some time. Literature data of veterinary medicines are summarised in Annex I-4.

3.4.1. Laboratory degradation in slurry, soil, and water

A biodegradation experiment was performed with UK sandy and clay soil and the three veterinary drugs, oxytetracycline, sulphachloropyridazine and tylosin at 20°C. The same sandy soil has been used in a lysimeter experiment and biodegradation and lysimeter experiment have been performed at the same time. The experiment is described by [157]. DT50s are 16 days for oxytetracycline, 2.8 days for sulphachloropyridazine and 97 days for tylosin fitting the data to a bi-exponential curve. The biodegradation experimented lasted for 64 days, so the DT50 for tylosin is an extrapolated value, which is considered indicative. Recalculation of the data with simple first order exponential kinetics results in the following DT50s.

Table 2 Estimated half-lifetimes of the three veterinary drugs in the sandy soil. Determined by non-linear fitting of first order exponential kinetics with GraphPad Prism.

	DT50 in days (20°C) in lysimeter soil	n	r^2	95% Confidence Interval
Oxytetracycline	27	3x7	0.68	19-50
Sulphachloropyridazine	3.3	3x9	0.88	2.5-4.5
Tylosin	81*	3x7	0.63	57-139

* extrapolated value

Table 3 Estimated half-lifetimes of the three veterinary drugs in the clay soil. Determined by non-linear fitting of first order exponential kinetics with GraphPad Prism.

	DT50 in days (20°C) in lysimeter soil	n	r^2	95% Confidence Interval
Oxytetracycline	30	3x7	0.74	22-50
Sulphachloropyridazine	6.3	3x9	0.83	4.7-9.5
Tylosin	84*	3x7	0.39	50-274

* extrapolated value

3.4.2. Laboratory sorption in slurry and soil

Oxytetracycline (OTC), Tylosin (TYL) and Sulfachloropyridazine (SCP)

Laboratory soil sorption isotherms (Freundlich) were determined for oxytetracycline, sulphachloropyridazine and tylosin at the Institute IRAS, Utrecht University [158]. The soil/water ratios were chosen according to the expected sorption capacities: OTC-clay $^1/_{300}$; OTC-sand $^1/_{150}$; TYL clay $^1/_{25}$; TYL sand ½ ; SCP clay ½ ; SCP sand ½ . Sodium azide was used at 10mM. Analytical recoveries of extraction method used were 25-40%.

The linearity of the sorption over a concentration-range (1.5-3 orders of magnitude) is expressed in the Freundlich exponent.

For oxytetracycline the K_{clay} are comparable to the values reported by [156], as well as the results for tylosin in the sand soil (when compared to the Kf). The sorption of tylosin in the clay soil is very much stronger when compared to the Kf reported by [156]. The low analytical recoveries are a point of concern and reduce the reliability of the reported Kf values.

Table 4 Kd values of OTC, TYL and SCP at 26 ± 1 ℃ calculated with Freundlich isotherms.

	Soil	pH	%oc	%clay	Kf (L/kg) at 1.0 mg/L (±SE)	N (±SE)
Oxytetracycline	Clay-top	7.40	2.2	25	1814 (±66.8)	0.65
	Clay-sub	7.26	0.7	38	887 (±19.8)	0.80
	Sand-top	7.47	1.3	10	655 (±35.9)	0.68
	Sand-sub	6.87	?	9.9	610 (±20.0)	0.79
Sulphachloropyridazine	Clay-top	6.6	2.2	25	2.04 (±0.07)	0.97
	Clay-sub	7.7	0.7	38	0.23 (±0.08)	0.90
	Sand-top	6.9	1.3	10	1.06 (±0.02)	0.91
	Sand-sub	7.5	?	9.9	0.54 (±0.09)	0.85
Tylosin	Clay-top	6.98	2.2	25	85.4 (±6.55)	1.13
	Clay-sub	7.01	0.7	38	993 (±50.0)	0.48
	Sand-top	6.82	1.3	10	6.83 (±0.22)	1.07
	Sand-sub	6.19	?	9.9	28.7 (±2.06)	0.74

3.4.3. UK lysimeter study

In the UK a lysimeter study is performed were oxytetracycline, sulphachloropyridazine and tylosin are applied simultaneously with pig manure [157]. Concentrations in the UK field and lysimeter study are 0.87, 1.18 and 4.49 kg/ha respectively in 45150 L manure/ha. Tylosin was used as a food additive, given to pigs at 100 g per ton feed. The slurry was stored for up to three months and subsequently spiked with oxytetracycline and sulphachloropyridazine and tylosin.

Lysimeters had a diameter of ~23.6 cm and contained an undisturbed sandy soil profile of about 50 cm deep. Soil characteristics are given in Annex III-2. The lysimeters were obtained just after harvest of winter cereals and some wheat stubble was present. All lysimeters were treated with 5 mm slurry containing a mixture of all three drugs plus bromide on 21 October 2002. Application rates were 0.87kg/ha oxytetracycline, 1.18 kg/ha sulphachloropyridazine, 4.49 kg/ha tylosin and 67 kg/ha Br^-. Lysimeters were artificially irrigated and covered between irrigation to prevent natural rainfall. Each lysimeter received 10 mm rainfall for ten consecutive days (equivalent to 440 mL per lysimeter per day). Lysimeter 1A-1D were irrigated on days 1-10, lysimeters 7A-7D were irrigated on days 7-16 and lysimeters 14A-14D were irrigated on days 14-23. Thus the experiment simulated moderately heavy rainfall over a 10 day period but with 0, 1 and 2 weeks delays after application and with 4 replicates for each scenario. Samples were collected on a daily basis if sufficient leachate was present. Volumes collected are recorded. Daily soil and air temperatures were recorded.

The amount of leachate collected from the lysimeters is presented in Figure-22. It is clear that the leaching period is more or less synchronous with the period of irrigation. So for lysimeter 1A-D leaching occurs between day 0 and 11 for lysimeter 7 leaching takes place between day 7 and 18 and in lysimeter 14 leaching takes place between day 14 and 25. At the last day of leachate collection the irrigation has stopped and the amount leachate is decreasing. The amount of leachate collected is larger than the amount of water applied (total 4400 mL). Because the lysimeters were covered to prevent rainfall another explanation must be found. A reasonable explanation is that rainfall is running down the outside of the lysimeters and then down the outside of the gravel filled funnel and then into the collection bottle. This could contribute to the observed variations in the cumulative amounts as well as to the observed discrepancy between applied and collected water. Other possibilities are described, and disproved in Annex III-3. Sample collection. The additional water collected causes a dilution of compounds and bromide leached. This complicates quantification of samples with

concentrations decreasing to values below the LOQ. Moreover it is an additional source of variation. The additional water, which has reached the collection bottles via the outside of the lysimeters, has not affected the leaching process itself and the cumulative mass leached will not be affected. Therefore it is expected that validation of the cumulative mass leached is more reliable than validation of concentrations.

Leaching of bromide is presented in Figure 23. A large variation in the total mass of Bromide leached has been observed. Moreover the theoretical total amount of 293 mg that has been applied to each lysimeter has not been recovered in the leachate. On average an amount of 39% of the applied amount has been found in the leachate. Since bromide is an inert tracer the remaining part must still be in the soil. It is concluded that the period and amount of irrigation was not long/Large enough to realise complete breakthrough of bromide.

Cumulative leaching of sulphachloropyridazine is presented in Figure 24. The variation of sulphachloropyridazine mass in the leachate has increased as compared to the variation observed in the amounts of water leached and the bromide breakthrough. This is not unreasonable because additional processes (biodegradation and adsorption) are an additional source of variation. In lysimeter 1A-D the mass in the leachate increases up to approximately 5.2 μg. In lysimeters 7A-D the same range is observed except for one huge outlier: lysimeter 7D. In lysimeter 7D a consistent increase of bromide leaching is observed, reaching a total amount of approx. 54 μg. It is suggested that a preferential flowpath is responsible for the rapid leaching of sulphachloropyridazine to the groundwater in lysimeter 7D. However interpretation possibilities are limited because the experiment was stopped before complete breakthrough of bromide was observed. A lysimeter experiment should preferably last long enough to replace three pore volumes of water, which is three times the breakthrough-time of bromide. In lysimeter 14A-D the mass of sulphachloropyridazine leached reaches a total value of 20 μg. The variation is large. Lysimeters 14A and 14C are in the same range as lysimeter 7 A, B and C and lysimeter 1A-D. Lysimeter 14B and 14D show deviating masses.

Concentrations of sulphachloropyridazine in the leachate are biased by dilution of additional water from outside the lysimeter. Nevertheless the concentrations are presented in Figure 25. The limit of quantification is approx. 1 μg/L. Concentrations are in the range of 0-4 μg/L for lysimeter 1, 0-17.5 μg/L for lysimeter 7 and 0-8.5 μg/L for lysimeter 14. Visual outliers are lysimeter 7D and to a lesser extent lysimeter 14D.

Concentrations of oxytetracycline and tylosin in the leachate are presented in Figure 26 and Figure 27. It is difficult to observe a pattern in the concentrations of both compounds. The findings seem to be randomly distributed in time and concentration level. Moreover relatively high concentrations are found in samples from day 1, which should still have background concentrations. This raises the suspicion of difficulties with the analysis of these compounds, in a concentration range close to the detection limit. Even lysimeter 7D that was assigned as a potential macropore system does not show a significant leaching pattern. The results for oxytetracycline and tylosin are not useful for validation of PEARL.

3.4.4. UK field study

The field study has been described in [159].
In a field study on two sites in the UK (Osgathorpe, Leicestershire, UK and Lockington, Derby, UK) performed in 2000-2002, spiked slurry was applied to agricultural soils [159].
In the sand soil, slurry was first applied in January 2001, without incorporation. The second year application was in January and in March the field was drilled. SCP was applied at a rate of 1.18 kg/ha, OTC at 0.87 kg/ha, and TYL was applied in slurry obtained from pigs fed with TYL continuously at 100 g/tonne of feed. This slurry had been stored for 0-3 months and applied at 33333 L/ha. Sampling: Year 1: no soil sampling. Year 2: 1, 6, 14, 21, 31, 59, 127 DAT. Sampling depth was 30 cm, sliced in 0-5, 5-10, 10-20 and 20-30 cm slices. The soil bulk density in the 0-30 cm layer was 1.68 g/cm^3.
In the clay soil, the application scheme was as follows. Year 1: Slurry applied to crop stubble on 19/10/00 and incorporated to 25 cm six days later. Soil then disked to 10cm the following day, drilled and disked again. Year 2: Slurry applied to disked soil on 05/09/01, incorporated 2 weeks later, then disked and drilled on 08/10/01. SCP was applied at 1.18 kg/ha, OTC at 0.87 kg/ha, and TYL was applied in slurry obtained from pigs fed with TYL continuously at 100 g/tonne of feed. This slurry had been stored for 0-3 months and applied at 33333 L/ha.
Soil samples taken at: Pre-application, 0 DAT (days after treatment), 1, 7, 14, 21, 30, 60, 90, 120, 240 and 360 DAT in both years. 50 cm soil cores were taken, and analysed in sections: 0-5, 5-10, 10-20, 20-30, 30-40 and 40-50cm. The soil has a bulk density of 1.3 kg/L in the upper 0-37 cm layer.
In both soils TYL was not detectable.
In the soils concentrations OTC ranging from the LOD to 1700 µg/kg were measured in the 5 or 10 cm slices. SCP was found at levels of the LOD to 986 µg/kg. Normalised to 0-20 cm the concentrations are given in Table 5 and Table 6.

Table 5 OTC and SCP concentrations in the sand field (UK) after treatment with 0.87 kg OTC/ha and 1.18 kg SCP/ha in slurry (normalised to 0-20 cm)

Time	mean	sd	mean	sd
	OTC		SCP	
Year 1	µg/kg		µg/kg	
DAT 1	122	144	416	226
DAT 6	94	87	98	74
DAT 14	55	22	46	21
DAT 127	18	7	6	-

Table 6 OTC and SCP concentrations in the clay field (UK) after treatment with 0.87 kg OTC/ha and 1.18 kg SCP/ha in slurry (normalised to 0-20 cm)

Time	mean	sd	mean	sd
	OTC		SCP	
Year 1	µg/kg		µg/kg	
DAT 1	74	31	59	47
DAT 21	141	244	49	63
DAT 289	207	290	<LOD	<LOD
Year 2	µg/kg		µg/kg	
DAT 1	71	79	37	20
DAT 14	80	96		
DAT 240	85	88	2	4
DAT 360			10	17

4. Tier I models

The parameters listed in chapter 2.5 above have not been parameterised by VICH and are left to the member states. The current models are deterministic in the sense that all parameters are fixed at reasonable (worst) case level, and all compartments have default properties.

4.1. Soil exposure models

4.1.1. Conceptual, algorithmic and software validation

Total residue concept

According to the VICH guidance, the calculated PECsoil based on a total residue is compared against the trigger value of 100 µg/kg. The total residue concept involves summing the parent drug with all related metabolites excreted by the treated animal. This assumes that 100% of the dose is excreted unless residue depletion data support a value less than 100%. The total residue approach is considered to be conservative in assessing effects in that it combines parent plus metabolites in calculating environmental concentrations, and metabolites generally have less biological activity than the parent compound. Results from degradation studies in manure and soils may be used to refine the estimate of the concentration of the VMP in soil [19,160].

It is according to the VICH guidance not compulsory to demonstrate the excretion of metabolites, but relevant data on depletion may be used. The implication is that the residue is treated as an unknown mixture of compounds, although the residue may be <100% of the dosage. In that respect, it is not possible to use degradation studies on the parent compound or a particular metabolite, unless the composition of the excreted residue is known and the fraction excreted for every compound is determined. For example, a rapid conversion of the parent compound to a metabolite in the slurry or soil cannot be used to modify the PEC, because it is unclear whether the parent compound or different metabolites were excreted in the slurry in the first place.

The incorporation of degradation terms in the models is hence not useful with respect to the phase I assessment, and may mislead the authorities in determining the residue level to be compared with the trigger.

With respect to the phase II assessment, aiming at a realistic case prediction of the exposure of identified and quantified residues, the model described by Montforts [57] contains transformation terms in slurry and in soil, and Spaepen et al. [23] also have introduced the possibility of using transformation terms. Though neither model provides the possibility of calculating concentrations of transformation products simultaneously with degradation of the parent compound.

Degradation is the only route of dissipation in the models. Volatilisation, drainage, leaching en plant uptake are not incorporated. The models aim at a worst case prediction of the exposure of identified and quantified residues, which supports the objectives at registration to minimise the risk to the environment and to select critical uses for further evaluation. However, most models employ a year-based immission pattern. The final concentration is the maximum concentration that may be reached within one year, and accumulation over the years is not considered. The VICH guidance on the other hand does not limit the time-window. In combination with the total-residue approach, a steady-state concentration would never be reached.

The annual slurry load in VetPec is mixed with the soil in four or five weekly batches over one month (concentrating the slurry application to 100% in one month). Also manure is stored at specific times of the year, and the final concentration is a steady state concentration where the residues being dosed every year. It is however claimed that VetPec was not designed for the PEC calculation in Phase I of the assessment (comparison with exposure trigger) [38].

In the second step in the VICH Phase I decision scheme natural substances can be exempted from further assessment. It is assumed that many natural substances are already present in the environment or are rapidly degraded upon entry into the environment, such that environmental exposure is not altered. VMPs likely to stop at this question include electrolytes, peptides, proteins, vitamins, and other compounds that occur naturally in the environment. In answering this question, the applicant documents and should give a reasoned case that use of the VMP will not alter the concentration or distribution of the substance in the environment. For other substances that occur naturally in the environment the trigger value applies. It is however not specified if, and what background concentration should be used in determining the exposure concentration.

In conclusion: the general concepts of soil exposure used in the models have been addressed and acknowledged by independent researchers. The consequences of the total risk approached are accepted (worst case). With respect to the phase I assessment based on a total residue approach according to VICH the incorporation of degradation terms is conceptually wrong, and at the same time the time-window was not specified. It is not clear how the background levels of naturally occurring substances should be incorporated in the calculations. The regulatory guidance document has not separately addressed the risk of persistency [17,19,161], although assessment of this criterion is required by the directive.
With respect to the phase 2 assessment, the models are conceptually correct, although it is unclear how the risk of accumulating residues is controlled within the complete risk assessment scheme.

Algorithmic validation of soil exposure models

Models algorithms for the FEDESA and Montforts model have been described extensively in the literature sources [57], [23] and have been compared in [162]. Scenarios for arable land and grassland were developed, using the specific situation to improve the worst-case exposure calculations to realistic worst-case calculations [57]. It is concluded here that the model concept is adequately translated into algorithms by Montforts (1999), but not by Spaepen et al. (1997), who suggest adding the mass of wet weight slurry to the mass of dry weight soil, which is conceptually and mathematically incorrect.

The VetPec model is based on the FEDESA model, but has set a different time-window for the immission into soil. Some validation of the VetPec model as a whole has been undertaken using information provided anonymously by VMD for four veterinary medicines. PECs estimated for soil and porewater were generally lower than calculated using the EMEA models. This was expected due to the way VetPec calculates the PECs using a ‘steady-state’ approach. Because of the way VetPec calculates a ‘steady-state’ PEC based on simulation over a number of years it should not be used to calculate the PECsoil in the Phase I assessment (because it is unlikely that sufficient data such as identity, degradation rates and soil adsorption data will be available to allow use of VetPec). It is suggested by VMD that the approach outlined in the Spaepen et al. (1997) model is used to calculate the PEC in Phase I [38]. It is concluded here that VetPec adequately translates the model concept into algorithms.

Software validation of soil exposure models

VetPec is the only model that is available in a software shell [38]. The soil sub-model is based on the Spaepen model and was validated against calculations made earlier assuming these latter calculations were correct, and using information provided anonymously by VMD for four veterinary medicines [163]. Soil input concentrations calculated by VetPec were reported to be the same as those calculated using the Spaepen model.

4.1.2. Parameterisation of soil exposure models

The soil exposure models contain several parameters that can assume different values under different circumstances. All models are capacity models, using year-based averaged parameter values. There are however no parameters that need to be derived indirectly. This reduces uncertainty but several parameters are accompanied with considerable variability, and others are delimited by the model concepts. Data variability may account for considerable spread in the results, as was demonstrated by [164].

Substance loading

If a product is used or not depends on the pathology (occurrence of diseases; frequency, scale). The model presupposition is that treatment occurs, thus pathology is not a parameter. Dosage is a parameter, which is 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. Depending on the model type (rate or capacity), this introduces the need to standardise body weights, treatments and dosages. Excretion of residues is also an input-parameter in the model. The data in 3.1 demonstrate that excretion patterns and cumulative excretion may differ depending on species, race, mode of application and dosage. This will result in a selection of an excretion value (deterministic) or a range of values.
If one would like to validate the model predictions with field measurements, where pathology, dosage and excretion are (variable) parameters, this should be kept in mind.

Animal husbandry

Values for the following animal husbandry parameters are needed (Table 7).

Table 7 General parameters for animal categories

General application inputs		
(averaged) body weight	m_{animal}	[kg_{bw}.animal^{-1}]
input for spreading of slurry		
number of cycli per year	$Ncyclus_{animal}$	[animal.place^{-1}.yr^{-1}]
number of housing days	$Thousing_{animal}$	[d.yr^{-1}]
manure production stable	$Pmanure_{animal}$	[kgwwt.place^{-1}.d^{-1}]
dirty water production stable	$Pdirty\ water_{animal}$	[kgwwt.place^{-1}.d^{-1}]
slurry production stable	$Pslurry_{animal}$	[kgwwt.place^{-1}.d^{-1}]
phosphate production	P_{P2O5} animal	[kg_{P2O5}. place^{-1}.d^{-1}]
nitrogen production	P_N animal	[kg_N. place^{-1}.d^{-1}]

Some animals are kept at their mature bodyweight, other are reared from a starting weight onwards. Pathology is expected to be related to a certain life –or husbandry- stage, thus limiting the range of body weights that is relevant for modelling. The number of cycli per year is used to interrelate pathology, body weight and manure production (number of treatments per cycle, number of cycles per year, manure quality) and produce outcomes as averages on a yearly basis. Data in EMEA (1997) and Spaepen are based on publications from different

countries dating from the period 1983 – 1993, including acts on nutrient emission and nutrient quality standards.
Data for the Netherlands situation in Montforts (1999) are based on information in [165] and on expert judgement. The number of cycles per year is based on the production periods including the days the pens stand empty, according to [165]. The different data sources yield different values for number of cycles, manure production and phosphate and nitrogen contents. This variability is the result of differences in feed type, animal health, race, climatic conditions and housing conditions. In order to come to worst case simulations, almost every parameter has been fixed into a default value. Depending on the region, these defaults might differ.

Agricultural practice

The concentration in soil depends on the soil properties, distribution processes and the application regime (timing, frequency, interval, and method of incorporation).
The area of soil affected is not described in the model concept. The model deals with the soil that is fertilised. Soil use, fraction of slurry contaminated, slurry production surplus and slurry allocation will have influence on the field situation in case of functional validation.
Season, climate, hydrology, volatilisation, and crop are not incorporated as parameters in the tier I models. They appear as boundary conditions for the capacity models (temperature at which transformation rates are determined) or were not defined in the concept.

Table 8 Parameterisation for spreading of veterinary medicinal product residues on grassland and arable land

Parameter	symbol	unit
mixing depth (land use type)	DEPTH	[m]
phosphate immission standard land use type	Q_{P2O5}	[$kg_{P2O5}.ha^{-1}.yr^{-1}$]
application interval manure	$T_{interval}$	[d]
storage time slurry before spreading	$T_{storage}$	[d]
number of spreading events	$N_{spreading}$	[yr^{-1}]
fraction of dosage chemical excreted in manure	$F_{excreted}$	[-]
biodegradation rate in slurry	$kdeg_{slurry}$	[d^{-1}]
biodegradation rate in soil	$kdeg_{soil}$	[d^{-1}]
dry bulk density of soil	RHOsoil	[$kg.m^{-3}$]
conversion factor for the area agricultural field	CONVarea field	[$m^2.ha^{-1}$]

Mixing depths and bulk densities, in practice, show a considerable variation (see Annex 1). Because land use (and associated use of incorporation techniques) is not modelled, no distribution of likely mixing depth can be made. All models use deterministic selections.
Mixing depths for grassland and arable land in Montforts were taken from agreements reached by the EMEA working group EMEA 1997. The mixing depth and bulk density are based on the values used for pesticide assessments and the TGD. Spaepen used a value of 25 cm in an example calculation; a value also used in VetPec.
The nutrient immission standards are based on legislation at the time. Currently, standards are harmonised for nitrogen across Europe although derogation has been applied for (e.g. the limit for grassland in the Netherlands is to be increased). However, the nitrogen immission limit only applies for designated areas under the Nitrate directive 91/676/EC. For those areas that are not under the jurisdiction of this directive, this standard does not apply and loads may be much higher. In the Netherlands the total area has been designated to the 91/676 /EC directive and nitrogen standard applies. However, phosphate is also restricted under the fertiliser acts and this mineral is the limiting factor for worst-case manure immission to soil.
The total residue approach of VICH suggests that the molar weight of the parent compound is transferred to the transformation products, which of course might be smaller or heavier. The $F_{excreted}$ is in this model based on parent equivalents.

The assumed storage time of slurry influences the dosage that will reach the soil. The number of animal cycles and hence the numbers of administrations that are made during a storage period are determined by the duration of an animal cycle. The residence time of a certain residue depends on the time of excretion by the animal and the time of disposal of the slurry. These parameters are either not modelled (excretion time is 1 day) or defined in the model concept (e.g. four spreading events). The model concept and time frame thus determine the parameter 'residence time'. VetPec and Montforts addressed this feature, Spaepen did not. The choices made by Montforts (1997) and VetPec are arbitrary, and other sources suggest a generally more simplified spreading regime [113,120,166], which depends on the agricultural practice and fertiliser regulations.

Substance behaviour

Several parameters that describe substance behaviour actually describe substance-environment interactions. Although e.g. degradation and sorption are directly established in experimental designs, there will be a considerable variation in results due to the difference in the soil types. Normalisation to standard conditions will alleviate this problem only partly [167].

4.1.3. Functional validation of slurry and soil models

Use and emission are the first steps in the contamination chain, followed by concentration (and dissipation) in slurry, and emission to soil. The models all use a deterministic approach in calculating the excretion to and concentration in slurry, using emission factors (default 100%) and year-averaged slurry production ratios (Chapter 2.2).

Literature data on occurrence of antibiotics is slurry and soil (Annex I) are compared to model predictions. A field study performed in the UK [159] and described in chapter 3.4.4 is also discussed.

Validation of literature data

For sulphonamides a comparison can be made between model calculations for sows, pigs and veal and one dosage (40 mg/kg for 5 days), and measurements in slurry (see Table 9). Data on transformation in slurry and soil (chapter 3.4.1) suggest a fairly rapid degradation of the compounds under aerobic conditions. The calculations assume that the slurry basin is emptied (or sampled) at 35 days after treatment, representing an average situation when degradation takes place.

Table 9 Results of PECslurry calculations (µg/kg ww) using the Montforts model on sulphonamides for various DT50 values in slurry, and the measurements reported by [168]

	sow	pig	veal
Model calculations			
no DT50 slurry	46	52	40
DT50 slurry 5 days	0.3	0.4	4
Measurements			
A sow; D pig; F veal	23.7	0.33	3.2
B sow; E pig	6.10	0.30	
C sow	3.45		

In Location A a specific treatment with sulfathiazole and sulfamethazine was going on. Assuming rapid degradation, sampling took place right after dosage, but perhaps not after total excretion. This situation is approached by assuming zero degradation in the model, which still yields a difference of a factor 2. Different dosages or retention/degradation in the animals may account for further deviations.

The relation between the predictions (not incorporating metabolites) and measurements in locations B-F is less straightforward, because information on dosage, excretion, time after treatment and exact DT50 are lacking.

For tetracyclines a comparison is made between model calculations for one animal type and one dosage, and measurements in slurry and soil. Per place only one sow per year is supposed to be present. Tetracycline (unspecified) is dosed with 40 mg/kg bw for 5 days. The excretion factor is set to 1.

Table 10 Agricultural parameters for sows

M_{sow}, set A	(averaged) body weight sow	240	[$kg_{bw}.animal^{-1}$]
M_{sow}, set B	(averaged) body weight sow	130	[$kg_{bw}.animal^{-1}$]
$M_{sow\ and\ litters}$, set A	(averaged) body weight sow and litters	100	[$kg_{bw}.animal^{-1}$]
$Pslurry_{sow}$, set A	slurry production sow in stable	7.75	[$kg_{ww}.place^{-1}.d^{-1}$]
$Pslurry_{sow\ and\ litter}$, set A	slurry production sow and litter in stable	13.5	[$kg_{ww}.place^{-1}.d^{-1}$]
$Pslurry_{sow}$, set B	slurry production sow (and litter) in stable	14.8	[$kg_{ww}.place^{-1}.d^{-1}$]
P_{P2O5} sow, set A	phosphate production sow in stable	0.0556	[$kg_{P2O5}.place^{-1}.d^{-1}$]
P_{P2O5} sow, set B	phosphate production sow in stable	0.0341	[$kg_{P2O5}.place^{-1}.d^{-1}$]
Tcyclus, sow	duration of cyclus sow	365	[d]
Tstorage, set A	average storage time slurry arable land	365	[d]
Tstorage, set B	average storage time slurry arable land	152	[d]
$N_{application}$	no. of applications per storage period	1	[-]
Q_{P2O5}	phosphate immission standard	110	[$kg_{P2O5}.\ ha^{-1}.yr^{-1}$]
RHOsoil	bulk density of soil	1500	[$kg.m^{-3}$]
DEPTHfield	mixing depth with soil	0.2	[m]
CONVarea field	conversion factor for the agricultural field	10,000	[$m^2.ha^{-1}$]

Data reported in Annex I suggest that total cumulative excretion may range from 0.2 to 1, and that DT50 for tetracyclines as a group may vary between 4 and 175 days, without correction for matrix, aeration or temperature. With the Spaepen model, soil concentrations from zero up to 75 µg/kg dw can be expected, with the Montforts model the upper limit is 207 µg/kg dw. Measurements for tetracyclines (TC, OTC, and CTC) reported in Annex I-4 are all within the latter range, but some are higher than predicted by the Spaepen model (Table 34)
The soil concentrations measured by Hamscher et al. (2002) [139] are a factor 2-4 higher than expected based on the corresponding measured slurry concentrations, indicating the presence of residues in the soil, additional release of bound residues between 2000 and 2001, higher application volumes, or higher concentrations in the slurry pit than in the slurry samples (incomplete mixing a sampling). See Figure 2.

The field experiment (Hamscher 2000-2001) with controlled slurry concentrations indicate that, although the nominal concentrations do not match with the actual concentrations, the model calculation presented by Spaepen are underestimating the initial concentrations. The Montforts model predicts a maximum concentration of 52 mg/L in slurry at a dosage of fourty mg/kg for 5 days (fattening pigs), which yields the 207 µg/kg. Compared to the data presented by Winckler and Grafe (2001), who found a mean concentration of 11.6 mg/L and a maximum of 66 mg/L, the measured concentrations in the ‘controlled’ slurry samples were indeed rather low (4 mg/L), and the worst-case predictions seem realistic.

Table 11 Results of PECsoil calculations (µg/kg dw) in arable land on oxytetracycline used in sows for two distribution scenarios and various DT50 values in slurry

Formula set	A Spaepen	B Montforts
No DT50 slurry	75	207
DT50 slurry 100 days	21	123
DT50 slurry 30 days	1.1	37

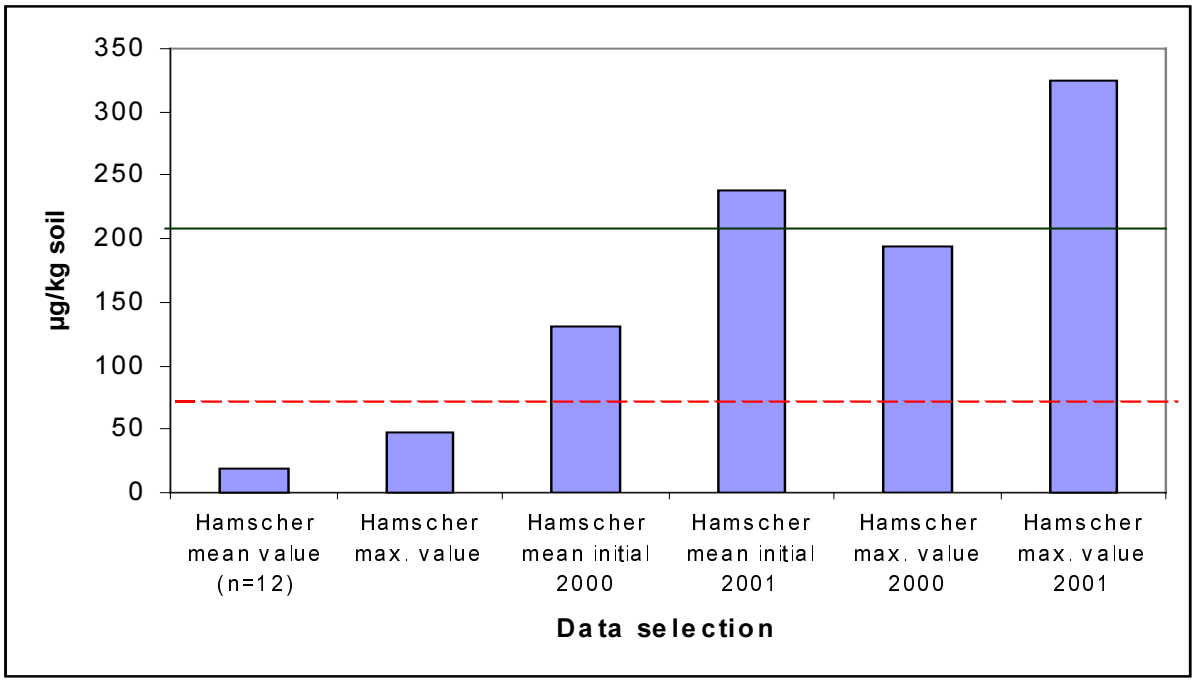


Figure 2 Graphical presentation of mean and maximum measurements in soil in Germany (1999-2001). Straight line: initial PEC according to Montforts (1999); dotted line: initial PEC according to Spaepen (1997) for fattening pigs dosed at 40 mg OTC/kg for 5 days (total residue approach)

Schlusener et al. (2003) found a tiamulin concentration of merely 43 µg/kg liquid pig manure, and calculated that about 98% of the dosage administered two months earlier (resulting in a concentration of 2 mg/kg) had disappeared [169]. This indicates that the slurry dissipation half-life for tiamulin was in the order of 10 days. In a study on oxytetracycline measured (<10 µg/kg) and predicted (12 µg/kg) concentrations (based on the general model, with location specific values for load and plough depth) in soil after manure addition were very close [170]. The manure was stored in heaps and the reported concentrations cannot be compared to those predicted for slurry pits [171].

Validation of the UK field soil concentrations

The experiment is described in chapter 3.4.3.

Sand soil

The nominal concentration for oxytetracycline in the standard models would be 290 µg/kg. Corrected for the bulk density the nominal concentration amounts to 259 µg/kg. This is based on a dilution of the dose per hectare over 20 cm with a soil bulk density of 1.5 kg/L (1.68 for sand soil). The measured initial concentration ranges from 18 to 287 µg/kg and concentration decrease in time, as expected (see Table 5). The average initial concentration (122 µg/kg) is about half the expected concentration, indicating a substantial loss of substance in the matrix or a very unlucky sampling.

The nominal concentration for sulphachloropyridazine in the standard models would result in 440 µg/kg. Corrected for the bulk density the nominal concentration amounts to 393 µg/kg. The measured initial concentration ranges from 232 to 669 µg/kg and concentration decrease in time, as expected. The average initial concentration (416 µg/kg) is also quite as expected.

Clay soil

The nominal concentration for oxytetracycline in the standard models assuming a mixing depth of 20 cm and a bulk density of 1500 kg/m^3 would result in 290 µg/kg. Corrected for the actual bulk density in the UK clay soil the nominal concentration amounts 335 µg/kg.
There is no evidence of decreasing concentrations in time nor increasing levels with repeated application (see Table 6). Therefore the collection of measurements is considered as a set of independent non-correlated observations, and consequently it is justified to consider a lognormal distribution. There are strong theoretical and empirical considerations to assume a lognormal distribution a priori for many physical entities [172]. The lognormal distribution is positively skewed, and has a domain ranging from zero to infinity.

The lognormal frequency distribution of the samples with oxytetracycline concentrations>LOD, is visualised in Figure 3. The range of oxytetracycline concentrations to 0-20 cm is <LOD-539 µg/kg. 90% of the measured concentrations falls in the range between 16 and 525 µg/kg. The nominal concentration of 335 µg/kg is the 88th percentile in this distribution, meaning that a random sample taken in the field has a probability of 88% to have a lower concentration then 335 µg/kg.

The nominal concentration for sulphachloropyridazine in the standard models would result in 440 µg/kg. Corrected for the actual bulk density in the UK field clay the nominal concentration amounts 500 µg/kg.

The range of SCP concentrations normalised to 0-20 cm is LOD-120 µg/kg. Assuming a log-normal distribution of the concentrations >LOD, 90% of the samples falls within the range between 8 and 145 µg/kg (Figure 4). The nominal concentration of 500 µg/kg is the 99.89th percentile in this distribution, meaning that a random sample taken in the field has a probability of 99.89% to have a concentration lower then 500 µg/kg. This implies that, based on the distribution of concentrations in the field, a nominal concentration of 500 µg/kg is very unlikely to occur, or that the worst case concept in the models is effective.

The simple dilution model (mixing of manure in a 20 cm soil layer) did not predict the field concentrations in clay very well. It seems that about 2/3 of the oxytetracycline and 90% of the sulphachloropyridazine is lost between spiking of manure and analysis of clay soil samples. Spatial heterogeneity may account for these deviations.

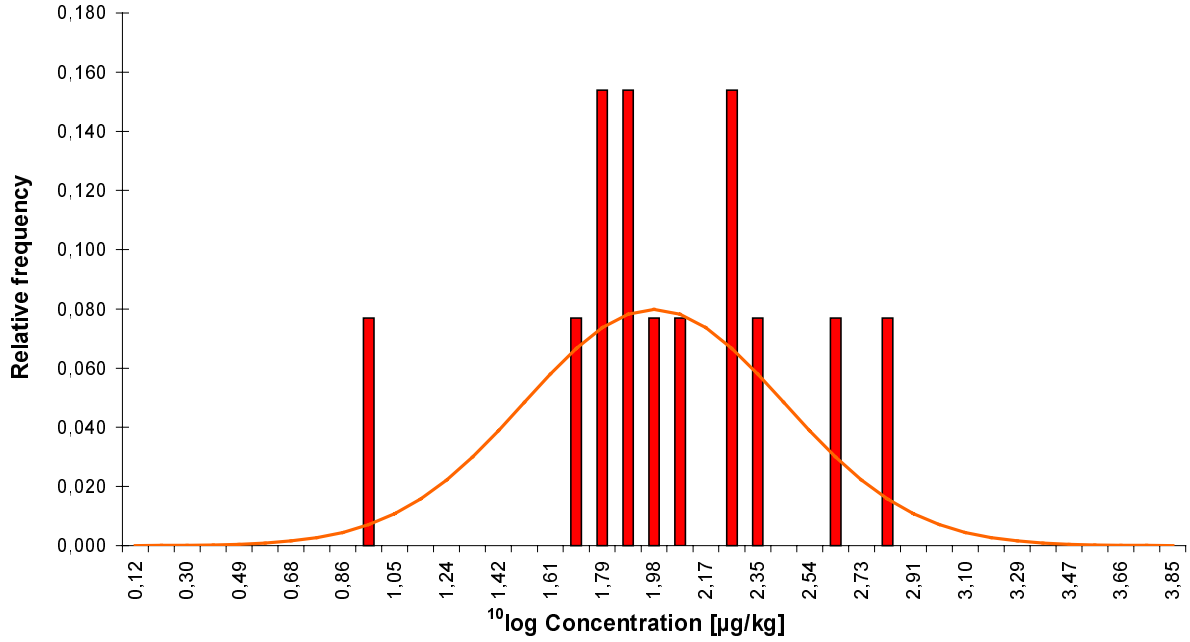


Figure 3 Frequency distribution of the normalised (0-20 cm) OTC samples >LOD (clay).

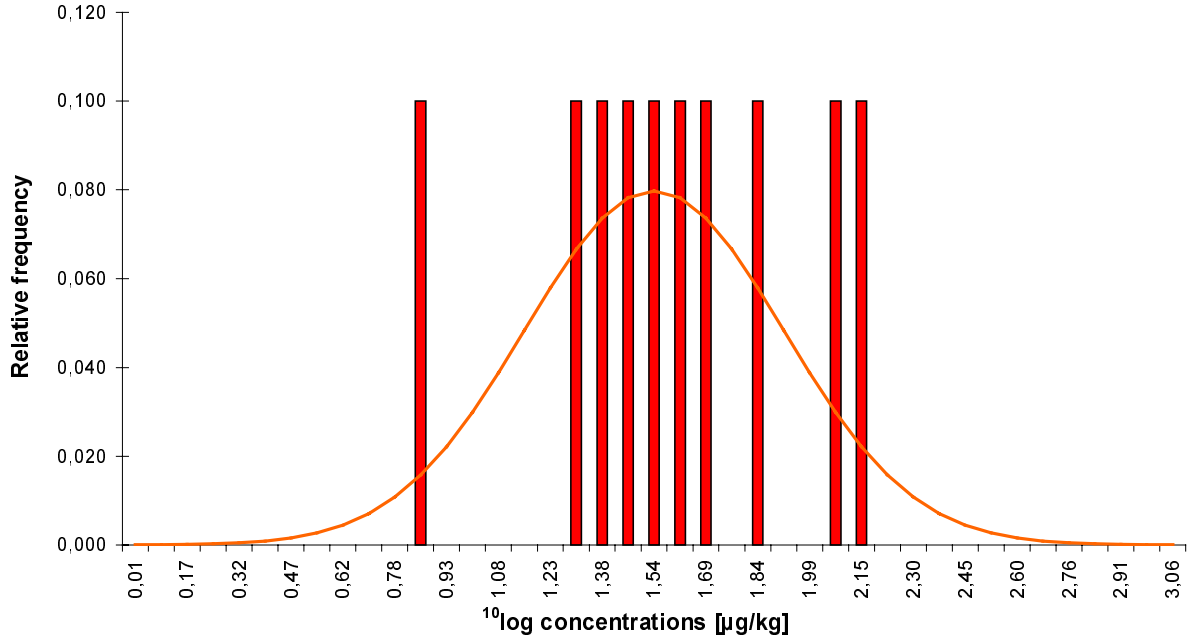


Figure 4 Frequency distribution of the normalised (0-20 cm) SCP samples >LOD (clay).

4.2. Surface water exposure models

This chapter describes the available and used models for surface water exposure and the existing research on validation. Together with the objectives of modelling as defined in chapter 2.1, recommendations for the field validation will be formulated.

The existing models cover a range of complexity; i.e. simple export coefficients, loading functions and mechanistic simulation models [91]. Several models have been validated in another context. A thorough comparison of model validations for several groundwater leaching models and surface water models with respect to regulatory purposes has been accomplished by the Forum for the Co-ordination of pesticide fate models and their Use (FOCUS) of DG Sanco [77,89,93]. The following models are discussed here: the EMEA model, the Montforts model, VetPec, PRZM and MACRO.

Surface water exposure is characterised by soil-water interaction, where the substance is transported from the soil compartment to the surface water compartment. The substance can be transported diluted by convection or diffusion, or adhered to soil particles. Transport can be over the soil surface or through the soil. The surface water can be described at different scales, ranging from natural catchment areas for the entire hydrological unit (e.g. the Rhine system) to a small cross-section in a river, stream or pond, both scales with their associated infiltration land areas. Several models are available that describe transfer of substances through (or over) soil to surface water. Some models have been suggested or explicitly designed to model slurry or manure components [29,38,58,91,113,120].
The model concepts should express the relation between surface water and land, the transport processes involved, and the inherent uncertainties vis-à-vis the decision making level in the process.

4.2.1. The EMEA surface water models

The EMEA model contains a transport model (soil-to-water transport rate) and a catchment model (distribution and concentration in surface water). Substances not adsorbed to soil particles may be present in the soil water and thus be prone to run-off during rainfall events. The concentration in surface waters will be influenced by the amount of rainfall relative to interstitial pore water, and subsequent dilution by the receiving water. The concentration of substance in the interstitial pore water can be estimated using the formula:

Ciw = Cs/Koc*foc
where
Ciw = concentration in interstitial water (µg/L);
Cs = concentration in soil (µg/kg dry soil) and
foc = fraction of organic carbon in soil (kg oc/kg dry soil) [17].

It is assumed that catchment areas tend to be proportional in size to the receiving stream therefore no account is taken of the size of the catchment or receiving water. It is assumed that soil moisture increases by 10% when run-off occurs. Further dilution occurs on entry of run-off into the receiving water. It can be assumed that two parts receiving water will dilute one part run-off water.
The underlying concept is that dissolved substances are available for transport, provided the Koc is <500 L/kg. Transport is fixed on a single event, transporting the equilibrium concentration of substance. This is a capacity type model: an export coefficient depending on the Koc of the substance. The EMEA guidance recommends for groundwater the EU TGD model (see 6.1.1), which also calculates a soil porewater concentration, but using relative compartment volumes. The partitioning model described above for surface water run-off ignores these dimensions. The EMEA guidance uses two models to describe the same process.
The 10% dilution of the pore water does not affect the amount of substance mobilised, as the partitioning code for pore water is not hampered by relative fractions of water, air and solids. In total the equilibrium concentration is diluted by a factor of 3.3 to give the surface water

concentration. The model assumes that all land surfaces are treated with slurry (catchment area is proportionate to receiving stream). The assumptions are not underpinned.

Montforts adapted the EMEA model as follows: see Table 12.

Table 12 Default settings for concentration in surface water due to run-off.

Parameter	symbol	unit	value
Dilution factor for run-off water reaching the surface water	DILUTIONrun-off	[-]	10

Calculation for concentration in surface water due to run-off.

$$PIECsw_{run-off} = \frac{PIECgw}{DILUTION_{run-off}}$$

Input			
PIECgw	predicted initial concentration in ground water	[mgc.l-1]	O
DILUTIONrun-off	dilution factor for run-off water reaching the surface water	[-]	D
Output			
PIECswrun-off	highest concentration in surface water	[mgc.l-1]	O

The 10% dilution of the pore water is not applied, and the dilution factor of the catchment is higher. The input soil pore water concentration is harmonised with the groundwater assessment (see 6.1.1). There is some confusion on the meaning of run-off vs. leaching and drainage.
Germany has developed a comparable export-coefficient model (see Table 13). Depending on the soil type 0.1 to 0.5% of the dosage is transferred to the surface water in a fixed amount of water [56].

Table 13 Parameter values for surface water exposure used by the German agency.

Run off		Direct entry
Arable land	grassland	
20 mm rainfall/24h (200 m3)	20 mm rainfall/24h (200 m3)	
100 m³ output	100 m³ output	
0.1% a.i.·ha-1	0.5% a.i.·ha-1	1% a.i.·ha-1

In terms of concept and algorithm validation all these models are more or less conceived around a simple algorithm (export coefficient). There is no software available and no functional validation has been attempted.

4.2.2. VetPec

A run-off model and an aquifer model accompany the soil concentration model in VetPec. Transport of chemicals in these models is fugacity-driven [96,173].
The aquifer model and run-off models have been validated for the pesticide isoproturon.
Some validation of the VetPec model as a whole has been undertaken using information provided anonymously by VMD for four veterinary medicines. PECs estimated for soil and porewater were generally lower than calculated using the EMEA models. This was expected due to the way VetPec calculates the PECs using a ‘steady-state’ approach. Because of the way VetPec calculates a ‘steady-state’ PEC based on simulation over a number of years it should not be used to calculate the PEC_{soil} in the Phase I assessment (because it is unlikely that sufficient data such as identity, degradation rates and soil adsorption data will be

available to allow use of VetPec). It is suggested that the approach outlined in the Spaepen et al. (1997) model is used to calculate the PEC in Phase I.
VetPec is only for use in situations where the treated animals are kept in housing during and after treatment. The species with which VetPec can be used have been pre-selected and user selection of species is not possible. The species available are those in the publication by Spaepen et al. (1997) plus turkeys. VetPec cannot be used for estimation of PECs from animals kept on pasture.
Concentrations in soil porewater and solids are calculated using fugacity models for percentages of land in the catchment area that are potentially available for slurry application. Next to the water bodies a 5 metre strip is supposed not to be treated.
The aquifer and catchment sub-models are capacity models consisting of cells with a limited number of variations in parameter properties, parameterised on English aquifers and river water catchments. Every timestep, substance degradation, inflow and outflow are calculated, after which a new equilibrium is obtained. The models have been validated on measurements on isoproturon, with a selection of substance properties on degradation and sorption. Hydrology as not validated. No validation on less mobile compounds has been performed.

4.2.3. Functional validation of surface water models

Some examples of research on surface water contamination by medicines and hormones via land are found in literature. Ivermectin has a very high sorption coefficient with K_{om} in the range of 4500-5500 L/kg based on radioactivity, and is rather persistent with DT50s (22°C) of 93 to 240 days (mean 187 days, n=4) in soil [174]. Under steady-state conditions the transport of ivermectin by drainage is considered negligible, and in a study on run-off from cattle feedlots, ivermectin was detected in some samples at levels <2 ng/L [175]. In contrast, the sorption of oestradiol-17β was associated with the surface area and/or the cation exchange capacity of the soil, with high correlations to particle size (clay) and organic matter. K_F values were in the range of 86 – 6670 L/kg, with high K_{om} values of 1800-72500 (median 2600) L/kg [176]. Nevertheless, from experimental plots treated with horse stall bedding or poultry litter, 20% and 30%, respectively, of the added amount of oestradiol-17β was transported in run-off directly following a simulated storm. In the poultry litter experiment, the total loss in a simulated storm event seven days later was 69% of the first loss, which is in proportion with the load remaining after the first event [177,178]. Several parameters, such as grazing by wild animals, variable litter applications and rainfall might contribute to changes of the levels of hormones in soil and consequently in run-off [179]. It is postulated that sorbed oestradiol-17β is transformed, and that oestradiol-17β remaining in the aqueous phase can be transported by preferential flow or colloid transport [176]. Analysis of poultry litter showed that 75% of the arsenic (from roxarsone) was readily soluble in water, although its leach rate is slow enough that it accumulates in the soil [180]. Oxytetracycline shows a strong adsorption behaviour in soils with logKoc of 4-5 L/kg [181]. In an Italian study, no oxytetracycline was detected in drainage ditches adjacent to treated fields (LOD 1 µg/L) [170], which is consistent with the model trigger that surface water is not exposed when Koc is >500 L/kg [182]. In an US investigation, in 31% of the water samples proximal to swine farms and 67% of the samples proximal to poultry farms were found to contain antimicrobial compounds used in these farms: chlor-, oxy,- and tetracycline, sarafloxacin, lincomycin, and sulfadimethoxine, generally at levels <4 µg/L [183]. The logKoc of sarafloxacin and related fluoroquinolones is reported to be 5 – 6 L/kg [184,185]. The concentrations of tetracyclines and sarafloxacin found occasionally (1-2 µg/L and 4 µg/L, respectively) do not support the above mentioned model trigger.

The lower tier models were subjected to a functional validation on the UK field experiment [186]. In the sand soil, soil porewater concentrations of SCP have been measured, and in the

clay soil, drain water concentrations are measured. The fate in the receiving compartments was not assessed. This also complicated the assessment of the fugacity models, as the transfer rates had to be compared and not the equilibrium concentrations. This excercise generated highly variable results. The results of the first year on clay are the result of preferential flow of both solutes and manure-associated particles through cracks following a massive rain event. The second year, the soil had been tiled and disked, thus cutting off the cracks that lead to the drain pipes from the top soil.

The EMEA and Montforts tier I model require input on soil concentration and on sorption coefficients (Koc). From the data in [158] on the field study site the following sorption parameters are used (Table 14).

Table 14. Kd values of OTC and SCP at 26 ± 1 °C calculated with Freundlich isotherms.

	Soil	pH	%oc	%clay	Kf (L/kg) at 1.0 mg/L (±SE)	N (±SE)	Koc
Oxytetracycline	Clay-top	7.40	2.2	25	1814 (±66.8)	0.65	82000
	Sand-top	7.47	1.3	10	655 (±35.9)	0.68	50000
Sulphachloropyridazine	Clay-top	6.6	2.2	25	2.04 (±0.07)	0.97	93
	Sand-top	6.9	1.3	10	1.06 (±0.02)	0.91	82

The Kd values are corrected for field %o.c. (Koc) and calculations are performed using both initial and nominal concentrations. These can be compared to the peak flows.
For the models, soil characteristics can be of importance. The clay soil has a water holding capacity of 32.2% w/w at saturation; the maximum $Fwater_{soil}$ is about 42% v/v. Given the bulk density of soil of about 1300 kg/m^3, the bulk density of the solids is about 1300/58% = 2250 kg/m^3. At field capacity, the $Fwater_{soil}$ is 36% v/v.
The sand soil has a water holding capacity of 28.6% w/w at saturation; the maximum $Fwater_{soil}$ is about 40% v/v. Given the bulk density of soil of about 1680 kg/m^3, the bulk density of the solids is about 1680/60% = 2800 kg/m^3. At field capacity, the $Fwater_{soil}$ is not determined, but should be near 17% v/v [62].
In VetPEC, for oxytetracycline a DT50 of 150 days was used, for sulphachloropyridazine a DT50 of 7.5 days (20°C) was used. VetPec calculates time-weighted concentrations, which makes it difficult to simulate the experimental field concentrations. First, the dosage to a dummy animal was calibrated to give the experimental concentration in soil, assuming no degradation. Then the substance DT50 was entered to calculate surface and groundwater concentrations. The effective annual rainfall was set to 1000 mm/y.

Table 15 Model calculations for surface water and peak drain flows in the UK field experiments.

VMP	Soil concentration [µg/kg]	VetPec Surface water groundwater Max. value [µg/L] SW	GW	EMEA surface water [µg/L]	Montforts surface water [µg/L]	German surface water [µg/L]	Peak flow from drainpipe (clay) or soil water concentrations (sand) (LOD 0.25 µg/L) [µg/L] Year 1	Year 2
SCP	Sand 393 nom	0.01	0.84	112	37	13	0	0
	Sand 416 ini	0.01	0.89	119	39	14		
	Clay 500 nom	0.01	0.78	74	26	13	589	6
	Clay 59 ini	0.00	0.09	9	3	1.5		
OTC	Sand 259 nom	0.00	0.00	0	0	9	0	0
	Sand 122 ini	0.00	0.00	0	0	4		
	Clay 335 nom	0.00	0.00	0	0	9	28	1
	Clay 74 initial	0.00	0.00	0	0	2		

Nom = nominal; ini = initially mean measured.

This exercise generated highly variable results. In the sand soil, soil porewater concentrations have been measured, and in the clay soil, drain water concentrations are measured. The models calculate surface water concentrations, either in the adjacent stream/ditch, or in the main aquifer in the catchment area.
The VetPec groundwater concentrations are closest to soil porewater concentrations. In the other models, porewater concentrations are supposed to be a factor 3-10 higher than the surface water concentrations. The field experiment was not designed to fully validate the VetPec catchment model. However, if the VetPec model intends to cover the ditch water quality, than this objective is not validated by these results.
What all models have in common is that soil porewater concentrations have been exaggerated compared to the results of the sand soils. Also for the drain water, which ought to be diluted by a factor 3-10, the results in the first year were a factor 2-3 underprotective, and up to 40 overprotective in the second year.
The results of the partitioning models also depend on the soil layer the measurements are standardised to. If a 5 cm layer was chosen instead of 20 cm, calculated concentrations would have been 4 times higher. The German mass transfer approach is not hampered by this parameter dependency.
The results of the first year on clay are considered due to preferential flow of both solutes and manure-associated particles through cracks following a massive rain event [159]. The second year, the soil had been tiled and disked, thus cutting off the cracks that lead to the drain pipes from the top soil. The presence of OTC in drain water is however a strong indication of transport of soil and or manure particles through the soil profile. The VetPec and EMEA models neglect this process by considering only solute transport or by setting the transport coefficient zero when Koc >500 L/kg. The German model does not consider this restriction and predicted the peak concentrations best. This model contains a mass loss parameter (see Table 16) that can be validated. Accepting that the first year drain event was extraordinary, the German mass loss parameter is clearly conservative.

Table 16 Validation of mass losses of applied SCP and OTC in the Clay field.

Clay soil drain pipe	Mass loss [%]		German model mass loss [%]
Compound	Year 1	Year 2	
SCP	0.47	0.01	0.1
OTC	0.011	0.00006	0.1

4.3. Groundwater exposure models

This chapter describes the models used by member states for groundwater exposure and the existing research on validation. Together with the objectives of modelling defined in chapter 2, recommendations for the field validation will be formulated.

The following tier I models are discussed here: the EMEA model, the TGD-model, VetPec.

In Phase I of EMEA the EU-approach[3] is used, where the concentration in the ground water is set equal to the concentration in the porewater. In this model partitioning depends on equilibrium sorption to solids, no saturation at binding places and steady-state conditions. Movement, dilution, desorption and transformation are not modelled. Soil parameters have been fixed. The EMEA scheme suggests that the PECsoil as calculated for the upper layer is the input term for the calculation. Conceptually the groundwater reaches to the mixing depth

[3] Chapter 2.3.8.6 ([270] p. 312)

defined by the PECsoil model, or supposes complete vertical transposition of the residue to a general depth. The algorithms and parameters are discussed below.

The model for calculation of the concentration in ground water is as follows.

$$PIECgw = PIECporewater$$

$$PIECporewater = \frac{PIECsoil \cdot RHOsoil}{K_{soil-water} \cdot 1000}$$

$$K_{soil-water} = Fair_{soil} \cdot K_{air-water} + Fwater_{soil} + Fsolid_{soil} \cdot \frac{Kp_{soil}}{1000} \cdot RHOsolid$$

$$Kp_{soil} = Foc_{soil} \cdot Koc$$

$$K_{air-water} = \frac{VP \cdot MOLW}{SOL \cdot R \cdot TEMP}$$

input			
PIECsoil	highest concentration in the soil	[mgc.kgsoil-1]	O
RHOsoil	fresh bulk density of soil	[kg.m-3]	D
RHOsolid	density of soil solids	[kg.m-3]	D
Fairsoil	fraction air in soil	[m3.m-3]	D
Fwatersoil	fraction water in soil	[m3.m-3]	D
Fsolidsoil	fraction solids in soil	[m3.m-3]	D
Focsoil	fraction organic carbon in soil (w/dw)	[kg.kg-1]	D
Koc	partition coefficient organic carbon - water	[dm3.kg-1]	S/O
VP	vapour pressure	[Pa]	S
MOLW	molar mass	[g.mol-1]	S
SOL	water solubility	[mg.l-1]	S
TEMP	temperature at air-water interface	[K]	D
R	gas constant	[Pa. m3.mol-1.K-1]	D
intermediate results			
Ksoil-water	partition coefficient solids and water in soil (v/v)	[m3.m-3]	O
Kpsoil	partition coefficient solids and water in soil (v/w)	[dm3.kg-1]	O
Kair-water	partition coefficient air and water in soil	[m3.m-3]	O
PIECporewater	predicted initial concentration in porewater	[mgc.l-1]	O
output			
PIECgw	predicted initial concentration in ground water	[mgc.l-1]	O

Table 17 Default settings of the module for ground water.

parameter	symbol	unit	value
bulk density of fresh soil (not dry soil!)	RHOsoil	[kg.m-3]	1700
density of soil solids	RHOsolidsoil	[kg.m-3]	2500
fraction air in soil	Fairsoil	[m3.m-3]	0.2
fraction water in soil	Fwatersoil	[m3.m-3]	0.2
fraction solids in soil	Fsolidsoil	[m3.m-3]	0.6
weight fraction organic carbon in soil	Focsoil	[kg.kg-1]	0.02
temperature at air-water interface	TEMP	[K]	285
gas constant	R	[Pa. m3.mol-1.K-1]	8.314

The CVMP guidance document does not discuss the relevance of the chosen parameter values and no claims are made with respect to its functionality or accuracy.
The EMEA and Montforts tier I model require input on soil concentration and on sorption coefficients (Koc). From the data in the field study site the sorption parameters from Table 17 are used. The experiment was described briefly in chapter 3.4.

Table 18 Kd values of OTC and SCP at 26 ± 1 °C calculated with Freundlich isotherms .

	Soil	KFreundlich (L/kg) at 1.0 mg/L (±SE)	NFreundlich (±SE)	pH (±SD)	%oc	Koc
OTC	Ctop	1814 (±66.8)	0.65 (±0.021)	7.40 (±0.11)	2.2	82000
OTC	Stop	655 (±35.9)	0.68 (±0.031)	7.47 (±0.04)	1.3	50000
SCP	Ctop	2.04 (±0.07)	0.97	6.6	2.2	93
SCP	Stop	1.06 (±0.02)	0.91	6.9	1.3	82

C stands for Clay Loam soil (Osgathorpe, UK), S stands for the Loamy Sand soil (Lockington, UK).

The model is corrected for field %o.c. and calculations are performed using both initial and nominal concentrations. These can be compared to the peak flows.
For the models, soil characteristics can be of importance. The clay soil has a water holding capacity of 32.2% w/w at saturation; the maximum $Fwater_{soil}$ is about 42% v/v. Given the bulk density of soil of about 1300 kg/m^3, the bulk density of the solids is about 1300/58% = 2250 kg/m^3. At field capacity, the $Fwater_{soil}$ is 36% v/v.
The sand soil has a water holding capacity of 28.6% w/w at saturation; the maximum $Fwater_{soil}$ is about 40% v/v. Given the bulk density of soil of about 1680 kg/m^3, the bulk density of the solids is about 1680/60% = 2800 kg/m^3. At field capacity, the $Fwater_{soil}$ is not determined, but should be near 17% v/v [62].
In VetPEC (see § 4.2.2), for OTC a DT50 of 150 days was used, for SCP a DT50 of 7.5 days (20°C) was used. VetPec calculates time-weighted concentrations, which makes it difficult to simulate the experimental field concentrations. First, the dosage to a dummy animal was calibrated to give the experimental concentration in soil, assuming no degradation. Then the substance DT50 was entered to calculate surface and groundwater concentrations. The effective annual rainfall was set to 1000 mm/y.

Table 19 Model calculations for groundwater and peak drain flows in the UK field experiments.

VMP	Soil concentration [µg/kg]	VetPec Max. value [µg/L]	EMEA groundwater [µg/L]	soil water concentrations (sand) (LOD 0.25 µg/L) [µg/L] Year 1	Year 2
SCP	Sand 393 nom	0.84	370	0	0
	Sand 416 ini	0.89	390		
OTC	Sand 259 nom	0.00	0.4	0	0
	Sand 122 ini	0.00	0.2		

Nom = nominal; ini = initially mean measured.

What all models have in common is that predicted soil porewater are higher than the measured concentrations in the sand soils. Apparently, the SCP in the top layer did not distribute to deeper layers.

4.4. Conclusions

The general concepts of soil exposure used in the regulatory models have been considered valid by independent researchers. The regulatory guidance document has not separately addressed the risk of persistency [17,19,161], although assessment of this criterion is required by the directive. With respect to the phase I assessment based on a total residue approach according to VICH the incorporation of degradation terms is conceptually wrong, and at the same time the time-window was not specified. The models have however been designed before the total residue concept was implemented. It is not clear how the background levels of naturally occurring substances should be incorporated in the calculations.

With respect to the phase 2 assessment, the models are conceptually correct, although it is unclear how the risk of accumulating residues is controlled within the complete risk assessment scheme.

It is concluded that Montforts (1999) and VetPec adequately translate the model concept into algorithms, but not by Spaepen (1997). The VetPec software has been validated against four example calculations.

The soil exposure models contain several parameters that can assume different values under different circumstances. All models are capacity models, using year-based averaged parameter values. There are however no parameters that need to be derived indirectly. This reduces uncertainty but several parameters are accompanied with considerable variability, and others are delimited by the model concepts. The different data sources yield different values for body weights, number of cycles, slurry production and phosphate and nitrogen contents. This variability is the result of differences in feed type, animal health, race, climatic conditions and housing conditions. In order to come to worst case simulations, almost every parameter has been fixed into a default value. Depending on the region, these defaults might differ. Season, climate, hydrology, volatilisation, and crop are not incorporated as parameters is the models. They appear as boundary conditions for the capacity models (temperature at which transformation rates are determined) or were not defined in the concept. The assumed storage time of slurry influences the dosage that will reach the soil. The number of animal cycles and hence the numbers of administrations that are made during a storage period are determined by the duration of an animal cycle. The residence time of a certain residue depends on the time of excretion by the animal and the time of disposal of the slurry. These parameters are either not modelled (excretion time is 1 day) or defined in the model concept (e.g. four spreading events). The model concept and time frame thus determine the parameter ‘residence time’. VetPec and Montforts addressed this feature, Spaepen did not. The choices made by Montforts (1997) and VetPec are arbitrary, and other sources suggest a generally more simplified spreading regime [113,120,166], which depends on the agricultural practice and fertiliser regulations.

There is considerable variation in the (experimental) field concentrations:
- field concentrations may vary a factor 30;
- patches of slurry may contain concentrations 30 time above those found in soil (Table 35).

The functional validations with (oxy)tetracycline and sulphonamides indicate that it is impossible to analyse the contribution of every single model parameter to the variability in the model predictions using random field samples. Not only variation in doses (a function of dosage and body weight) and excretion factors, dilution, degradation, slurry application rates, and soil variability, but also factors such as representative sampling in slurry and soil, and field residue history, complicate the validation of this part of the model.

There are indications that the soil concentration model by Spaepen is under-protective when compared to the normalised results of the German and UK field experiments. The Montforts model was more successful in predicting maximum (nominal) values. It can be concluded that:
- slurry or nutrient concentrations should be related to a realistic time frame in which the contaminated slurry is produced and diluted in order to optimise the worst case predictions (see [187]);
- the available field data do not allow for validation of the parameter selection in the models;
- field concentrations may vary a factor 30 within one field.

5. Tier II models

In this chapter the performance of advanced groundwater models for leaching of veterinary medicines is described. Three model compounds oxytetracycline sulphachloropyridazine and tylosin have been used for comparison with computed concentrations. The performance is built up in three steps.

First an a priori calculation of PEC_{gw} has been done, using average input for DT50 and Kom obtained from available literature, using three FOCUS models: PEARL, PRZM and PELMO in combination with nine FOCUS groundwater scenarios. The results are presented in chapter 5.1.

Secondly a lysimeter has been simulated, which requires site-specific soil and weather data. Simulation serves two goals. First it gives an indication of the functional validity of the model and it gives a quantity (simulation error) that enables correction of model calculations for scenarios of interest. The output of the computed concentrations is directly compared with concentrations measured in the lysimeter study. The ratio computed/measured concentration, called the simulation error [188], is used as a measure for the relative deviation between model and measurements. The results are presented in chapter 0.

In the third step the simulation error (obtained in the previous step) is treated as a substance specific parameter and has been used to correct/refine initial calculations in the FOCUS scenarios.

5.1. $PEC_{groundwater}$ calculations using FOCUS scenarios

5.1.1. Scenarios

PEC calculations using FOCUS models and scenarios require only input with respect to dosage and application time of the compound and the chemical properties of the compounds. Properties of soil, crop and climate are included in the standard scenarios (Table 20). The soil and meteorological properties of the FOCUS scenarios are specified in Table 47. The FOCUS models require precise input on application time and dosage on the soil. There is no module included that enables the input of treatment rate per animal, type of animal and manure production.
Therefore an initial step is to estimate what amount of veterinary drugs is applied to the soil and how often. The considerations involved for calculation of concentration of veterinary medicines in slurry has been discussed in chapter 4.1.2. Another complication can be the increase of the organic matter content in the top soil due to manure applications. Organic matter is very influential on the distribution of hydrophobic compounds. Grass and maize are considered as most relevant crops for manure spreading in first instance. As a third variant the application of manure on winter cereals is considered, because this crop was used in the field experiment, that is used for validation.

It is assumed that the organic matter content in the tillage layer does not increase due to the annual manure applications.

Table 20 Overview of crop, application time and tillage option for model calculations.

No.	Crop	Application time	Tillage
1	Grass	January 15	no
2	Maize	May 15	20 cm on May 16
3	Winter cereals	October 15	no

5.1.2. Substances

The FOCUS model outcomes are very sensitive to the adsorption coefficient and the biodegradation rate. A representative value for these input parameters is of major importance. A list of substance properties considered is given in Table 48 to Table 53. The ultimate choice of input values is discussed in the following paragraphs.

Oxytetracycline

Oxytetracycline is used in food producing cattle against pneumonia and is administered most widely as intramuscular or intravenous injections at a dose of 6.6-20 mg/kg body weight daily. The amount of oxytetracycline that will enter the environment is dependent on many factors like manure storage, number of treated animals, number of spreading events [162]. In a risk assessment of the FDA [189] a worst case level of oxytetracycline residue in excreta is 0.61 mg/kg and manure spreading of 4500 kg/acre are assumed, resulting in an initial concentration of 0.03 mg/kg soil (6-inch depth).
In a field experiment in Denmark, the level of oxytetracycline was estimated to be within the range of 300-500 mg/kg dry weight manure [190]. The manure was stored for 3 months. To ensure measurable concentrations the manure was fortified to target concentrations in soil of 30-100 µg/kg.
In a field experiment in the UK fortified manure was applied at a level of 0.87 kg oxytetracycline/ha ([191]), which is approximately 0.29 mg oxytetracycline/kg soil (20 cm deep).
To enable a comparison with measurements in the UK field study, for the model calculations an application rate of 0.87 kg/ha is used.

The adsorption of oxytetracycline is expected to be dependent of the pH in the soil. Oxytetracycline is a weakly acidic compound with three pKa values of 3.5, 7.6 and 9.2. This means that the molecule has one or two negative charges at current agricultural pHs. Experimental Kom values range from $16*10^3$ to $74*10^3$ l/kg. The average experimental Kom is 39215 l/kg. The Kom of oxytetracycline is much higher than expected based on its low logKow value of –1.1. This suggests that besides hydrophobic interactions other binding processes take place. When the Kom value is compared with the clay content or pH a significant relationship between Kom and clay content is visible (r^2 =0.86, p=0027) see Figure 20.The expected pH-effect however is statistically insignificant (r^2 = 0.59, p=0.48).
The relation between Kom and clay can be expressed with the following formula:

Formula 1

Kom=13820+1575*%Clay

Unfortunately none of the FOCUS models has the possibility to enter relationships like this. The scenario soils have clay contents in the top soil varying from 3.6% in Jokioinen to 25% in Thiva (see Table 47). Using Formula 1 the Kom can be calculated for each scenario, resulting in Kom values in the range of 19490 – 53195 l/kg, average 34785 l/kg. We would like to enter one set of substance input data, which can be used in combination with 9

different scenarios. Although this introduces an input error for some scenarios there are two reasons why the use of one clay independent value is justified:
1. Using one Kom for all the scenarios is current practice,
2. Kom values in the observed range indicate an immobility to such an extent that the exact Kom value is not relevant for the determination of the concentration in the groundwater. (concentrations are expected to be <<0.001 µg/L. Therefore an average Kom of 34785 L/kg will be used for the calculations.

Biodegradation of oxytetracycline has been studied in water, water-sediment, water-sludge and manure. Usually a rapid disappearance of oxytetracycline is observed. However a distinction between biodegradation and sorption could not be made. Because of the high Kom value, the fate calculations are not very sensitive to small changes in the biodegradation rate. Biodegradation rates between 4 and 46 days under aerobic conditions have been reported, a worst-case DT50 of 46 days at 20°C will be used for PEC calculations.

Sulphachloropyridazine

In a UK field experiment ([191] sulphachloropyridazine was applied at a rate of 1.18 kg/ha, which is approximately 0.4 mg sulphachloropyridazine/kg soil (20 cm deep). This dosage rate is also used for the FOCUS modelling.

Sulphachloropyridazine is a relatively new compound and not much is known about it. The solubility is high (5 g/L), but the NH_2-group and other nitrogen groups in the molecule suggest a potential for strong adsorption. Moreover it is expected that the compound has a weak acidic (due to the SO_2-group) as well as weak alkaline character, but pKa nor pKb values have been reported.

The adsorption of sulphachloropyridazine is studied by [192] in a clay loam and a sandy loam. Kd values have been translated into Kom values for top soil of 54 and 48 L/kg are available (see Table 50). Due to the small amount of data a potential relation of the K_{om} with pH or clay can not be checked. An average Kom of 51 L/kg will be used for the model calculations.

Biodegradation studies of sulphachloropyridazine in soil have been performed at the IRAS (University of Utrecht) and the RIVM [193]. The half-life of sulphachloropyridazine was dependent of the initial concentration (see Table 41). The DT50 determined at the lowest concentration of 0.3 mg sulphachloropyridazine/kg soil was considered most relevant. Therefore a DT50 at 25°C of 11.3 days, similar to a DT50 of 16.9 days at 20°C will be used for the modelling.

Tylosin

Tylosin is used in two field experiments in the UK ([191]) and Denmark ([190]. In the UK a lysimeter study is performed were oxytetracycline, sulphachloropyridazine and tylosin are applied simultaneously with pig manure. Concentrations of tylosin in the UK field and lysimeter study is 4.49 kg/ha respectively in 45150 L manure/ha.
In the UK field and lysimeter experiments tylosin was used as a food additive, given to pigs at 100 g per ton feed. The slurry was stored for 0-3 months.
In the Danish field experiment the tylosinA concentration in soil at t=0 was approximately 30-35 µg/kg. Because the UK experiment will be used for validation of the PEARL model a dosage rate of 4.47 kg/ha will be used also for the FOCUS modelling.

Tylosin has a pKa of 7.1-7.7 so the adsorption is expected to be pH dependent. The relation between pH and K_{om} is not been confirmed by the data (r^2=0.066, p=0.62). A relation between clay and K_{om} or K_f is not obvious (see Table 49). The spread in results is remarkable (factor 10) and the selection made here should be considered preliminary. The worst-case Kom values of 89, 108, 255, 321, 327, 447 and 528 L/kg, are selected for sorption in soil [150,181]. A median Kom of 321 L/kg will be used for model calculations.

The biodegradation is soil has been determined in two field experiments in Denmark. In Lundgaard DT50 values of 45, 52 and 54 days were established (average 50 days). In Askov DT50 values of 58 and 76 days were found (average 67 days). It is assumed that during the test, which lasted from May till October an average temperature of 15°C prevailed. An average DT50 of 59 days at 15°C will be used for the modelling, which is equal to 39.5 days at 20°C.

Table 21 Overview of major substance related input for FOCUS groundwater modelling

	Oxytetracycline	Sulphachloropyridazine	Tylosin
Dosage (kg/ha)	0.87*	1.18*	4.47*
K_{om} (l/kg)	34785**	51**	321**
DT50 at 20°C (days)	46***	16.9*	39.5**

* realistic, ** average, *** worst case

5.1.3. Results

The FOCUS scenarios are derived to represent realistic worst case situations for major agricultural areas in Europe. In the framework of FOCUS realistic worst case is defined as the 90th percentile vulnerable situation, with vulnerability attributed equally to soil and climatic conditions.

For oxytetracycline the FOCUS target concentration in the groundwater was 0.000 µg/L, for grass, maize and winter cereals in all the scenarios, which is not surprising considering the high K_{om} values.

Table 22 FOCUS target concentration of sulphachloropyridazine in the groundwater(µg/L) of 9 FOCUS scenarios calculated with FOCUS PEARL, FOCUS PRZM and FOCUS PELMO.

	FOCUS_PEARL1.1.1			FOCUS_PRZM 2.4.1			FOCUS_PELMO 3.3.2		
Location	Grass	Maize	Winter cereals	Grass	Maize	Winter cereals	Grass	Maize	Winter cereals
Châteaudun	0.002	0.046	0.002	0.000	0.000	0.000	0.000	0.000	0.000
Hamburg	0.018	0.124	0.092	0.001	0.001	0.028	0.000	0.000	0.001
Jokioinen	0.000	-	0.002	0.000	-	0.000	0.000	-	0.000
Kremsmünster	0.003	0.140	0.039	0.000	0.000	0.001	0.000	0.000	0.000
Okehampton	0.020	0.327	0.139	0.003	0.000	0.039	0.000	0.000	0.001
Piacenza	0.305	0.350	0.338	0.038	0.008	0.212	0.011	0.001	0.022
Porto	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Sevilla	0.000	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000
Thiva	0.000	0.044	0.035	0.000	0.000	0.000	0.000	0.000	0.000

For SCP the FOCUS target concentrations are given in Table 22 and for tylosin in Table 23. If the FOCUS calculations are treated as a first tier assessment similar to pesticide risk

assessment, the scenarios with concentrations >0.1 µg/L are considered not safe. Maize is most vulnerable, probably because the applied compounds were incorporated in the top soil, whereas in the other crops an application to the soil surface is assumed.

It is recognised that predicted concentrations with PEARL are higher than computations with PRZM and PELMO. This phenomenon is known and occurs when FOCUS parameterisation is used. When PEARL, PRZM and PELMO have the same parameterisation the models have similar outputs [194]. PELMO shows the lowest concentrations.

Table 23 FOCUS target concentration of tylosin in the groundwater(µg/L) of 9 FOCUS scenarios calculated with FOCUS PEARL, FOCUS PRZM and FOCUS PELMO.

	FOCUS_PEARL1.1.1			FOCUS_PRZM 2.4.1			FOCUS_PELMO 3.3.2		
Location	Grass	Maize	Winter cereals	Grass	Maize	Winter cereals	Grass	Maize	Winter cereals
Châteaudun	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Hamburg	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Jokioinen	0.000	-	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Kremsmünster	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Okehampton	0.000	0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Piacenza	0.001	0.081	0.001	0.000	0.000	0.000	0.000	0.000	0.000
Porto	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Sevilla	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Thiva	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

5.2. Simulation of a lysimeter experiment

5.2.1. General simulation procedure

Field leaching and lysimeter studies are performed when laboratory experiments indicate a potential risk for leaching. Field and lysimeter studies provide information on leaching under more realistic conditions. A limitation of field and lysimeter studies however is that the observed leaching has no general validity because leaching is very dependent on the field and meteorological conditions. The experimental conditions can not be controlled and are not always relevant for the scenario of concern to the risk assessment. Simulation of the field or lysimeter study is a step that enables the extrapolation of results of a particular study to other scenarios. The method is described in detail by [188]. A simulation error is derived by comparing measured leaching with calculated leaching; SE =Calculated leaching/Measured leaching. The simulation error is a measure for over- or underestimation by the simulation model. Computations for other scenarios of interest can be corrected with the observed simulation error. The method fulfils the need for additional interpretation methods for field and lysimeter studies. Field and lysimeter studies are very expensive and time-consuming, and it is not desirable to require such experiments for each scenario in the EU. Lysimeter experiments are more suitable for simulation than field experiments because amounts of percolated water and the concentrations of solutes in it are recorded at regular time intervals.

A simulation error is a lumped parameter containing uncertainties and variations, as well as processes not described and experimental analytical and modelling errors. The availability of detailed information on the performance of the experiment, environmental conditions and site-specific degradation rates and coefficients is required, to decrease the contribution of uncertainty to the simulation error. Also, the experiments must last for at least three times the

breakthrough of the inert tracer and preferably long enough to observe total breakthrough of the veterinary chemical.

The first step in the simulation of a field or lysimeter study is to compare the water balance of the initial run with site-specific input with the measured water balance. Two aspects of the water balance are considered: the total amount of water leached and the leaching pattern in time. Certain hydrological parameters in the model can be calibrated to fit the water balance. Guidance for calibration of lysimeter studies is described elsewhere [76,188].
As a second step the concentration of an inert tracer in the leachate is considered. Hydrological parameters are tuned to fit the tracer concentration as closely as possible. When the fit of the water balance and the tracer concentration is satisfactory the values of the hydrological parameters are fixed. Subsequently simulation of the veterinary chemical is performed. The computed accumulated mass of the chemical leached is compared with measured accumulated leaching. As a measure of the goodness of fit the simulation error is computed.

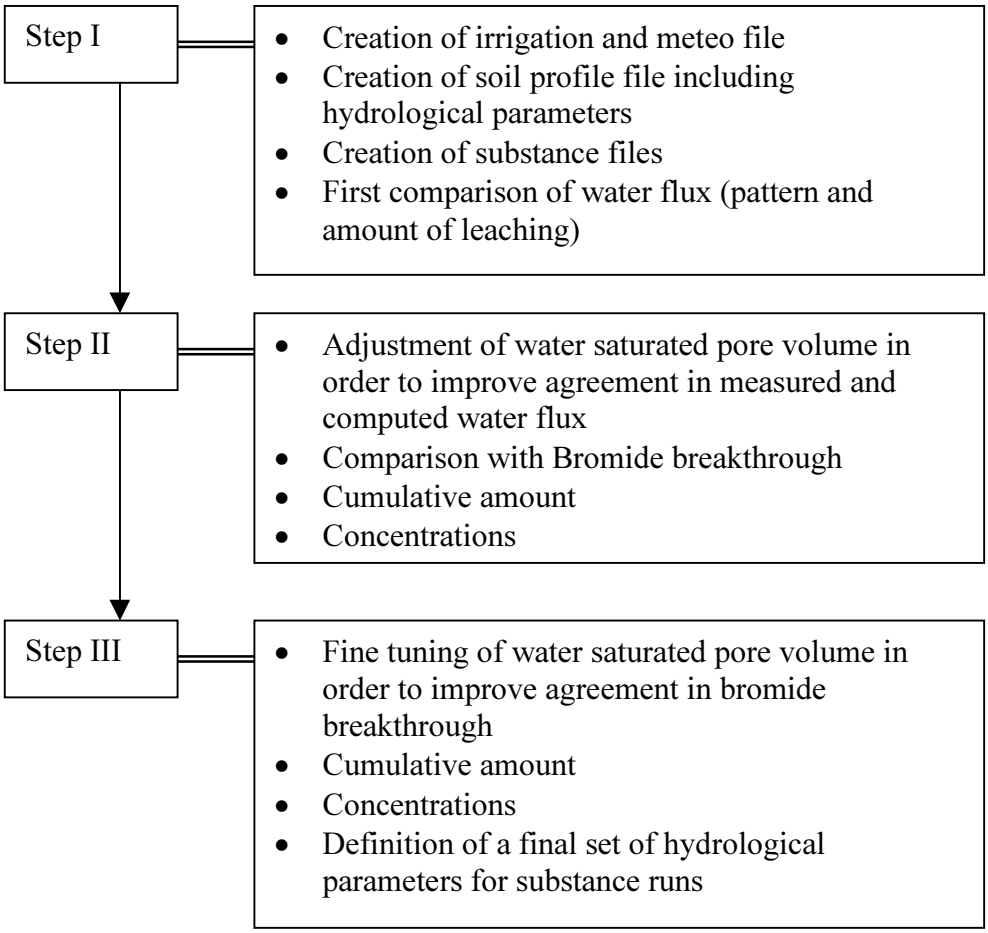


Figure 5 Schematic view of the validation procedure

5.2.2. Measurements in the lysimeter soil

The results of the lysimeter experiment were described in chapter 3.4.3 and Annex III-4 contains figures of the water balance, bromide concentrations, and concentrations of oxytetracycline, sulphachloropyridazine and tylosin.

It is difficult to observe a pattern in the concentrations of oxytetracycline and tylosin. The findings seem to be randomly distributed in time and concentration level. Moreover relatively high concentrations are found in samples from day 1, which should still have background concentrations. This raises the suspicion of difficulties with the analysis of these compounds, in a concentration range so close to the detection limit. The results for oxytetracycline and tylosin are considered not useful for validation of PEARL.

Halflife times and K_{om} were determined by [157] and [192]. The results are summarised in Table 24.

Table 24 Estimated halflife time of the veterinary drug SCP in the lysimeter soil. Determined by non-linear fitting of first order exponential kinetics with GraphPad Prism.

		n	r^2	95% Confidence Interval	value used in FOCUS scenarios
DT50 in days (20°C)	**3.3**	3x9	0.875	2.5-4.5	16.9
Kom (l/kg)	**48**				51

Compared with DT50 values used for calculation in combination with FOCUS scenarios, the DT50s of sulphachloropyridazine in the lysimeter soil were significantly lower, resulting in a decreased potency for leaching. When concentrations in the leachate of the lysimeter are lower than FOCUS calculations it does not necessarily implicate a model error, because in this case also a lower DT50 in the lysimeter soil is responsible for lower concentrations in the leachate.
The variation between lysimeters was quite large. To demonstrate the procedure of the lysimeter simulation the results of one lysimeter have been used. Lysimeter 1C was chosen because the water balance, bromide concentration and the sulphachloropyridazine concentration show a consistent pattern without fluctuations. Later an average fit on all the lysimeters is presented.

5.2.3. Simulation of the lysimeter study

Lysimeter 1C showed a more or less consistent range of increasing sulphachloropyridazine concentrations. This lysimeter is used as an example to demonstrate the simulation method. Lysimeter 1C is used including water balance, Br-concentrations and sulphachloropyridazine concentrations. The major difference with lysimeters 7 A-D and lysimeter 14 A-D is the delayed breakthrough; due to postponed irrigation. These situations are not so suitable because it is likely that sulphachloropyridazine has partly disappeared due to biodegradation before leaching can occur.

Calibration of the water balance

The water balance is dependent on the soil profile (the values of hydrological parameters) and the meteorological conditions. On site meteorological conditions are used for the simulation. Initial values of the hydrological parameters were estimated based upon the texture of the soil profile. The help-function of FOCUS_PEARL contains tables with soil water characteristics for unsaturated soils. The van Genuchten parameters θ_{sat} and θ_{res}, the water-saturated pore volume and the residual pore volume are changed from their initial guesses 0.44 and 0.38 to best guesses 0.35 and 0.25 in order to obtain the fit shown in Figure 6. Also, an equilibration period of one month before substance application with daily water input of 10 mm was necessary in order to simulate the leaching pattern.

The amount of leachate collected exceeds the amount of irrigation water added. Because the lysimeters were covered to prevent rainfall another explanation must be found. A reasonable explanation is that rainfall is running down the outside of the lysimeters and then down the outside of the gravel filled funnel and then into the collection bottle.

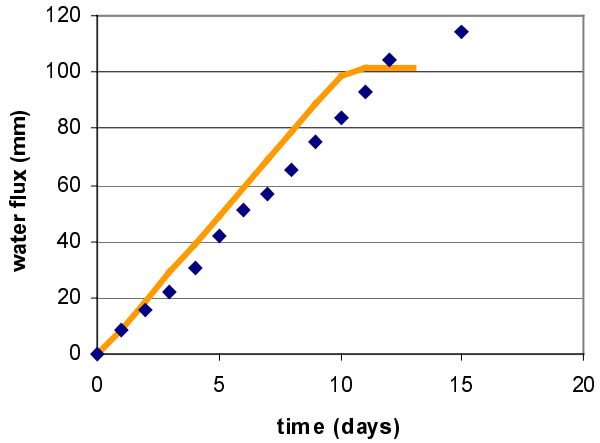


Figure 6 Cumulative water flux at lower boundary of lysimeter 1C; measured (dots) and computed (line)

Calibration of bromide breakthrough

With these parameters obtained by calibration of the water balance the fit of the bromide breakthrough is satisfactory as well (see Figure 7). The ultimate PEARL input files are given in Annex II-6. The calibration steps and fitting to all the lysimeters in given in Annex III, Figure 27.

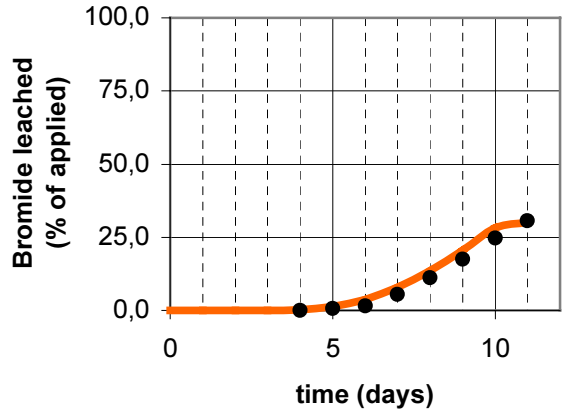


Figure 7 Breakthrough of bromide in lysimeter 1C. Measurements (dots) versus computations (line).

Finally the leaching of sulphachloropyridazine was computed with using a DT50 of 3.3 days at 20°C and a Kom of 48 L/kg measured in the lysimeter soil. The result is shown in Figure 8.

The y-axis has a logarithmic scale. It is observed that computed mass leached is lower than the measured mass leached.

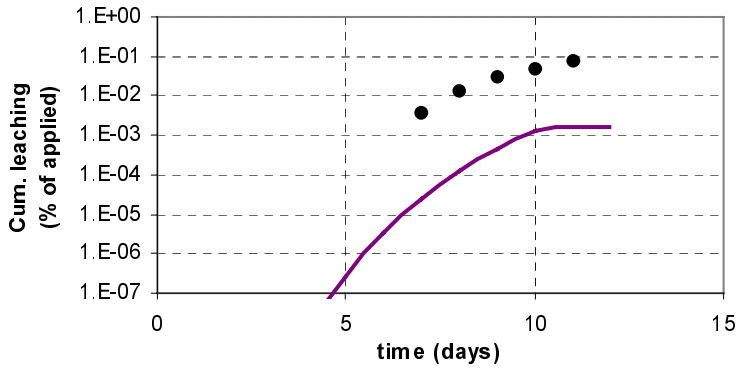


Figure 8 Simulation of SCP leaching. Measurements (dots) versus computations (line).

For each measurement the simulation error can be calculated with:

$$SE = C_{computed} / C_{measured}$$

In Table 25 the simulation errors of bromide and sulphachloropyridazine are listed. A simulation error of 1 implicates an ideal simulation (no error). A simulation error < 1 means that computed values are lower than the measurements and a simulation error > 1 means that computations overestimate measured values. The overall simulation error for bromide is the geometrical mean of individual time points. The simulation tends to a small overestimation of bromide breakthrough and a significant underestimation of sulphachloropyridazine leached.

Table 25 Simulation errors based on cumulative mass leached of bromide and sulphachloropyridazine.

time(days)	Br	SCP
7	1.44	7.02E-03
8	1.21	8.79E-03
9	1.17	1.41E-02
10	1.14	2.66E-02
11	0.98	2.20E-02
Average	1.19	0.02

Overall, the simulation of bromide is quite good with simulation errors in the vicinity of 1. For SCP were additional processes like biodegradation and adsorption come into play the simulation error is significant with values in the range of 0.007 to 0.022. This means that the measured mass leached is on average approx. a factor 50 times higher than computed values.

5.3. Refined calculations of PEC_{gw}

The PEC_{gw} values calculated a priori for the 9 FOCUS models can be refined with the simulation error. The correction is only valid for computations with PEARL, because the simulation error was derived with PEARL. Correction of the output of PELMO and PRZM can only be done with simulation errors derived with the particular model involved. The simulation error is considered to be substance specific. When a priori calculations indicate an concentration <0.001 µg/L a refined estimation is not relevant, because the performance of a lysimeter study wil not be triggered by tier I. The simulation error for sulphachloropyridazine derived for lysimeter C was 0.02. The refined PEC_{gw} is calculated with:

$$PECgw_{refined} = C_{a\ priori} / SE$$

Table 26 A priori and refined FOCUS target concentrations in groundwater.

	A priori PECgw (µg/L)			Refined PECgw (µg/L)		
Location	Grass	Maize	Winter cereals	Grass	Maize	Winter cereals
Châteaudun	0.002	0.046	0.002	0.1	2.3	0.1
Hamburg	0.018	0.124	0.092	0.9	6.2	4.6
Jokioinen	<0.001	-[1]	0.002		-[1]	0.1
Kremsmünster	0.003	0.14	0.039	0.15	7	1.95
Okehampton	0.02	0.327	0.139	1	16.35	6.95
Piacenza	0.305	0.35	0.338	15.25	17.5	16.9
Porto	<0.001	<0.001	<0.001			
Sevilla	<0.001	0.001	0.001		0.05	0.05
Thiva	<0.001	0.044	0.035		2.2	1.75

[1] maize/Jokioinen is not an existing FOCUS scenario

5.4. Discussion

The lysimeter experiment was performed to study the effect of short periods with high rainfall on the leaching of veterinary drugs. It was investigated whether the occurrence of preferential flow could determine transport of compounds to the groundwater. In PEARL substance transport is a result of chromatographic transport; preferential flow is not a process that is accounted for. Since PEARL was able to describe the bromide breakthrough well, with realistic hydrological parameter values it can be concluded that preferential flow was not a significant process in this sandy loam lysimeter. As a consequence the observed concentrations oxytetracycline and tylosin can not be the result of preferential flow. Leaching is not a realistic explanation because of the very high adsorption coefficient of oxytetracycline. Difficulties with the analysis of these compounds is a plausible explanation for the 'random' scatter of these compounds.

From the viewpoint of validation of PEARL for veterinary drugs, the experimental set-up is not optimal. The experimental period with water input is too small to observe complete breakthrough of bromide. As a consequence the chromatographic breakthrough of an adsorbing, degrading veterinary drug is not expected within the duration of the experiment. Concentrations of sulphachloropyridazine at this short time interval are a result of dispersion,

and are very low. Simulation of these concentrations is possible as can be seen in Figure 8, because dispersion ís described by PEARL. However, a simulation is more robust when peak concentrations are measured during the experiment, and when all relevant processes have had time to be effective. The problem of uncertainty when an experiment is stopped too early is visualised in Figure 9 and Figure 10. The simulation errors resulting from these simulations are overestimated, because of many uncertainties (analytical and mathematical) in this early breakthrough phase. In this experiment with low sulphachloropyridazine concentrations error at the analytical side as well as the inaccuracy of the model at concentration <0.001 µg/L contribute to the simulation error.

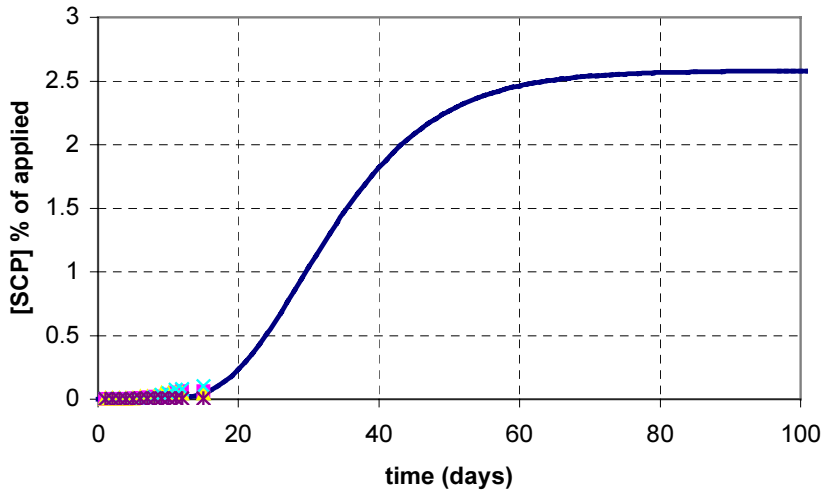


Figure 9 Computed mass of sulphachloropyridazine leached (% of applied) in lysimeters 1A-D at continued irrigation (dots are measurements, line is computed).

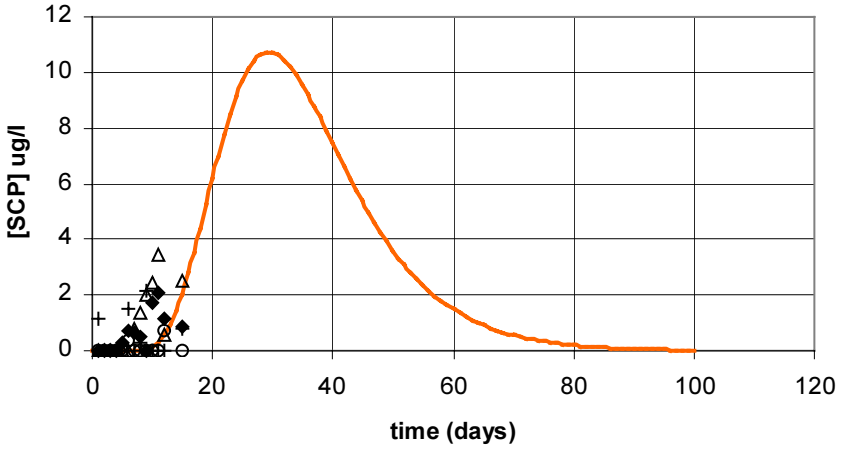


Figure 10 Computed concentrations of sulfachloropyridazine in the leachate at continued irrigation in comparison with available measurements of lysimeter 1A-D (dots are measurements, line is computed).

An additional point of concern is the reliability of the important substance input parameter; the Kom. In this case a Kom value of 48 has been used as PEARL input. The reliability of this parameter could not be assessed by criteria used in the pesticide assessments [195] because the original data have not been published. Moreover several studies indicated that

besides partitioning to organic matter interactions with mineral parts of the soil is also possible. This would invalidate the K_{om}-concept. In Figure 11 it is demonstrated that with a K_{om} of 25 l/kg the simulation error approaches 1 quite closely. However it is not correct to conclude that the sorption coefficient should be 25 L/kg because other uncertainties also contribute to the simulation error. However one has to keep in mind that small mistakes in the K_{om} have a large impact on the computed leaching and consequently on the simulation error.

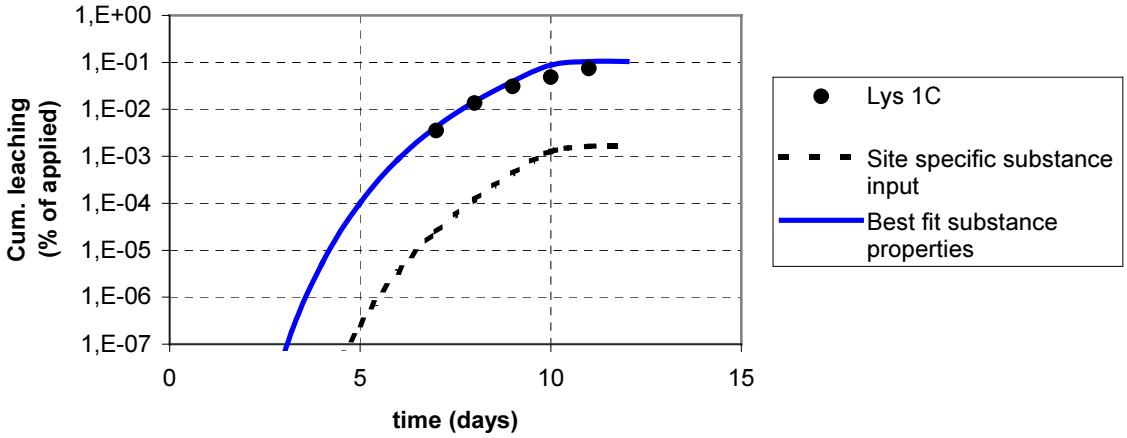


Figure 11 Simulation of sulphachloropyridazine mass leached with a K_{om}= 25 L/kg (best fit) and K_{om}=48L/kg (measured in lysimeter soil).

This case shows that the premature end of the lysimeter study resulted in a relatively high simulation error. The fact that the most relevant processes of downward transport have not come to full expression limits the usefulness of the simulation error. Refined PEC_{gw} concentrations are therefore considered not reliable. It was possible to exclude preferential flow as a potential process for downward transport, based on simulation of bromide concentrations.
Despite the shortcomings of the case study the potential of pesticide leaching models in general and of PEARL in particular is demonstrated.

6. Discussion and conclusions

Strictly speaking, a model cannot be validated in the sense that the validation proves the model is true. All that can be done is to show how small the probability is that the model has been refuted by a statistical comparison with measurements. This implicates that eventually the policy maker must decide how much difference between reality and model results is acceptable. This level of acceptability depends on the purpose of the model. Several authors have discussed statistical criteria for test performance and validation status, but it appears that other issues, such as model documentation and user friendliness, are more important in selecting appropriate models for the regulatory framework. The following elements of model validation are discerned [46,97,101]:

1. Documentation considerations: Availability of source code, version control, support and training, user manual (language, model description -concept, -mathematics, -parameterisation);
2. Modeller skills: problems in using models in the regulatory process include: lack of clearly defined objectives or standard procedures when using models, misuse of models by untrained staff, input data handling, output data handling, and inaccurate interpretation of modelling results.
3. System features: hardware requirements, reliability, clarity of error messages, the limited accuracy of models, graphical user interfaces (GUI).
4. Model science (concept, algorithm, and software):
   - Conceptual validation: to make explicit the consequences of the choices on what variables and relationships in the natural system are formalised in the model; assess the motivation for the applied definitions and choices; assess the applicability, usefulness and accuracy of the conceptual model; assess the demands to which the model input must comply.
   - Algorithmic validation: establish the extent to which the procedures for computation (codes, boundary conditions and parameter values) represent the conceptual model. Software validation includes all activities, which build confidence in the quality of the software implementation, particularly its correctness with respect to the algorithmic implementation, but also in the data handling.
5. Most validation studies do not refer to the way a model is put together, but regard it at a black box: an input-output function, which might represent the system the researcher is interested in. Some studies simply compared results with field observations. This approach is denoted *functional* validation. In fact, most validation studies are of the functional type, but hardly ever result in rejection of the models.

With respect to the regulatory objectives, validation contributes to a better understanding of the information generated in the risk assessment and thus to the transparency of the decision making process.

The registration process of products is primarily be concerned with the level of no effect and the risk that this level will be exceeded. This protection goal is generally pursued by determining a reasonably worst case situation, thus assuming that either the chance on a negative impact is reasonably small and/or that the affected fraction of the area (nation, water catchment, crop area) and the impact itself are acceptably small. Both the model and the validation exercise should target the regulatory benchmarks: levels of no concern. Modelling at such low levels requires a rigorous understanding of all relevant transport and fate processes, or requires sufficient safety factors. Evidently, there should be good agreement

between the protection goal and the methodology used to assess the impact, in the sense that it should be clear what situations the methodology represents, and what level of certainty the predictions have.
The scale of the model (one-dimensional, regional, catchment area, site specific, GIS-based) is not fastened down. The same applies for the time-window. Various scales might serve the regulatory objective, given the scenario and decision making criterion.
The guidance on the PEC calculations in soil, surface water and groundwater provided by the EMEA, Montforts, and VetPec are used by the regulatory assessors. The assessors were not familiar with underlying models PESTAQ and PESTCAT or other catchment area models designed for manure and slurry like GWLF, also used for the distribution model of *Cryptosporidium* oocytes in manure [120,196], the EGPE Model, nor with specific fugacity models like the Mackay model as applied in SoilFug or the Stella diagram. Emission and distribution models used in other agricultural or environmental research areas were also not considered [113,123].
None of the existing models have been functionally validated for veterinary medicines yet. Functional validation should target different steps in the exposure models, all of which are related:

1) Emission
   a) pathology and remediation: occurrence of infections and diseases throughout the year(s)
   b) Dose administered
   c) Duration of treatment
   d) excretion of residues by animals
2) Storage
   a) Slurry production
   b) storage time
   c) storage conditions
   d) slurry removal
   e) slurry quality
3) Substance behaviour in slurry
   a) degradation in slurry
   b) concentrations in slurry
4) Immission into soil
   a) Dosage applied
   b) repetitions
   c) soil management
5) Substance behaviour in soil and water
   a) degradation in soil
   b) concentrations in soil
6) Environmental conditions
   a) climate
   b) soil
   c) hydrology
   d) topography.

Given the sheer quantity of variables, it is virtually impossible to control all variables and examine one parameter at the time. The nature of the available models and the way they are implemented will be discussed in the following chapters. The extent to which the models used for veterinary medicines were capable of predicting field observations is investigated. A field data base stretching 2 years (2000-2001) on soil leaching, drainage and run-off for three veterinary medicines applied in slurry is available.

The functional validations with (oxy)tetracycline and sulphonamides indicate that it is impossible to analyse the contribution of every single model parameter to the variability in the model predictions using random field samples. Not only variation in doses (a function of dosage and body weight) and excretion factors, dilution, degradation, slurry application rates, and soil variability, but also factors such as representative sampling in slurry and soil, and field residue history, complicate the validation of this part of the model.
There are indications that the soil concentration model by Spaepen is under-protective when compared to the normalised results of the German and UK field experiments. The Montforts model was more successful in predicting maximum (nominal) values. It can be concluded that:

- slurry or nutrient concentrations should be related to a realistic time frame in which the contaminated slurry is produced and diluted in order to optimise the worst case predictions;
- the available field data do not allow for validation of the parameter selection in the models;
- field concentrations may vary a factor 30 within one field.

The field experiment performed in the UK focused on the emission and distribution: leaching, drainage and run-off. The fate in the receiving compartments was not assessed. Surface water concentrations were not followed, therefore a validation of models like TOXSWA and PESTCAT were not attempted. This also complicated the assessment of the fugacity models, as the transfer rates had to be compared and not the equilibrium concentrations.

A lysimeter study was used to investigate the functional validity of the groundwater model PEARL for leaching of sulphachloropyridazine. The datasets of oxytetracycline and tylosin were not suitable for simulation because many measurements were below the limit of determination and the leaching pattern was too much scattered. A simulation error of 0.02 was assessed for sulphachloropyridazine, which means that computed values underestimated the measured values by a factor 50. Tracing back parameters, which are responsible for the observed simulation error, is complicated. The simulation error is a lumped parameter containing uncertainties and variations as well as processes not described and experimental analytical and modelling errors. In this study two major factors for uncertainty in the simulation are discerned. First, the untimely ending of the lysimeter hampered the full expression of downward transport. As a consequence sulphachloropyridazine has not shown breakthrough, which reduces the reliability of the simulation considerably. Second, the uncertainty in the sorption process and parameter is of major importance for a reliable simulation. In the simulation performed for this study a equilibrium partitioning to soil organic matter is assumed. It is mentioned in the literature that veterinary drugs are liable to binding to mineral surfaces also, a process which was not considered in our simulation. Several studies indicate that veterinary drugs do not behave solely according to equilibrium partitioning, but also electrostatic interactions and covalent binding to mineral surfaces play a role. This is based on the discrepancy between lipophilic characteristics and observed Kom values.
The simulation made clear that the sorption mechanism must be determined and the simulation model must be able to describe the appropriate sorption mechanism. Moreover, the lysimeter experiment should fulfil certain criteria. Measured concentrations must be well above the detection limit and leaching must continue at last until peak concentrations of the tracer and the compound under investigation have been observed.
Endpoint of a simulation should be the simulation error, which can be used to adjust *a priori* calculations for any scenario of interest.

The functional validity of pesticides leaching models for veterinary drugs can easily be assessed. However, the models will not be rejected soon because:

- Statistical criteria are not common to evaluate model performance.
- The comparison between model outcome and measurements is semi-qualitative. Agreement between shape of the curves, breakthrough moment and total mass leached contribute to an overall impression of the model performance.
- The simulation error is a parameter that is fed not only by model errors but also by uncertainties at the experimental side.

A rejection of a model based on conceptual grounds is more likely. For instance, when the sorption mechanism is obviously different then the one described by the model. The simulation error should not be used to mask conceptual errors.

Literature

1. Montforts MHMM. 1999. Environmental Risk Assessment for Veterinary Medicinal Products. 1. Other than GMO-containing and Immunological Products. RIVM Bilthoven the Netherlands Report 601300001.
2. WRc-NSF (2001) VetPec. Veterinary Medicines Directorate, UK.
3. Soulides DA, Pinck LA, Allison FE. 1962. Antibiotics in soils: V. Stability and release of soil adsorbed antibiotics. Soil Science 94: 239-244.
4. Tabak HH, Bunch RL. 1970. Steroid hormones as water pollutants. I. Metabolism of natural and synthetic ovulation-inhibiting hormones by microorganisms of activated sludge and primary settler sewage. Dev Ind Microbiol 11: 367-376.
5. Zondek B, Sulman F. 1943. Inactivation of estrone and diethylstilbestrol by microorganisms. Endocrinology 33: 204-208.
6. Berland BR, Maestrini SY. 1969. Study of bacteria associated with marine algae in culture. II. Action of antibiotic substances. Mar. Biol. 3: 334-335.
7. Manten A. 1971. De betekenis van antibiotica in het milieu. Ned. Tijdschr. Geneesk. 115: 1844-1848.
8. Blume RR, Younger RL, Aga A, Myers CJ. 1976. Effects of residues of certain anthelmintics in bovine manure on *Onthophagus gazella*, a non-target organism. Southwest. Entomologist 1: 100-103.
9. Rurainski RD, Theiss HJ, Zimmermann W. 1977. Über das Vorkommen von natürlichen und synthetischen Östrogenen im Trinkwasser. GWF-Wasser/Abwasser 118: 288-291.
10. Roij ThAJM, De Vries PHU. 1980. Milieutoxicologische aspecten van het gebruik van veevoederadditieven en therapeutica. Persistentie in dierlijke excreta en milieu. The Hague, The Netherlands: Ministry of Environments. Report no. Reeks Bodembescherming nr. 4.
11. Daughton CG and Jones-Lepp TL. 2001. Pharmaceuticals and Personal Care Products in the Environment: Scientific and Regulatory Issues American Chemical Society, Washington, D.C.
12. Dietrich D. 2002. Special Issue on pharmaceuticals in the environment Toxicology Letters 131.
13. Römbke J, Knacker T and Stahlschmidt-Allner P. 1996. Umweltprobleme Durch Arzneimittel - Literaturstudie-. Umweltbundesamt , Berlin, Germany.
14. Ternes TA. 1999. Special Issue on Pharmaceuticals in the Environment Science of the Total Environment 225.
15. Jorgensen SE, Halling-Sørensen B. 2000. Special issue on pharmaceuticals in the environment. Chemosphere 40.
16. Kümmerer K. 2001. Pharmaceuticals in the Environment. Springer Verlag, Germany.
17. EMEA. 1997. Note for Guidance: Environmental Risk Assessment for Veterinary Medicinal Products Other Than GMO-Containing and Immunological Products. EMEA, London, UK.
18. EMEA. 2000. Dicussion Paper on Environmental Risk Assessments for Non-Genetically Modified Organism (Non-GMO) Containing Medicinal Products for Human Use. EMEA, London, UK.
19. VICH. 2000. Environmental Impact Assessment (EIAs) for Veterinary Medicinal Products (VMPs) - Phase I. CVMP/VICH, London.

20. Länge R, Dietrich D. 2002. Environmental risk assessment of pharmaceutical drug substances—conceptual considerations. Toxicology Letters 131: 97-104.
21. Römbke J, Knacker T, Teichmann H. 2001. Ecotoxicological evaluation of pharmaceuticals. In: Kümmerer K (ed) Pharmaceuticals in the environment. Springer Verlag, Germany, pp. 123-141
22. Schowanek D, Webb S. 2002. Exposure simulation for pharmaceuticals in European surface waters with GREAT-ER. Toxicology Letters 131: 39-50.
23. Spaepen KRI, Van Leemput LJJ, Wislocki PG, Verschueren C. 1997. A uniform procedure to estimate the predicted environmental concentration of the residues of verterinary medicines in soil. Environmental Toxicology and Chemistry 16: 1977-1982.
24. Römbke J, Knacker T, Teichmann H. 2001. Environmental risk assessment of pharmaceuticals: a proposal with special emphasis on European aspects. In: Daughton CG, Jones-Lepp TL (eds) Pharmaceuticals and Personal Care Products in the Environment: Scientific and Regulatory Issues. American Chemical Society: Washington, D.C., pp. 304-319
25. De Knecht JA, Montforts MHMM. 2001. Environmental risk assessment of veterinary medicine products: an evaluation of the registration procedure. SETAC Globe 2: 29-31.
26. Gärtner S. 1998. Arzneimittel in der Umwelt: Umweltschutz im Arzneimittelrecht. Zeitschrift für Umweltchemie, Ökotoxikologie 10: 154-156.
27. Koschorrek J, Koch C, Rönnefahrt I. 2002. Environmental risk assessment of pharmaceutical drug substances—conceptual considerations. Toxicology Letters 131: 117-124.
28. Montforts MHMM, De Knecht JA. 2002. European medicines and feed additives regulation are not in compliance with environmental legislation and policy. Toxicology Letters 131: 125-136.
29. Jorgensen SE, Lützhoft HC, Halling-Sørensen B. 1998. Development of a model for environmental risk assessment of growth promotors. Ecological Modelling 107: 63-72.
30. Cranor CF. 1997. The normative nature of risk assessment: features and possibilities. RISK: Health, Safety and Environment 8: 1-4.
31. Van Leeuwen CJ. 1995. General introduction. In: Van Leeuwen, CJ Hermens, JLM, eds. Risk Assessment of Chemicals: An Introduction. Dordrecht/Boston/London, Kluwer Academic Publishers. 1. pp.1-18.
32. DG Enterprise. 2000. Pharmaceuticals in the European Union. Office for Official Publications of the European Communities, Luxembourg.
33. TK. 1989. Milieucriteria ten aanzien van stoffen ter bescherming van bodem en grondwater, TK21021. Proceedings of the Dutch Parliament 21021:
34. Health Council. 2001. Milieurisico's Van Diergeneesmiddelen. Health Council, The Hague.
35. 91/414/EEC. 1991. Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market.
36. 98/8/EC. 1998. Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market.
37. NW4. 1998. Fourth Action Plan Watermanagement. Ministry of Traffic and Public Works, The Netherlands.
38. WRc-NSF (2001) VetPec. Veterinary Medicines Directorate, UK.
39. IAHR. 1994. Guidelines for documenting the validity of computational modelling software. Delft, The Netherlands: IAHR/AIHR.

40. Brouwer WWM, Boesten JJTI, Linders JBHJ, Van der Linden AMA. 1994. Dutch guidelines for laboratory studies on behaviour in soil and their evaluation. Pesticide Outlook October: 23-28.
41. Resseler H, Shäfer H, Görlitz G, Hermann M, Hosang J, Kloskowski R, Marx R, Sarafin R, Stein B, Winkler R. 1997. Recommendations for conducting simulation calculations for the registration procedure. Nachrichtenblatt des Deutschen Pflanzenschutzdienstes 49: 305-309.
42. Uffink GJM and Van der Linden AMA. 1998. Dilution of Pesticides in Groundwater During Advective Dispersive Transport. RIVM, Bilthoven, The Netherlands.
43. Van der Linden AMA and Van Beek CGEM. 1999. Beoordeling Van Het Gedrag Van Bestrijdingsmiddelen in De Verzadigde Zone Van De Bodem. RIVM, Bilthoven.
44. TCB. 1990. Advies bodembescherming en bestrijdingsmiddelen. Leidschendam, The Netherlands: Technical Committee on Soil Protection. Report no. Report TCB A89/05.
45. Kolluru RV. 1996. Risk assessment and management, a unified approach. In: Kolluru, R, Bartell, S, Pitblado, R, Stricoff, S, Risk assessment and Management Handbook, New York, USA: McGraw-Hill. 1.
46. Dee DP. 1995. A pragmatic approach to model validation. In: Lynch, DR Davies, AM, eds. Quantitative skill assessment for coastal ocean models., Washington DC, USA: American Geophysical Union. 1.
47. 2001/79/EC. 2001. Commission Directive 2001/79/EC of 17 September 2001 amending Council Directive 87/153/EEC fixing guidelines for the assessment of additives in animal nutrition (Text with EEA relevance.).
48. 2001/82/EC. 2001. Directive 2001/82/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to veterinary medicinal products.
49. Heyvaert V. 1999 Coping With Uncertainty. The Regulation of Chemicals in the European Union. Law Department, European University Institute, Florence.
50. 76/464/EEC. 1976. Council Directive 76/464/EEC of 4 May 1976 on pollution caused by certain dangerous substances discharged into the aquatic environment of the Community.
51. 80/68/EEC. 1979. Council Directive 80/68/EEC of 17 December 1979 on the protection of groundwater against pollution caused by certain dangerous substances.
52. 2000/60/EC. 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy.
53. 98/83/EEC. 1998. Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption.
54. Wösten MAD, Blok J and Van de Plassche E. 2001. International Environmental Quality Standard Setting. RIVM / Royal Haskoning, Nijmegen.
55. Van Rijswick HFMW. 2001. De Kwaliteit Van Water (The Quality of Water). Thesis, Utrecht University, Utrecht.
56. De Knecht JA, Van Vlaardingen PLA, Montforts MHMM, Linders JBHJ. 2001. Report on the European workshop on the ERA of VMPs, 25-26 January 2001. RIVM Bilthoven:
57. Montforts MHMM. 1999. Environmental Risk Assessment for Veterinary Medicinal Products. 1. Other than GMO-containing and Immunological Products. RIVM Bilthoven, the Netherlands Report 601300001.

58. Addison JB. 1984. Antibiotics in sediments and run-off water from feedlots Residue Reviews Vol. 92.: 1-28.
59. Boesten JJTI. 2000. Modeller subjectivity in estimating pesticide parameters for leaching model using the same laboratory data set. Agricultural Water Management. 44: 389-409.
60. Tiktak A. 2000. Application of nine pesticide leaching models to the Vredepeel dataset. Pesticide fate. Agricultural Water Management 44: 119-134.
61. Pontolillo J, Eganhouse RP. 2001. The search for reliable aqueous solubility (S_w) and octanol-water partition coefficient (K_{ow}) data for hydrophobic organic compounds: DDT and DDE as a case study. Reston, Virginia, USA: US Department of the Interior, US Geological Survey. Report no. Water-Resources Investigations Report 01-4201.
62. Mensink BJWG, Montforts MHMM, Wijkhuizen-Maslankiewicz L, Tibosch H, Linders JBHJ. 1995. Manual for Summarising and Evaluating the Environmental Aspects of Pesticides. RIVM, report 679101022, Bilthoven, The Netherlands, 1995. Bilthoven, The Netherlands: RIVM. Report no. 679101022.
63. Luttik R, Van Raaij MTM. 2001. Factsheets for the (eco)toxicological risk assessment strategy of the National Institute of Public Health and the Environments (RIVM). RIVM Bilthoven, The Netherlands, Report 601516007.
64. Linders JBHJ, Jager D.T. 1997. USES 2.0 The Uniform System for the Evaluation of Substances, version 2.0. The Netherlands' supplement to EUSES. RIVM Bilthoven, The Netherlands, Report 679102037.
65. Van der Poel P. 2000. Supplement tot the methodology for risk evaluation. Proposal for the formats of names, parameters, variables, units and symbols to be used in emission scenario documents. RIVM Bilthoven, the Netherlands, Report 601450007.
66. Tiktak A, Berg Fvd, Boesten JJTI, Kraalingen Dv, Leistra M, Linden AMAvd. 2000. Manual of FOCUS PEARL version 1.1.1. RIVM Bilthoven, the Netherlands, Report 711401008.
67. Klein M. 1995. Pesticide Leaching Model, User Manual Version 2.01. Fraunhofer-Institut für Umweltchemie und Ökotoxikogie.
68. *PRZM information avaialable from the Centre for Exposure Assessment Modelling.* [Web Page] www.epa.gov/ceampubl Accessed May 2003.
69. Carsel RF, J.C. Imhoff, P.R. Hummel, J.M. Cheplick and A.S. Donigian Jr.. 1998. PRZM-3, A Model for Predicting Pesticide and Nitrogen Fate in the Crop Root and Unsaturated Soil Zones: Users Manual for Release 3.0. U.S. EPA, Athens, USA.
70. Jarvis NJ. 1995. Simulation of water dynamics and herbicide persistence in a silt loam soil using the MACRO model Ecol. Model. 81: 97-109.
71. *MACRO homepage.* [Web Page] http://www.mv.slu.se/bgf/macrohtm/macro.htm Accessed May 2003.
72. FOCUS Leaching Modelling Workgroup. 1995. Leaching Models and EU Registration.
73. FOCUS Leaching modelling workgroup. 2000. FOCUS Groundwater Scenarios in the EU Review of Active Substances.
74. FOCUS Leaching modelling Workgroup. 2000. Generic Guidance for FOCUS Groundwater Scenarios.
75. *FOCUS website* [Web Page] http://viso.ei.jr.it/focus/ Accessed May 2003.

76. Dubus I, Beulke S, Brown CD. 2002. Calibration of pesticide leaching models: critical review and guidance for reporting. Pest Management Science 58: 745-758.
77. FOCUS. 1995. Leaching models and EU registration. Brussels, Belgium: EC DG Sanco. Report no. 4952/VI/95.
78. Addiscot TM, Wagenet RJ. 1985. Concepts of solute leaching in soils: a review of modelling approaches. Journal of Soil Science 36: 411-424.
79. Jager T, Vermeire TG, Rikken MGJ, Van der Poel P. 2001. Opportunities for a probabilistic risk assessment of chemicals in the European Union. Chemosphere 43: 257-264.
80. Aldenberg T, Jaworska J. 2000. Uncertainty of the hazardous concentration and fraction affected for normal species sensitivity distributions. Ecotoxicology and Environmental Safety 46: 1-18.
81. Traas TP. 2001. Guidance document on deriving Environmental Risk Limits. RIVM Bilthoven, the Netherlands, Report 601501012.
82. Aldenberg T, Luttik R. 2002. Extrapolation factors for tiny toxicity data sets from species sensitivity distributions with known standard deviation. In: Posthuma, L, Suter II, GW, Traas, TP, eds. Species sensitivity distributions in ecotoxicology., Boca Raton, USA: Lewis Publishers.
83. Hart A. 2001. Probabilistic Risk Assessment for Pesticides in Europe. Sand Hutton, York, United Kingdom: Central Science Laboratory.
84. ECOFRAM. 1999. Ecological Committee on FIFRA (Federal, Insecticide, fungicide and Rodenticide Act) Risk Assessment Methodologies. Aquatic Draft Report. Washington D.C., USA: U.S. Environmental Protection Agency. Report no. Report available at http://www.epa.gov/oppefed1/ecorisk/index.htm.
85. 65/65/EEC. 1965. Council Directive 65/65/EEC of 26 January 1965 on the approximation of provisions laid down by Law, Regulation or Administrative Action relating to proprietary medicinal products.
86. 793/93. 1993. Council Regulation (EEC) No 793/93 of 23 March 1993 on the evaluation and control of the risks of existing substances.
87. 93/67/EEC. 1993. Commission Directive 93/67/EEC of 20 July 1993 laying down the principles for assessment of risks to man and the environment of subtances notified in accordance with Council Directive 67/548/EEC.
88. Rautmann D, Streloke M, Winkler R. 2001. New basic drift values in the authorisation procedure for plant protection products. In: Forster, R Streloke, M, Workshop on Risk Assessment and Risk Mitigation Measures in the Context of the Authorization of Plant Protection Products (WORMM)., Berlin-Dahlem, Germany: Biologischen Bundesanstalt für Land- und Forstwirtschaft.
89. FOCUS. 2000. FOCUS groundwater scenarios in the EU plant protection product review process. Brussels, Belgium: EC DG Sanco. Report no. Sanco/321/2000. 197 pp.
90. Linders J, Mensink H, Stephenson G, Wauchope D, Racke K. 2000. Foliar interception and retention values after pesticide application. A proposal for standardized values for environmental risk assessment. Pure and Applied Chemistry 72: 2199-2218.
91. Haith DA. 1987. Generalized watershed loading functions for stream flow nutrients. Water Resour.Bull. 23: 471-478.

92. Vega MM, Carbonell G, Pablos MV, Ramos C, Fernández C, Ortiz JA, Tarazona JV. 2001. Evaluación ambiental de residuos ;procinos y gestión agrícola de purines mediante el modelo informático EGPE. Invest. Agr. Prod. Sanid. Anim. 16: 165-180.
93. FOCUS. 2001. Surface water models and EU registration of plant protection products. Final report of the Regulatory Modelling Working Group on Surface Water models of FOCUS. Draft 21-12-2001. Brussels, Belgium: EC DG Sanco.
94. Jarvis N, Brown CD, Granitza E. 2000. Sources of error in model predictions of pesticide leaching: a case study using the MACRO model. Agricultural Water Management 44: 247-262.
95. Bergström LF, Jarvis NJ. 1994. Evaluation and comparison of pesticide leaching models for registration purposes. Journal of Environmental Science and Health
96. Bajracharya K, Barry D. 1993. Mixing cell models for nonlinear, equilibrium, single species adsorption and transport. Journal of Contaminant Hydrology 12:
97. Vanclooster M, Boesten JJTI, Trevisan M, Brown CB, Capri EVO, Eklo OM, Gottesburen B, Gouy V, Van der Linden AMA. 2000. A European test of pesticide leaching models: methodology and major recommendations. Agricultural Water Management 44: 1-19.
98. Addiscott TM. 1998. Modelling concepts and their relation to the scale of the problem. Nutrient Cycling in Agroecosystems. 50: 239-245.
99. Barra R, Vighi M, Maffiolo G, Di Guardo A, Ferrario P. 2000. Coupling SoilFug model and GIS for predicting pesticide pollution of surface water at watershed level. Env. Sci. Tech. 34: 4425-4433.
100. Gustafson DI, Holden LR. 1990. Nonlinear pesticide dissipation in soil: a new model based on spatial variability. Environ. Sci. Technol. 24: 1032-1038.
101. Russel MH, Layton RJ, Tillotson PM. 1994. The use of pesticide leaching models in a regulatory setting: an industrial perspective. Journal of Environmental Science and Health A29:
102. Rekolainen S, Felding G, Sorensen JB, Mogensen BB, Salo S. 1995. Pesticide loss in surface runoff - a model test. TemaNord 558: 138-143.
103. Styczen M, Villholt K. 1995. Pesticide modelling and models. Denmark: Danish Environmental Protection Agency. Report no. 9.
104. Beltman WHJ, Adriaanse PI. 1999. User's manual TOXSWA 1.2. Simulation of pesticide fate in small surface waters. Wageningen, The Netherlands.: Alterra. Report no. Technical Document 54.
105. Rasmussen D. 1995. Modelling of leaching of pesticides - PESTLA and MACRO. Denmark: Danish Environmental Protection Agency. Report no. 310.
106. Jorgensen PR, Schroder T, Felding G, Helweg A, Spliid NH, Thorsen M, Refsgaard JC, Jacobsen OH. 1998. Validation and development of pesticide leaching models. Denmark: Danish environmental Protection Agency. Report no. 47.
107. Rasmussen D. 1995. Surface water model for pesticides - SLOOT.BOX. Denmark: Danish Environmental Protection Agency. Report no. 311.
108. Klein M, Müller M., Dust M, Görlitz G, Gottesbüren B, Hassink J, Kloskowski R, Kubiak R., Resseler H, Schäfer H, Stein B, Vereecken H. 1997. Validation of the pesticide leaching model PELMO using lysimeter studies performed for registration. Chemosphere 35: 2563-87.
109. Tiktak A, van der Linden AMA, van der Pas L. 1998. Application of the pesticide transport assessment model to a field-study in a humic sandy soil in Vredepeel. Pesticide Science 52: 321-336.

110. Addiscot T, Smith J, Bradbury N. 1995. Critical evaluation of models and their parameters. J. Environ. Qual. 24: 803-807.
111. Salo S, Posch M, Rekolainen S. 1994. Testing the modfied CREAMS/GLEAMS model for pesticide concentration in soil. Agricultural Science in Finland. 3: 59-67.
112. Westein E, Jansen MJW, Adriaanse PI, Beltman WHJ. 1998. Sensitivity analysis of the TOXSWA model simulating fate of pesticides in surface waters. Wageningen, The Netherlands: Alterra. Report no. Report 154.
113. Mooren MAM, Hoogervorst NJP. 1993. CLEAN, the RIVM agricultural model. Part 1. Modelstructure version 1.0. RIVM Bilthoven, The Netherlands, Report 259102005.
114. Van Staalduinen LC, Van Zeijts H, Hoogeveen MW, Luesink HH, Van Leeuwen TC, Prins H, Groenwold JG. 2001. Het landelijk mestoverschot 2003. Methodiek en berekening. The Hague, The Netherlands: LEI Reeks Milieuplanbureau 15.
115. Roij ThAJM, De Vries PHU. 1983. Veevoederadditieven, diergeneesmiddelen en het milieu. Tijdschrift Voor De Diergeneeskunde. 108: 665-671.
116. LAC . 1986. Environmental risks of veterinary medicines (in Dutch). The Hague, The Netherlands: Landbouwadviescommissie Milieukritische Stoffen, Ministry of Agriculture and Fisheries.
117. Stappen Rv, Huysman F, Verstraete W. 1989. Microbiële indicatoren van varkensmestapplicatie in landbouwgronden. Landbouwtijdschrift - Revue De L' Agriculture, België 42: 1087-1099.
118. Zuidema M and Klein AE. 1993. Bacteriële Antibiotische Resistentie En Bodemkwaliteit. Technische Commissie Bodembescherming, The Hague.
119. Breimer T, Smilde KW. 1986. De effekten van organische mestdoseringen op de zware metaalgehalten in de bouwvoor van akkergronden. Lelystad, The Netherlands: PAGV. Report no. Themaboek nr. 7.
120. Walker FR, Stedinger JR. 1999. Fate and transport model of *Cryptosporidium*. Journal of Environmental Engineering. 125: 325-333.
121. Van der Poel P and Bakker J. in press. Emission Scenario Document for Biocides. Emission Scenarios for All 23 Product Types of the Biocidal Products Directive (EU Directive 98/8/EC). RIVM, Bilthoven, The Netherlands.
122. Montfoort JA, Van der Poel P, Luttik R. 1996. The use of disinfectants in livestock farming. Bilthoven, The Netherlands: National Institute for Public Health and the Environment. Report no. RIVM Report 679102033.
123. Mills WB, Lew CS, Hung CY. 1999. Sensitivity of concentration and risk predictions in the PRESTO and MMSOILS Multimedia models: regression technique assessment. Risk Analysis 19: 511-525.
124. Walker FR. 1997. A fate and transport model of *Cryptosporidium* in the New York city water supply watersheds. Ithaca, USA: Cornell University. Report no. Thesis.
125. Groen KP 1997. Pesticide leaching in polders. Field and model studies on cracked clays and loamy sands. Lelystad, the Netherlands: Ministry of Traffic and Public Works.
126. Beusen AHW, Boekhold AE, Makaske GB, Van der Linden AMA. 1997. Validation of the PESTLA model: comparison of PRZM-1, PELMO, LEACHP and PESTLA using the input data set for the Dutch standard scenario. Bilthoven, The Netherlands: RIVM. Report no. RIVM Report 715801006.
127. Pennel KD, Hornsby AG, Jessup RERPSC. 1993. Evaluation of five simulation models for predicting alicarb and bromide under field conditions. Water Resource Res. 26: 2679-2693.

128. Diekkrüger B, Söndgerath D, Kersebaum KC, McVoy CW. 1995. Validity of agro ecosystem models: a comparison of results of different models applied to the same data set. Ecological Modelling 81: 3-29.
129. Simmonds LP, Nortcliff S. 1998. Small scale variability in the flow of water and solutes, and implications for lysimiter studies of solute leaching. Nutrient Cycling in Agroecosystems 50: 65-75.
130. Tiktak A, Swartjes F, Sanders R, Janssen P. 1994. Sensitivity analysis of a model of pesticide leaching and accumulation. In: Grasman, J Van Straten, G, Eds. Predictability and nonlinear modelling in natural sciences and economics., the Netherlands: Kluwer Academic Publ. pp.471-484.
131. Vose, D. (1996) Quantitative Risk Analysis, a Guide to Monte Carlo Modelling., John Wiley and Sons, United Kingdom.
132. Van der Linden AMA, Boesten JJTI, Brouwer WWM, Leistra M, Linders JBHJ, Pol JW, Verschoor AJ. in prep. New Dutch decision tree for evaluating leaching of pesticides. Interim report. Bilthoven, The Netherlands: National Institute for Public Health and the Environment. Report no. RIVM Report 601506 006.
133. Verschoor AJ, Boesten JJTI, Leistra M, Van der Linden AMA, Linders J.B.H.J., Pol JWW. 2001. Evaluation of pesticide leaching in lysimeter and field studies. Parent compounds. Bilthoven, The Netherlands: RIVM. Report no. 601506007.
134. Tiktak, A., D. de Nie, J. Pineros Garcet, A. Jones and M. Vanclooster XII International Symposium on Pesticide Chemistry, Pesticides in Air, Plant, Soil and Water Systems., Piacenza, Italy.
135. Poels J, van Assche P, Verstraete W. 1984. Effects of disinfectants and antibiotics on the anaerobic digestion of piggery waste. Agricultural wastes. 9: 239-247.
136. Thayer DW, Unfred DW, Andor RK. 1974. Effect of DES in feedlot associated bacteria. Bull. Environ.Contam. Toxicol. 11: 554-562.
137. Vischetti C, Businelli M, Marini M, Capri E, Trevisan M, Del Re AAM, Donnarumma L, Conte E., Imbroglini G. 1997. Characterisation of spatial variability structure in three seperate field trials on pesticide dissipation. Pesticide science 50: 175-182.
138. Hamscher G, Sczesny S, Abu-Quare A, Höper H. 2000. Stoffe mit pharmakologischer Wirkung einschliesslich hormonell aktiver Substanzen in der Umwelt: Nachweis von Tetrazyklinen in güllegedüngten Böden. Deutsche Tierärztliche Wochenschrift 107: 332-334.
139. Hamscher G, Sczesny S, Höper H, Nau H. 2002. Determination of persistent tetracycline residues in soil fertilized with liquid manure by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. Analytical Chemistry 74: 1509-1518.
140. Al-Ahmad A, Daschner FD, Kümmerer K. 1999. Biodegradability of cefotiam, ciprofloxacin, meropenem, penicillin G, and sulfamethoxazole and inhibition of waste water bacteria. Archives of Environmental Contamination and Toxicology 37:
141. Kümmerer K, Al-Ahmad A. 1997. Biodegradability of the anti-tumour agents 5-fluorouracil, cytarabine and gemcitabine: impact of the chemical structure and synergistic toxicity with hospital effluents. Acta Hydrochim Hydrobiol 25: 166-172.
142. Kümmerer K, Al-Ahmad A, Steger-Hartmann T. 1996. Epirubicin hydrochloride in the aquatic environment - biodegradation and bacterial toxicity. Umweltmed Forsch Prax 1: 133-137.

143. Bull DL, Ivie GW, MacConnell JG, Gruber VF, Ku CC, Arison BH, Stevenson JM, VandenHeuvel WJA. 1984. Fate of avermectin B1a in soil and plants. J Agric Food Chem. 32: 94-102.
144. Halley BA, Jacob ThA, Lu AYH. 1989. The environmental impact of the use of ivermectin: environmental effects and fate. Chemosphere 18: 1543-1563.
145. Pramer D, Starkey RL. 1972. Decomposition of streptomycin in soil and by an isolated bacterium. Soil Sci. 114: 451-455.
146. Hübener B, Dornberger K, Zielke R, Gräfe U. 1992. Abbau von cyclosporin A durch Bodenmikroorganismen. UWSF-Z. Umweltchem. Ökotox 4: 227-230.
147. Marengo JR, Kok RA, Velagaleti R, Stamm JM. 1997. Aerobic degradation of 14C-sarafloxacin hydrochloride in soil. Environmental Toxicology and Chemistry 16: 462-471.
148. Schiffer B, Daxenberger A, Meyer K, Meyer HHD. 2001. The fate of trenbolone acetate and melengestrol acetate after application as growth promoters in cattle: environmental studies. Environmental health persepectives 109: 1145-1151.
149. Weerasinghe CA, Towner D. 1997. Aerobic degradation of virginiamycin in soil. Environmental Toxicology and Chemistry 16: 1873-1876.
150. Ingerslev F, Halling-Sørensen B. 2001. Biodegradability of metronidazole, olaquindox, and tylosin, and formation of tylosin degradation products in aerobic soil/manure slurries. Ecotoxicology and Environmental Safety 48: 311-320.
151. Yeager RL, Halley BA. 1990. Sorption/desorption of 14C[efromycin] with soils. J Agric Food Chem. 38: 883-886.
152. Lange IG, Daxenberger A, Schiffer B, Witters H, Ibarreta D, Meyer HHD. 2002. Review: sex hormones originating from different livestock production systems: Fate and potential disrupting activity in the environment. Analytica Chimica Acta DOI: 10.1016/S0003-2670(02)00748-1:
153. Finlay-Moore O, Hartel PG, Cabrera ML. 2000. 17ß-Estradiol and testosterone in soil and runoff from grasslands amended with broiler litter. J. Environ. Qual. 29: 1604-1611.
154. Nichols DJ, Daniel TC, Moore PA, Edwards DR, Pote DH. 1997. Runoff of estrogen hormone 17ß-estradiol from poultry litter applied to pasture. J. Environ. Qual. 26: 1002-1006.
155. Nowara A, Burhenne J, Spiteller M. 1997. Binding of fluoroquinolone carboxylic acid derivatives to clay minerals. J Agric Food Chem. 45: 1459-1463.
156. Rabølle M, Spliid NH. 2000. Sorption and mobility of metronidazole, olaquindox and oxytetracycline and tylosine in soil. Chemosphere 40: 715-722.
157. Blackwell P. in prep. A lysimeterstudy with OTC, SCP, TYL in a UK sandy loam.
158. Ter Laak Th. 2002. Data on sorption of oxytetracycline, tylosine, and sulfachloropyridazine in various soils. Utrecht, The Netherlands: IRAS, Utrecht University. Report no. Unpublished.
159. Boxall ABA, Blackwell P, Cavallo R, Kay P, Tolls J. 2002. The sorption and transport of a sulphonamide antibiotic in soil systems. Toxicology Letters 131: 19-28.
160. Bouwman GM, Reus JAWA. 1994. Persistence of medicines in manure. Utrecht, The Netherlands: Centrum voor Landbouw en Milieu. Report no. CLM-163-1994.
161. EMEA. 1996. Note for guidance: environmental risk assessment for immunological veterinary medicinal products.
162. Montforts MHMM, Kalf DF, Van Vlaardingen PLA, Linders JBHJ. 1999. The exposure assessment for veterinary medicinal products. Science of the Total Environment 225: 119-133.

163. Boxall ABA, Watts CD, Ripley P. 1997. The application of predictive models for the environmental risk assessment of ECONOR. Journal of Veterinary Pharmacology and Therapeutics 20: 331.
164. Kelly LA, Taylor MA, Wooldridge MJA. 2003. Estimating the predicted environmental concentration of the residues of veterinary medicines: should uncertainty and variability be ignored? Risk Analysis 23: 489-496.
165. Verhoek A. 1996. Kwantitatieve Informatie Veehouderij 1996-1997. Lelystad, The Netherlands: Praktijkonderzoek Rundvee, Schapen en Paarden (PR).
166. Hoogervorst NJP, Van der Hoek KW. 1991. Het landbouw-scenario in de nationale milieuverkenning 2; uitgangspunten en berekeningen. Bilthoven, The Netherlands: RIVM. Report no. Report number 251701005.
167. Wauchope RD, Yeh S, Linders JBHJ, Kloskowski R, Tanaka K, Rubin B, Katayama A, Kördel W, Gerstl Z, Lane M, Unsworth JB. 2002. Pesticide soil sorption parameters: theory, measurement, uses, limitations and reliability. Pesticide Management Science 58: 419-445.
168. Haller MY, Müller SR, McArdell CS, Alder AC, Suter MJ. 2002. Quantification of veterinary antibiotics (sulfonamides and trimethoprim) in animal manure by liquid chromatography-mass spectometry. Journal of Chromatography A 952: 111-120.
169. Schlusener MP, Bester K, Spiteller M. 2003. Determination of antibiotics such as macrolides, ionophores and tiamulin in liquid manure by HPLC-MS/MS. Analytical and Bioanalytical Chemistry 375: 942-947.
170. De Liguoro M, Cibin V, Capolongo F, Halling-Sørensen B, Montesissa C. 2003. Use of oxytetracycline and tylosin in intensive calf farming: evaluation of transfer to manure and soil. Chemosphere 52: 203-212.
171. Montforts MHMM, Kalf DF, Van Vlaardingen PLA, Linders JBHJ. 1999. The exposure assessment for veterinary medicinal products. Science of the Total Environment 225: 119-133.
172. Seiler FA, Alvarez JL. 1996. On the selection of distributions for stochastic variables. Risk Analysis 16: 5-29.
173. MacKay D, Paterson S, Schroeder W. 1986. Model describing the rates of transfer processes of organic chemicals between atmosphere and water. Env. Sci. Tech. 20: 810-816.
174. Halley BA, Jacob ThA, Lu AYH. 1989. The environmental impact of the use of ivermectin: environmental effects and fate. Chemosphere 18: 1543-1563.
175. Nessel RJ, Wallace DH, Wehner TA, Tait WE, Gomez L. 1989. Environmental fate of ivermectin in a cattle feedlot. Chemosphere 7-8: 1531-1541.
176. Casey FXM, Larsen GL, Hakk H, Simunek J. 2003. Fate and transport of 17*b*-estradiol in soil-water systems. Environmental Science & Technology 37: 2400-2409.
177. Busheé EL, Edwards DR, Moore PA. 1998. Quality of runoff from plots treated with municipal sludge and horse bedding.. ASAE 41: 1035-1041.
178. Nichols DJ, Daniel TC, Moore PA, Edwards DR, Pote DH. 1997. Runoff of estrogen hormone 17ß-estradiol from poultry litter applied to pasture. J. Environ. Qual. 26: 1002-1006.
179. Finlay-Moore O, Hartel PG, Cabrera ML. 2000. 17ß-Estradiol and testosterone in soil and runoff from grasslands amended with broiler litter. J. Environ. Qual. 29: 1604-1611.
180. Rutherford DW, Bednar AJ, Garbarino JR, Needham R, Staver KW. 2003. Mobility of arsenic in soils amended with poultry litter. Environmental Science and

Technology 37: 1515-1520.
181. Rabølle M, Spliid NH. 2000. Sorption and mobility of metronidazole, olaquindox and oxytetracycline and tylosine in soil. Chemosphere 40: 715-722.
182. EMEA. 1997. Note for Guidance: Environmental Risk Assessment for Veterinary Medicinal Products Other Than GMO-Containing and Immunological Products. EMEA, London, UK.
183. Campagnolo ER, Johnson KR, Karpati A, Rubin CS, Kolpin DW, Meyer MT, Esteban JE, Currier RW, Smith K, Thu KM, McGeehin M. 2002. Antimicrobial residues in animal waste and water resources proximal to large-scale swine and poultry feeding operations. Science of the Total Environment 299: 89-95.
184. AHI. 1997. Analysis Of Data And Information To Support A PEC_{soil} Trigger Value For Phase I (A Retrospective Review of Ecotoxicity Data From Environmental Assessments Submitted to FDA/CVM to Support the Approval of Veterinary Drug Products in the United States From 1973-1997). Animal Health Institute, Environmental Risk Assessment Working Group., USA.
185. Nowara A, Burhenne J, Spiteller M. 1997. Binding of fluoroquinolone carboxylic acid derivatives to clay minerals. J Agric Food Chem. 45: 1459-1463.
186. Boxall ABA, Blackwell P, Cavallo R, Kay P, Tolls J. 2002. The sorption and transport of a sulphonamide antibiotic in soil systems. Toxicology Letters 131: 19-28.
187. Montforts MHMM and Tarazona JV. 2003. Environmental Risk Assessment of Veterinary Medicinal Products. Part 4. Exposure Assessment Scenarios RIVM, Bilthoven, The Netherlands.
188. Verschoor AJ, Boesten JJTI, Leistra M, van der Linden AMA, Linders JBHJ and Pol JWW. 2001. Evaluation of Pesticide Leaching in Lysimeter and Field Studies. Parent Substances. RIVM, Bilthoven.
189. US Food and Drug administration, Center for Veterinary Medicine *Environmental assesment report for NADA 113-232.* [Web Page] (1989) www.fda.gov/cvm/efoi/ea/EA_files/113-232bEA.PDF Accessed May 2003.
190. Halling-Sørensen B, Jacobsen AM, Jensen J, Sengeløv G, Vaclavik E, Ingerslev F. 2003. Fate and effects of chlortetracycline, tylosin and sulphonamide in two different soils- A field scale study in Southern Denmark. in Prep.
191. Blackwell P. in prep. Field experiment with oxytetracycline, sulphachloropyridazine and tylosine.
192. Van der Laak T. 10/10/2002. Sorption results of oxytetracycline (OTC), tylosine (TYL) and sulphachloropyridazine (SCP) Unpublished Results
193. Schmitt H. October 2002. Biodegradation rates of sulphachloropyridazine in soil Unpublished Results
194. Boesten, J.J.T.I. (2002) Book of Abstracts of 10th IUPAC International Congress on the Chemistry of Crop Protection, Vol. 2, P. 150.
195. Mensink BJWG, Montforts M, Wijkhuizen-Maslankiewicz L, Tibosch H and Linders JBHJ. 1995. Manual for Summarising and Evaluating the Environmental Aspects of Pesticides. National Institute of Public Health and the Environment (RIVM), Bilthoven, The Netherlands.
196. Walker MJ, Montemagno CD, Jenkins MB. 1998. Source water assessment and nonpoint sources of acutely toxic contaminants: A review of research related to survival and transport of *Cryptosporidium parvum*. Water Resources Research 34: 3383-3392.

197. Panter GH, Thompson RS, Beresford N, Sumpter JP. 1999. Transformation of a non-oestrogenic steroid metabolite to an oestrogenically active substance by minimal bacterial activity. Chemosphere 38: 3579-3596.
198. Henschel KP, Wenzel A, Diedrich M, Fliedner A. 1997. Environmental hazard assessment of pharmaceuticals. Regulatory toxicology and pharmacology. 25: 220-225.
199. Ramazza V, Zucchi M, Lanzoni A, Bianchi C. 1996. Presence of oxytetracycline in pig farming after high doses and longer administration times in comparison to the suggested ones. Proc. Euro Residue Conference III, Veldhoven, The Netherlands 2: 814-818.
200. Winckler C, Grafe A. 2001. Use of veterinary drugs in intensive animal production: evidence for persistence of tetracycline in pig slurry. Journal of soils and sediments 1: 66-70.
201. Sommer C, Steffansen B. 1993. Changes with time after treatment in the concentrations of ivermectin in fresh cow dung and in cow pats aged in the field. Veterinary Parasitology 48: 67-73.
202. Wardhaugh KG, Rodriguez-Menendez H. 1988. The effect of the antiparasitic drug, ivermectin, on the development and survival of the dung breeding fly *Orthelia cornicia* and the scarabeine dung beetles, *Copris hispanus, Bubas bubalus and Onitis belial*. J Applied Entomology 106: 381-389.
203. Lumaret J, Galante E, Lumbreras C, Mena J, Bertrand M, Bernal JL, Cooper JF, Kadiri N, Crowe D. 1993. Field effects of ivermectin residues on dung beetles. J Applied Ecology 30: 428-436.
204. Strong L, Wall R, Woolford A, Djeddour D. 1996. The effect of faecally excreted ivermectin and fenbendazole on the insect colonisation of cattle dung following the oral administration of sustained-release boluses. Veterinary parasitology 62: 253-266.
205. Nessel RJ, Wallace DH, Wehner TA, Tait WE, Gomez L. 1989. Environmental fate of ivermectin in a cattle feedlot. Chemosphere 1531-1541.
206. Herd R. 1995. Endectocidal drugs: ecological risks and counter-measures. International Journal for Parasitology 25: 875-885.
207. Short CR, Barker SA, Hsieh LC, Ou S-P, McDowell T, Davis LE, Nerff-Davis CA, Korim G, Bevill RF, Munsiff IJ. 1987. Disposition of fenbendazole in cattle. Am J Vet Res 48: 958-961.
208. ADAS. 1998. Animal manure practices in the beef industry. ADAS Market Research Team report for MAFF. 20 pp.
209. Berende PLM. 1998. Praktische kengetallen over fokkerij, huisvesting, voeding, lichaamssamenstelling, urine- en fecesproductie en toediening van diergeneesmiddelen bij het rund. Wageningen, The Netherlands.: Rikilt-DLO. Report no. 98.10.
210. Berende PLM. 1998. Praktische kengetallen over fokkerij, huisvesting, voeding, lichaamssamenstelling, urine- en fecesproductie en toediening van diergeneesmiddelen bij het schaap. Wageningen, The Netherlands: Rikilt-DLO. Report no. 98.002.
211. Van Eerdt M. 1998. Mestproductie, mineralenuitscheiding en mineralen in mest, 1997 Mndstat Landb (CBS) 12: 52-62.
212. ADAS. 1998. Animal manure practices in the dairy industry. ADAS Market Research Team report for MAFF. 20 pp.
213. ADAS. 1997. Animal manure practices in the pig industry. ADAS Market Research Team report for MAFF. 22 pp.

214. ADAS. 1997. Animal manure practices in the poultry industry. ADAS Market Research Team report for MAFF. 18 pp.
215. Tijmensen MJA, Van den Broek RCA, Wasser R, Kool A, De Mol RM, Hilhorst MA. 2002. Mestvergisting op boerderijschaal in bestaande opslagsystemen. ECOFYS, CLM, IMAG, The Netherlands Rapport 373002-0230:
216. Hoeksma P, Poelma HR, Van Zadelhoff A. 1987. Koude vergisting van mengmest; mogelijkheden voor praktijktoepassing. IMAG Wageningen, The Netherlands
217. Novem. 1991. Commersialisering van koude vergisting van varkensdrijfmest onder stal met behulp van kapjessysteem. NOVEM/RIVM/Haskoning. No. 9134: Nijmegen, The Netherlands.
218. Qiang Z. 1999. In-Barn Evaluations Of Manure Pit Additives For Odour Reduction. Manitoba Agriculture and Food, Canada.
219. Arogo J, Zhang RH, Riskowski GL, Day DL . 1999. Mass transfer coefficient for hydrogen sulfide emission from aqueous solutions and liquid swine manure. Transactions Of The ASAE 42: 1455-1462.
220. Richard T, Harmon J, Honeyman M, Creswell J. 1998. Hoop structure bedding use, labor, bedding pack temperature, manure nutrient content, and nitrogen leaching potential. Iowa State University ASL-R1499:
221. Moreira, V. *Manure handling and storage effects on nitrogen losses of dairy farms.* [Web Page] (2001) http://dfrc.wisc.edu/powell/ Accessed May 2003.
222. Jenkins, M.B., Bowman, D.D., Walker, M.J. and Ghiorse W.C. 7th International Coccidiosis Conference, London, UK.
223. Parker, D., Williams, D., Cole, N.A., Auvermann, B. and Posey, J.S. (2000) Demonstration of Biogas Production Using Low Moisture Content Beef Cattle Manure., West Texas A&M University, Canyon, Texas, USA., 2000.
224. Pitts CW, Tobin PC, Weidenboerner B. 1998. In-house composting to reduce larval house fly, Musca Domestica L., populations. Journal of Applied Poultry Research 7: 180-188.
225. Konings, V. and Beke, M. *Onderzoeksplan melkvee en milieu 1996* [Web Page] (1996) www.agris.be/nl/rundvee/mededeling/med83_1.htm Accessed November 2002.
226. ADAS. 1997. Animal manure practices in the pig industry. ADAS Market Research Team report for MAFF. 22 pp.
227. ADAS. 1997. Animal manure practices in the poultry industry. ADAS Market Research Team report for MAFF. 18 pp.
228. WRc-NSF. 2000. The development of a model for estimating the environmental concentration (PECs) of Veterinary medicines in soil following manure spreading. London, UK.: MAFF. Report no. VM0295.
229. WRc-NSF. 2000. The development of a model for estimating the environmental concentration (PECs) of Veterinary medicines in soil following manure spreading. London, UK.: MAFF. Report no. VM0295.
230. Warner NL, Godwin RJ. 1988. An experimental investigation into factors influencing the soil injection of sewage sludge. *Journal of Agricultural Engineering Research* 39: 287-300.
231. ADAS. 1998. Animal manure practices in the beef industry. ADAS Market Research Team report for MAFF. 20 pp.
232. ADAS. 1998. Animal manure practices in the dairy industry. ADAS Market Research Team report for MAFF. 20 pp.

233. Stevens, E. *Enquete mestinjectie* [Web Page] (2002) http://www.limburg.be/provincialelandbouwdienst/mestinjectie.html Accessed May 2003.
234. Hilpert R, Winter J, Hammes W, Kandler O. 1981. The sensitivity of archaebacteria to antibiotics. Zbl. Bakt. Hyg. I C 2: 11-20.
235. Gavalchin J, Katz SE. 1994. The persistence of fecal-borne antibiotics in soil. J. AOAC Int. 77: 481-485.
236. Warman PR, Thomas RL. 1981. Chlortetracycline in soil amended with poultry manure. Canadian journal of soil science. 61: 161-163.
237. Meyer MT, Bumgarner JE, Thurman EM, Hostetler KA, Daughtridge JV. 1999. A radioimmunoassay method to screen for antibiotics in liquid waste at confined livestock operations, with confirmation by liquid chromatography/mass spectrometry. West Trenton, New Jersey USA: Proceedings US Geological Survey. Report no. Water-Resources investigations report 99-4018B.
238. Meyer TM, Bumgarner JE, Daughtridge JV, Kolpin D, Thurman EM, Hostetler KA. 1999. Occurrence of antibiotics in liquid waste at confined animal feeding operations and in surface and ground water. Fort Collins, Colorado, USA: Proceedings US Geological Survey. Report no. OF 00-0204.
239. Holm JV, Rugge K, Bjerg PL, Christensen TH. 1995. Occurrence and distribution of pharmaceutical compounds in the groundwater downgradient of a landfill. Environmental science & technology 29: 1415-1420.
240. Kuhn EP, Suflita JM. 1989. Anaerobic biodegradation of nitrogen-substituted and sulfonated benzene aquifer contaminants. Hazardous Waste & Hazardous Materials 6: 121-133.
241. Kühne M, Ihnen D, Möller G, Aghte O. 2000. Stability of tetracycline in water and liquid manure. J. vet. med. A 47: 379-384.
242. Winckler C, Grafe A. 2001. Charakterisierung und Verwertung van Abfällen aus der Massentierhaltung unter Berücksichtigung verschiedener Böden. Stoffeintrag in Böden durch Tierarzneimittel und pharmakologisch wirksame Futterzusatzstoffe unter besonderer Berücksichtigung von Tetrazyklinen. Berlin, Germany: UBA. Report no. UBA report 29733911 - UBA-FB 000074.
243. Loke ML, Jespersen S, Vreeken R, Halling-Sørensen B, Tjørnelund J. 2003. Determination of oxytetracycline and its degradation products by high-performance liquid chromatography-tandem mass spectrometry in manure-containing anaerobic test systems. Journal of Chromatography B 783: 11-23.
244. Loke ML, Tjørnelund J, Halling-Sørensen B. 2002. Determination of the distribution coefficient (logKd) of oxytetracycline, Tylosin A, olaquindox and metronidazole in manure. Chemosphere 48: 351-361.
245. ISO. 1993. Soil quality -- Sampling -- Part 6: Guidance on the collection, handling and storage of soil for the assessment of aerobic microbial processes in the laboratory. International Organisation for Standardisation. Report no. 10381-6.
246. Ingerslev F, Halling-Sørensen B. 2000. Biodegradation properties of sulfonamides in activated sludge. Environmental Toxicology and Chemistry 19: 2467-2473.
247. Ingerslev F, Baun A, Nyholm N. 1998. Aquatic biodegradation behaviour of pentachlorophenol assessed through a battery of shake flask die-away tests. Environmental Toxicology and Chemistry 17: 1712-1719.
248. Ingerslev F, Toräng L, Loke M, Halling-Sørensen B, Nyholm N. 2001. Primary biodegradation of veterinary antibiotics in aerobic and anaerobic surface water simulation systems. Chemosphere 44: 865-872.
249. Vej-Hansen B, Bundgaard H, Kreilgård B. 1978. Kinetics of degradation of

oxytetracycline in aqueous solution. Arch. Pharm. Chemi. Sci. Ed. 6: 951-963.
250. Schmitt H. Verification of soil concentrations of SCP in lab exposed soil samples. in Prep.
251. Schmitt H. Verification of soil concentrations of TYL in lab exposed soil samples in Prep.
252. Boesten JJTI. 1990. Influence of solid/liquid ratio on the experimental error of sorption coefficients in pesticide/soil systems. Pesticide Science 30: 31-41.
253. Beusen AHW, Boekhold AE, Makaske GB and van der Linden AMA. 1997. Validatie Van Het Model PESTLA; Vergelijking Van De Modellen PRZM-1, PELMO En LEACHP Met PESTLA Voor Het Nederlandse Standardscenario. RIVM, Bilthoven, the Netherlands.
254. Boekhold AE, van den Bosch H, Boesten JJTI, Leistra M, Swartjes FA and van der Linden AMA. 1993. Validation of the PESTLA Model: Definitions, Objectives and Procedure. RIVM, Bilthoven, the Netherlands.
255. Boekhold AE, Swartjes FA, Hoogenboom FGG and van der Linden AMA. 1993. Validation of the PESTLA Model: Field Testing Using Data From a Sandy Soil in Schaijk (the Netherlands). RIVM, Bilthoven,the Netherlands.
256. Tiktak A. 1999. Modelling Non-Point Source Pollutants in Soils. Applications to the Leaching and Accumulation of Pesticides and Cadmium., PhD Thesis, University of Amsterdam.
257. Pinoceros Garcet JD, De Nie D, Vanclooster M, Tiktak A, Klein M and Jones A. 2002. Validation of the Scenarios Designed for the EU Registration of Pesticides. EGS General Assembly, Nice, France.
258. Vanclooster M, Pinoceros-Garcet JD, Boesten JJTI, van den Berg E, Leistra M, Smelt J, Jarvis N, Burauel P, Vereecken H, Volker A., Fernandez E, Trevisan M, Capri E, Klein M, Tiktak A, van der Linden AMA, Bidoglio G, Baouroui G, Jones A, Armstrong A and Bontoux L. 2001. Effective Approaches for Assessing the Predicted Environmental COncentrations of Pesticides. A Project Supporting the Harmonised Registration of Pesticides in Europe. EGS General Assembly, Nice, France.
259. Trevisan M, E. Capri., A.A.M. del Re. 1993. Pesticide soil transport models; model comparisons and field evaluation Tox. Environ. Chem. 40: 171-81.
260. Strek HJ. 1998. Fate of chlorsulfuron in the environment. 2. Field evaluations Pesticide Science 53: 52-70.
261. Sadeghi AM, A.R. Isensee, A.Sshirmohammadi. 1995. Atrazine movement is soil;compasrison of field observations and PRZM simulations. Journal of Soil Contamination 4: 151-161.
262. Klein M, J. Hosang, H. Schäfer, B. Erzgräber, H. Resseler. 2000. Comparing and evaluating pesticide leaching models. Results of simulations with PELMO. Agricultural Water Management 44: 263-281.
263. Tree Tech *Material safety data sheet, OTC Calcium complex.* [Web Page] www.treetech.net Accessed 2003.
264. Xian Hengtong Guanghua Pharmaceutical *Tylosin.* [Web Page] (2003) www.ebigchina.com Accessed MAy 2003.
265. Elanco Animal Health *Tylan soluble* [Web Page] (1999) www.elanco.com/us/pdf/msds/tylan_soluble.pdf Accessed May 2003.
266. Loke M-L, Tjørnelund J, Halling-Sørensen B. 2002. Determination of the distribution coefficient (logKd) of oxytetracycline, tylosin A, olaquindox and metronidazole in manure Chemosphere 48: 351-361.

267. Rabølle M, Spliid NH. 2000. Sorption and mobility of metronidazole, olaquinodox,oxytetacycline and tylosin in soil. Chemosphere 40: 715-722.
268. Ingerslev F, Halling-Sørensen B. 2001. Biodegradation of metronidazole, olaquindox, and tylosin and formation of tylosin degradation products in aerobic soil-manure slurries. Ecotoxicology and Environmental Safety, Environmental Research , Section B 48: 311-320.
269. Ingerslev F, Toräng L, Loke M-L, Halling-Sørensen B, Nyholm N. 2001. Primary biodegradation of veterinary antibiotics in aerobic and anaerobic surface water simulation systems. Chemosphere 44: 865-872.
270. ECB. 1996. Technical Guidance Documents in Support of the Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances and the Commission Regulation (EC) 1488/94 on Risk Assessment for Existing Substances. European Chemicals Bureau, Ispra, Italy.

Annex I. Literature review of environmental residues of veterinary medicines

I-1 Use and emission of veterinary medicines

Some infectious diseases are expected to occur at every breeding cycle. In the models a default value for body weight is assumed, a constant for every cycle. Average body weights are selected for meat production animals, even though the growth is exponential, and maximum weights for breeding and milking animals. Pathology and body weights at administration might vary throughout the years. The maximum number of cycles is seven (broilers); body weights increase during the cycle with a factor of up to 400 (broilers). The doses are based on the prescription dosage and the default body weights. Medicines that are administered by feed or water might be emitted in higher amounts than assumed due to spillage of feed and water.

Although the parent compound may be transformed into transformation products, the non-oxidised glucuronides may be reverted in the slurry to active compounds [197,198] and others may have some activity themselves [22]. Excretion of metabolites may hence contribute to the effects of the parent compound. A total residue approach addresses this uncertainty.

Excretion of the administered dose is not instantaneous, some residue may be retained in animal tissue, and some may be mineralised in the gut. Excretion may thus be prolonged and this might interfere with the moment the manure tank is emptied and the slurry is spread. The slurry might not contain the theoretical maximum amount of residue, or may contain residues from a 'previous' treatment. Examples (oxytetracycline, ivermectin and fenbendazole) are discussed below.

After administration of oxytetracycline in feed at low concentrations (30-50 ppm), residues are excreted in 3-4 days, but with high doses (800-1600 ppm) it persists in bones and edible tissues after more than 7 days, and was excreted up to 100 days in urine [162] [199]. It was found that wethers (castrate sheep) excrete at least 21% of the oral dosage oxytetracycline and that young bulls excrete 17 - 75% of an oral dosage chlortetracycline as the parent compound [10]. Orally applied tetracycline were rapidly excreted via faeces and urine, with 72% of the five-days dosage being recovered until the second day after the end of the application. Individual animals contributed to excrete residues over a longer period of time [200].

Ivermectin is hardly excreted in urine (<2%), and c. 90% of a dosage is excreted via faeces in the 7 to over 14 days following administration, depending on the route of administration (oral, subcutaneous) [144] [201]. It was found that larvae of the dung fly *Neomyia cornicia* did not develop in dung from cattle collected up to 32 days after injection with ivermectin [202], whereas after oral treatment all ivermectin residues are excreted after one week (see Table 27).Measured concentrations in dung (voided after 5 days) from cattle (ca. 276 kg bw) dosed subcutaneously with 0.2 mg/kg bw contained 0.42 mg/kg wet weight [203]. Cattle (ca. 300 kg) dosed subcutaneously with 0.2 mg/kg bw voided after 2 days 0.58 mg/kg wet weight [201]. These measurements are consistent with the theoretical excretion profile. Sheep (unspecified body weight) dosed intraruminally with 0.3 mg/kg bw voided after 1-3 days an average of 0.75 mg/kg wet weight of ivermectin and drug-like metabolites [144], data consistent with those for cattle with a highest concentration of 0.66 mg/kg wet weight [204]. However, steers (unspecified body weight) dosed subcutaneously with 0.3 mg/kg bw voided after 2-5 days an average of 0.30 mg/kg wet weight of ivermectin and drug-like metabolites,

and pigs (unspecified body weight) dosed subcutaneously with 0.4 mg/kg bw voided after 1-7 days an average of 0.32 mg/kg wet weight of ivermectin and drug-like metabolites. Moreover, steers (ca. 365 kg) dosed subcutaneously with 0.2 mg/kg bw voided after 3 days only 0.055-0.075 mg/kg wet weight [205]. These values are lower than expected.
An oral dosage of 0.2 mg/kg body weight to a horse resulted in 1.70 mg/kg wet weight ivermectin in the manure, en no excretion after 4 days (LOD 0.01 mg/kg wet weight) [206]. Oral application of fenbendazole to cattle resulted in an elimination of only 44.8% of the dose after 6 days [207].

Table 27 The excretion of labelled ivermectin by cattle after oral dosage [162]

days after application of the dose	fraction excreted r.a.	fraction ivermectin	excreted ivermectin as a fraction of the applied dose
1	0.37	0.97	0.359
2	0.23	0.94	0.216
3	0.23	0.84	0.193
4	0.11	0.72	0.079
5	0.04	0.61	0.025
6	0.01	0.51	0.006
7	0.008	0.43	0.004
11	<0.001	<0.3	<0.0001

I-2 Slurry storage, production and removal

The excreta consist of faeces and urine. In the field these two components are dispersed separately, whereas in the stable they are mixed. 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 final concentrations are not exclusively determined by the treated animals. Slurry production and quality is monitored more or less intensively in EU countries due to the restrictions in the use of fertilisers, and especially in The Netherlands. The figures in the publications are not always comparable, as they may refer to some (adult) individuals or to the total husbandry system (including young) and are not always identical (due to differences in feed, race and housing conditions). There may be a great variation in temperature, redox potential, pH, and storage time. Spreading events are monitored as well at regional levels [114,165,208-214].

In the Netherlands, 90% of the manure storage capacity is provided by pits (50%), silos (14%) and combination systems (37%). In the period June-September, the pit temperature for cattle 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 [215].
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 [216], cited in [217]. Pig manure temperatures under rearing facilities in Canada were reported to range from 16°C-23°C over the year [218]. Typical values of air temperature (15-35°C), 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 [219]. 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) [220]. 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 [221]. 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 [222]. 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 [223]. 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 [224].

The input, storage and outflow of contaminated and uncontaminated slurry determine the loads that will reach the soil. Residues can be excreted during a time period in which the slurry basin is emptied. The residence time after excretion and before the basin is emptied differs for every situation. Slurry storage, production and removal should thus be defined in scenarios.

Slurry is collected and either applied directly to fields or stored. Slurry will be applied (injected) on grassland generally as soon as the field is accessible and possibly again after the first cutting. On arable land slurry will be applied before (and after) cropping in the period autumn-winter-spring [225], in one or two runs.

In the UK, a significant proportion is applied directly to land (Table 28). Different storage methods exist for slurry. In the UK cattle industry, one quarter of all farmers have one or more slurry stores on a farm (ADAS, 1998a; 1998b). The most common type of store is a tank or structure below ground, other types of stores comprise above ground circular tanks, earth bank lagoons and weeping wall stores. Most farms only have the capacity to store slurry for less than one month (51% in the dairy industry and 70% in the beef industry). Only 6% and 4% of dairy and beef farmers respectively have the capacity to store slurry for more than six months. Many farms with stores have slurry in them all of the time (58% of dairy farms and 38% of beef farms). On dairy farms, few slurry stores were empty for more than four months whilst on beef farms one quarter of respondents had empty stores for 5-6 months a year. Around half of cattle farmers stirred their slurry occasionally and less than 10% once a week, the remainder never stirred slurry lagoons. A small amount of cattle manure and slurry is transported off the farm area, the remainder being spread on fields on farm.
Over half of pig farmers that 57% have on farm stores [226], the majority being below ground tanks and the rest comprising earth bank lagoons or circular tanks above ground. Slurry from pig houses is ether collected monthly, daily or weekly. The capacity of stores varies with pig farmers storing slurry for less than one month to more than nine months. The majority of pig farmers (87%) have slurry in their stores all of the time. Generally, the lagoons are unstirred although a small proportion (6%) are stirred weekly. Only 8% of pig farms transported any of their slurry off the farm.
In the poultry industry 37% (broilers) and 46% (layers) of holdings did not store any manure after its removal from poultry houses [227]. If manure was stored, the majority of it was

placed in the yard or field, where it may be covered. The manure was stored for 1-6. Generally, manure was removed from the cages weekly, although on some holdings it was removed at the end of the production cycle or daily. A relatively large proportion of poultry manure is transported off site.

Table 28 Percentage of farm yard manure spread directly to land in the UK (ADAS, 1997-1998).

Proportion of total manure	% of dairy farms	% of beef farms	% of pig farms
Less than 25%	36	26	41
25 - <50%	10	10	9
50% - <75%	16	22	13
75% or more	38	42	37

Another source reports the following data on slurry storage and application rates (Table 29). It is not specified to what animals the data apply [228].

Table 29 Amounts of slurry stored and applied, time of application and length of storage [229].

	% applied	Quantity stored (gallon)	storage time (months)	application rate (kg/ha/y)	plough depth (cm)	waste content (kg P/m3)	waste content (kg N/m3)
slurry							
Average	100	1042571	9.1	21212.88	16.49	0.68	2.69
SD	0	1462894	9.84	23015.33	7.56	0.55	2.41
min	100	0	0	80	0	0.12	0.99
max	100	5000000	50	100000	28	1.5	7
manure		(tonne)				(tonne)	(tonne)
average	98.44	2358.5	8.06	12636.89	16.53	1.07	2.11
SD	10.83	14150.5	6.1	14252	7.92	na	na
min	25	0	0	3	0	107	2.11
max	100	110000	48	55000	28	1.07	2.11

For the Netherlands the storage capacity of slurry basins depend on the slurry surplus and are expressed in terms of storage time: this varies between 2 and 12 months depending on the fate of the slurry (e.g. use on own farmland; export). The periods in which the spreading of manure (i.e. stable manure, slurry and sludge) is allowed are different for indicated and non-indicated areas. For indicated areas this period is February 1-August 31 for grassland and arable land. For non-indicated areas this period is February 1-September 15 for grassland and the whole year for arable land [165]. On grassland, and arable land on sandy soil, the growing season is the period in which slurry is spread the most, compared to the autumn (75:25). Arable land on clay or peat soil is manured almost exclusively in autumn and winter. One or two spreading events within a half year are most common. Liquid manure is mostly injected into the soil (grassland) or spread and incorporated within 24 hours (arable land) [166].
In Flanders, Belgium, it is not allowed (decree of May 11th 1999) to use slurry on grassland between September 21 and January 31, and on arable land between September 21 and February 21, regardless of the soil texture [225].

The total load to the field soil depends on the dosage applied with every spreading event (governed by the factors addressed above), and the number of spreading events within the period of concern. In the Netherlands the amount of slurry used is limited by the phosphate content and phosphate immission standards, not by nitrate. Since 2000 the allowed immission

of phosphate via slurry is 80 kg/ha [114]. This quotum is presumably spread over the respective spreading events.

The soil management after and during spreading determines the distribution of the slurry and the concentration of the residue in soil.
Four main types of slurry distribution system exist, each of which can be fitted to a vacuum tanker, pumped tanker or used with an umbilical system. Self-travelling irrigators cannot be used with injectors:
Broadcast spreader (splash plate or nozzles) – the slurry is forced under pressure through a nozzle, often onto an inclined plate to increase the sideways spread, resulting in widespread distribution of slurry across the land surface;
Band spreader – the boom of the spreader has a number of hoses connected to it, distributing the slurry in strips close to the ground (Figure 12) It is fed with slurry from a single pipe, thus relying on the pressure at each of the hose outlets to provide even distribution. Advanced systems use rotary distributors to proportion the slurry evenly to each outlet.

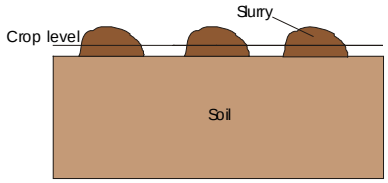


Figure 12 Slurry deposition using a band spreader

Trailing shoe spreader – this is a similar configuration to the band spreader with a shoe added to each hose allowing the slurry to be deposited under the crop canopy onto the soil (Figure 13).

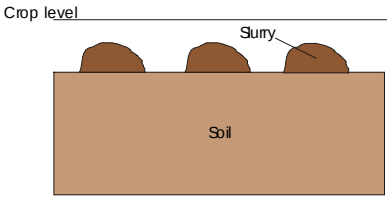


Figure 13 Slurry deposition by trailing shoe spreader.

Injector – slurry is injected under the soil surface. There are various types of injector but each fits into one of two categories: either open slot shallow injection, up to 50 mm deep (Figure 14) or deep injection over 150 mm deep (Figure 15), up to around 330 mm. The width of each strip of slurry injected with an open slot will be approximately 40 mm whilst closed slot injection produces a band of slurry with a base of about 280 mm [230].

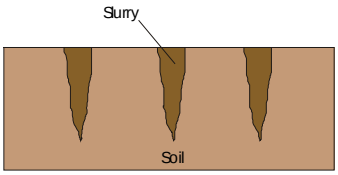


Figure 14 Slurry application by open slot injection

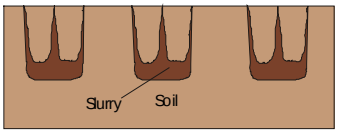


Figure 15 Slurry deposition by closed slot injection

Solid manure may be spread using a rotaspreader, rear discharge spreader or dual purpose spreader, all of which will result in the manure being spread in a similar fashion over the land surface. It is recommended that, following application, manure and slurry are incorporated into the soil by ploughing within 24 hours (MAFF, undated).
This, however, may not be carried out in practise as shown by Table 30. Relatively few farms incorporate within the recommended period and a significant number do not plough the waste in at all.

Table 30. Incorporation of manure and slurry into soil (ADAS, 1997a; 1997b; 1998a;1998b).

Incorporation	Slurry				FYM			
	Dairy	Beef	Pig	Poultry	Dairy	Beef	Pig	Poultry
None	47	68	38	-	26	35	12	31
Same day	5	10	13	-	5	9	13	13
< week	32	12	26	-	46	37	55	43
> week	16	10	22	-	24	19	20	13

The timing of application of manure and slurry varies in the UK (Table 31). Waste from cattle is applied predominantly between February and April, whilst manure from beef cattle is spread in greater volumes in the August to October period. However, significant amounts of cattle manure are also spread from late summer through winter. Waste from pigs and poultry is spread in the greatest quantities between August and October, particularly manure. Taking all manure and slurry types into account, it is evident that the dominant spreading period is from late summer through to the end of the following spring. The data collected by WRc-NSF support these findings [228].

Table 31 Timing of manure and slurry applications as a percentage of total applied [226,227,231,232].

Months	Dairy cattle		Beef cattle		Pigs		Poultry	
	Slurry	FYM	Slurry	FYM	Slurry	FYM	Layers	Broilers
Feb-Apr (%)	40	40	46	28	27	17	21	26
May-July (%)	10	10	13	10	18	7	16	9
Aug-Oct (%)	24	25	20	42	35	56	44	50
Nov-Jan (%)	26	26	21	20	20	19	19	15

Incorporation depths have been recorded in an inventory by WRc-NSF (Table 29). Plough depths for slurry and solid manure were 0-28 cm, with averages of 16.5 cm.

In the Netherlands and in Belgium, over 95% of the agricultural (arable) land is manured one to three times per year. In the Netherlands there is a strong preference to restrict manuring

before the growing season. 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 (personal communication E. van Well, Centre for Agriculture and Environment (CLM), Utrecht, The Netherlands). On heavy (clay) soils (as opposed to sandy soils) a substantial number of farmers prefers to spread the liquid fractions before the growing season, and the solid fractions before the winter. In the Belgium province of Limburg, 20-60% of the farmers manure once per year, and 30-45% twice, and 2-30% three times [114,233].

I-3 Substance behaviour in slurry

Elmund (1971) found that therapeutic levels of chlortetracycline selects for a microbial population relatively inefficient in the stabilisation process (at 14 mg/kg in slurry), and apparently alters the digestive processes in the animal, resulting in manures which are less biodegradable as measured by standard BOD5 procedures. Hilpert and Winter (1984) demonstrated influence of lasalocid, monensin, spiramycin, tylosin (-20%; -33; -35% at 3; 10; 100 mg/L), virginiamycin, arsanilic acid, furazolidone and olaquindox on methanogenesis in anaerobic sewage sludge down to levels of 1 mg/L. Flavomycin and sulfamethazine did not affect the process up to 50 and 100 mg/L, respectively. Poels et al. (1984) demonstrated influence of virginiamycin and bacitracin on methanogenesis (-86%) and species patterns in a piggery waste fermentor (at 16 and 33 mg/L, respectively). The following antibiotics did not disturb the digestion: chlortetracyclin, tylosin, erythromycin and chloramphenicol. On the other hand, the application of the highest dose for bacitracin and virginiamycin gave a large decrease in biogas production. After 2 days a decrease of 49% for virginiamycin and 19% for bacitracin was registered. The decrease of gas production was coupled with an increase in volatile fatty acids (VFA). Thayer et al. (1974) demonstrated a susceptibility of cellulose decomposing bacteria to DES; Hilpert et al. (1981) demonstrated susceptibility of methanogens to virginiamycin [234].

I-4 Residues of veterinary medicines in soils

Tetracycline, oxytetracycline, chlortetracycline and tylosin.
The distribution and persistence of the frequently used tetracyclines (oxy-, tetra-, chlortetracycline) and tylosin in soils fertilised with liquid manure was investigated in Germany by Hamscher et al. (2000; 2002) [138,139].
In a pilot study concentrations of tetracycline (TC), oxytetracycline (OTC), chlortetracycline (CTC) and tylosin (TYL) were determined in field soils. Two fields served as controls, having received no organic fertilisers for at least five years. Two fields received swine or cattle slurry in September 1999. Ten fields were sampled without a recorded history of slurry treatment. In the controlled fields soil samples were taken at 0-90 cm; pore water at 80 cm and 120-140 cm. Other field soils were sampled 0-30 cm. Sampling in February 2000 in duplicate.

Table 32 Measured concentrations in selected fields. Results are averages of duplicates. – indicates below LOD.

Field	Depth [cm]	TC [µg/kg]	CTC [µg/kg]
3 pig slurry	0 – 10	10.3	3.3
	10 – 20	32.2	5.6
	20 – 40	17.8	2.5
4 cattle slurry	0 – 10	1.4	3.8
	10 – 20	2.0	2.6
	20 – 40	<1	<1
5	0 – 10	<1	<1
	10 – 20	1.2	1.5
	20 – 30	1.8	2.8
6	0 – 10	26.9	5.9
	10 – 20	17.7	5.9
	20 – 30	10.5	5.0
7	0 – 10	12.2	25.4
	10 – 20	5.0	12.9
	20 – 30	13.4	26.4
8	0 – 10	8.3	8.1
	10 – 20	10.1	14.6
	20 – 30	16.1	21.8
9	0 – 10	1.0	1.2
	10 – 20	<1	1.2
	20 – 30	1.0	1.6
10	0 – 10	-	-
	10 – 20	-	-
	20 – 30	-	-
11	0 – 10	-	<1
	10 – 20	-	1.5
	20 – 30	-	-
12	0 – 10	5.1	8.6
	10 – 20	2.2	4.6
	20 – 30	7.0	6.1
13	0 – 10	2.5	3.3
	10 – 20	2.9	2.6
	20 – 30	2.2	1.4
14	0 – 10	2.8	7.3
	10 – 20	2.1	2.9
	20 – 30	2.9	1.2

Extraction and analysis were described briefly, and is most likely identical to the method described in [139]. Recoveries and RSD were not reported. LOD 1 µg/kg; LOQ 5 µg/kg for all substances. Soil parameters were not reported (density, water content, %o.c.). Results are listed below (Table 34). None of the porewater samples contained any residues. None of the soil samples contained OTC or TYL. No residues in samples 40-90 cm. One random field contained no residues.

Results varied per field and substance. However, because no data on concentrations in slurry are available, the usefulness for model validation is limited.
In a follow-up study, slurry (unspecified) was spread out in an agricultural field in an area of intensive livestock farming in northern Germany in April 2000 at a rate of 40 m^3/ha and in April 2001 at a rate of 30 m^3/ha [139]. The farmer performed sampling of slurry; it was not specified if slurry was mixed before sampling. In May 2000, November 2000 and May 2001, soil samples were collected. Within a field of 5 ha, samples were collected in four areas of 16 x 16 m at depths of 0-10, 10-20, 20-30, 30-60 and 60-90 cm below the soil surface. Also, dried liquid manure aggregates (from cattle and pig) from the soil surface, as well as soil samples underneath the aggregates at 0-10, 10-20 and 20-30 cm depths, were also obtained in May 2000 from the same field. Samples were stored in the dark at 4^oC prior to analysis. Samples of the liquid manure from April 2000 and 2001 were analysed for antibiotics. Groundwater was sampled in May 2000, November 2000 and June 2001.
Climatic conditions (air, soil and precipitation) were not documented.

Extraction procedures: One gram of wet soil and liquid sample was buffered (1 M citrate buffer (pH 4.7)) and eluted with 6 ml of ethyl acetate (twice); organic phases were combined and evaporated to dryness under vacuum at 40°C. The dry residue was redissolved in 200 µL (1 mL for liquid manure samples) of 90% acetonitrile/10% 100mM ammonium acetate in water. Extraction of water samples was performed on a Baker column processing system SPE-12 G. Determination of antibiotics was performed with LC-MS-MS (HPLC employing a simple gradient system combined with ESI-MS-MS). The limit of quantification was 5 µg/kg for all compounds in the soil. Recovery for soil samples: the recovery rate was determined with control sandy soil spiked at the 5, 10, 25, 50 and 100 µg/kg level. Mean total organic carbon content of the control sandy soil was 2%. Day-to-day variation of the method was tested with eight sandy soil samples containing tetracycline and chlortetracycline, using five to six independent experiments. Recovery studies for liquid manure were carried out with residue-free liquid manure at the 0.2 and 1 mg/kg level. Statistical analysis: the potential influence of soil depth and sampling or storage time on the antibiotic concentration in the soil was determined with two-way ANOVA.

Table 33 Recoveries ± standard deviations and the corresponding relative standard deviations of the studies compounds in soil, water, and liquid manure[a]

	oxytetracycline		**tetracycline**		**chlortetracycline**		**tylosin**	
Conc.	recovery [%]	RSD [%]	recovery [%]	RSD [%]	recovery [%]	RSD [%]	recovery [%]	RSD [%]
				In Soil				
5 µg/kg	66.7 ± 19.2	28.8	33.8 ± 9.7	28.6	76.0 ± 15.8	20.8	63.9 ± 8.2	12.9
10 µg/kg	74.2 ± 15.1	20.3	33.7 ± 8.0	23.6	57.3 ± 11.0	19.2	67.4 ± 8.4	12.4
25 µg/kg	85.9 ± 14.7	17.1	46.5 ± 5.1	11.1	66.4 ± 10.0	15.0	63.2 ± 9.6	15.2
50 µg/kg	71.2 ± 6.1	8.5	33.2 ± 2.5	7.5	7.2 ± 4.7	6.5	66.1 ± 2.5	3.8
100 µg/kg	73.0 ± 8.4	11.6	41.2 ± 3.5	8.6	75.7 ± 5.8	7.7	60.3 ± 4.8	7.9
				In Water				
0.2 µg/L	90.0 ± 17.4	19.3	98.2 ±11.0	11.2	86.6 ± 15.2	17.6	68.6 ± 11.1	16.2
1 µg/L	76.9± 9.5	12.4	86.1 ± 14.2	16.5	89.7 ± 9.2	10.3	72.0 ± 13.0	18.1
				In Liquid manure				
0.2 mg/kg	87.3 ± 6.0	6.9	82.3 ± 5.9	7.2	94.1 ± 8.5	9.1	b	b
1 mg/kg	100.2 ± 2.1	2.1	104.2 ± 3.4	3.3	127.1 ± 9.7	7.6	b	b

[a] Values are the mean of eight independent determinations at each concentration
[b] Extraction procedure not suitable for the analysis of tylosin in liquid manure

Mean recovery in soil of tetracycline = 37.7% and of chlortetracycline = 69.2%. Mean recovery in soil of oxytetracycline = 74.2% and of tylosin = 64.2%. The relative standard deviations (RSDs) calculated from the experiments to determine the day-to-day variation in samples containing tetracycline and chlortetracycline ranged from 20.5 to 48.9% for tetracycline and from 10.1 to 49.7% for chlortetracycline and were independent of the concentrations range.

Oxytetracycline and tylosin could not be detected in any liquid manure or soil sample investigated. Tetracycline and chlortetracycline could not be detected at depth below 30 cm, indicating that tetracyclines are bound to the topsoil and that transfer to the subsoil does not occur.
The concentrations of tetracycline in samples of May 2001 at 0-10, 10-20 and 20-30 cm depth were significantly higher ($p<0.05$) than the concentrations in samples of May 2000 and November 2000. The concentrations of tetracycline in samples collected at all three dates at 0-10 cm were at all times significantly lower ($p<0.5$) than in the soil depths of 10-20 and 20-

30 cm. The possible reversible degradation product, 4-epi-tetracycline was present in liquid manure at 10-15% of the parent drug. The degradation product may be present in the soil but it was not looked for in the experiments.

Table 34. Mean values of tetracycline and chlortetracycline[a] after spreading of liquid manure containing 4 mg/L tetracycline and 0.1 mg/L chlortetracycline in April 2000 and 3.2 mg/L tetracycline and 0.09 mg/L chlortetracycline in April 2001

	sample collection				
soil depth [cm]	in May 2000	in Nov 2000	in May 2001	In May 2000, stored for 6 months at 4°C	In May 2000, stored for 12 months at 4°C
			Tetracycline [µg/kg]		
0-10	56.4 ± 20.1	43.4± 2.2	86.2 ± 15.3	49.8 ± 11.3	73.6 ± 19.7
10-20	100.5 ± 68.8	94.2 ± 40.3	198.7 ± 84.0	61.8 ± 22.6	119.4 ± 86.5
20-30	90.5 ± 35.4	59.0 ± 21.7	171.7 ± 65.4	101.5 ± 51.5	141.3 ± 82.6
Average 0-30	83	65	152		
Nominal	38		61		
			Chlortetracycline [µg/kg]		
0-10	4.6 ± 1.1	5.1 ± 1.4	6.1 ± 2.0	3.7 ± 1.0	6.6 ± 1.4
10-20	4.7 ± 0.3	6.0 ± 1.2	7.1 ± 3.4	4.4 ± 1.0	6.8 ± 1.5
20-30	4.8 ± 1.2	5.0 ± 1.0	5.8 ± 3.2	4.2 ± 1.4	7.3 ± 3.2
Average 0-30	4.7	5.3	6.3		
Nominal	0.96		1.5		

[a] values have been corrected for mean recovery (37.7% for tetracycline and 69.2% for chlortetracycline)

The concentrations of chlortetracycline in samples (0-10, 10-20 and 20-30 cm) of May 2000 stored at 4°C for 12 months at were significantly higher than that of May 2000 without storage and with storage for only 6 months.
High concentrations of tetracycline and chlortetracycline were determined by LC-MS-MS in dried liquid manure (Table 35).

Neither tetracyclines nor tylosin were detected in any water sample obtained at a depth of 80 cm, or in groundwater at a depth of 200-240 cm below soil surface.

Table 35. Tetracycline and chlortetracycline concentrations[a] in dried liquid manure aggregates deposited in May 2000 and in the underlying soil.

dried liquid manure/soil depth [cm]	tetracycline [µg/kg]	chlortetracycline [µg/kg]
area A (liquid manure from pigs) (n=1)	349.3	1435.0
0-10	33.2	59.9
10-20	50.1	12.0
20-30	30.3	14.9
area B (liquid manure from pigs) (n=1)	117.1	4.9
0-10	4.0	1.7
10-20	2.6	2.6
20-30	2.6	2.9
area C (liquid manure from cattle (n=4)	6.6 ± 3.2	10.9 ± 3.9
0-10	5.0	7.2
10-20	4.6	6.6
20-30	2.3	3.3

[a] corrected for mean recovery

The theoretical doses were 160 g/ha and 96 g/ha for OTC and 4 and 2.7 g/ha for CTC in 2000 and 2001, respectively. Divided over 30 cm soil with a bulk density of 1400 kg.m^{-3}, the nominal concentrations are 38 and 23 µg/kg OTC and 0.96 and 0.64 µg/kg CTC. The measured concentrations are a factor 2-4 higher, indicating the presence of residues in the

soil, additional release of bound residues between 2000 and 2001, higher application volumes, or higher concentrations in the slurry pit than in the slurry samples. Compared to Winckler and Grafe (2001), the concentrations in the slurry samples were rather low.

Tetracycline
In a study by Winckler and Grafe (2001), 181 random pig slurry samples were obtained from commercial farms. Of the random samples 24% contained tetracycline in concentrations >0.6 mg/L. The mean concentration was 11.6 mg/L, the maximum was 66 mg/L [200].

Chlortetracyclin
In one study by Gavalchin and Katz (1994), a standard solution of chlortetracyclin was mixed with chicken faeces to obtain a final concentration of 5.6 mg/kg soil [235]. After 30 days 44% remained at 30°C, 88% at 20°C, and no degradation occurred at 4°C. Another study investigated the effect of chlortetracycline in litter dung on microbial respiration in amended soil [236]. Two groups of White Leghorn chicks were maintained on a 20% chick starter ration with the experimental group receiving 11 µg chlortetracycline (CTC) as Aurofac 10 and 125 µg Amprolium Plus/g feed. Manure from the control groups is referred to as untreated manure (UT), while the manure from chicks fed the control ration plus additives is referred to as treated manure (T). CTC was extracted from poultry manure and manure-soil mixtures with HCl-acetone and bioassayed by a method developed to measure CTC in animal feeds. CTC was added as Aurofac 10 to untreated manure at rates of 240 and 480 µg/g; pure tetracycline HCl was added to untreated manure at 250 µg/g; and amprolium at 1.25 mg/g plus CTC as Aurofac 10 at 240 µg/g was added to untreated manure. Treated manure was added to control soil at ratios of 1:5, 1:10, 1:40, and 1:200. These ratios correspond to 4.5, 2.3, 1.1, 0.6, 0.1 µg CTC/g of soil, respectively. The manure plus soil was mixed, incubated for 2 h and extracted for CTC. To determine if there was any effect on soil biological activity a Warburg respirometer was used to determine if CTC in treated manure affected respiration of the soil micro-organisms. Treated manure was added to soil at manure to soil ratios of 1:40 and 1:200 (0.6 µg and 0.1 µg CTC/g). The respiration pattern was determined in triplicate for up to 24 days in three separate experiments. In addition, to test the soil biological system severely, pure tetracycline HCl was added to soil at 25 and 50 µg/g and the respiration followed for 2 days. Recovery of antibiotic added to untreated manure was 34% for pure tetracycline and 22-28% for CTC in Aurofac 10. The presence of amprolium did not affect the recovery of CTC. Manure from CTC fed birds contained 22.5 µg CTC/g compared to the 11 µg/g in the feed. Of the five manure-soil mixtures, only the 1:5 and 1:10 mixtures gave positive results in the bioassay and these were equivalent to 2.7 and 0.74 CTC/g soil, the recoveries were 60% for the 1:5 mixture and 33% for the 1:10 manure to soil mixture. The CTC in treated manure had no affect on soil respiration over 24 days at the manure-soil ratio of 1:40 and 1:200. The high rates of pure tetracycline also did not affect soil respiration. The low determination of CTC is probably not the result of degradation, but of adsorption to the manure, or alteration to a non-detectable form.
In six selected swine slurry samples in the US chlortetracycline was detected with a radioimmunoassay method (with LC-MS; LOQ 1 µg/L) in concentrations of <1-800 µg/L: <1, 1, 5, 20, 90 and 800 µg/L. Of the 17 surface and ground water samples, in one surface water samples chlortetracycline was present in a concentration below the LOQ [237,238].

Sulphonamides
Analysis of six grab samples taken in Switzerland from manure pits on farms where medicinal feed had been applied revealed total sulphonamide concentrations of 0-20 mg/kg slurry [168].
In one location (A) a treatment of the animals with a mixture of trimethoprim, sulfamethazine and sulfathiazole in the ration 2:5:5 was going on. Dosages were however not recorded. No information on treatments in other locations.
Samples were collected from pig and cattle farms after stirring the slurry in the pits for a minimum of half an hour. Ca. 4 L was sampled three times and mixed. Next, 500 ml samples were ground in a kitchen blender before storing at –20°C. Before extraction, samples were thawed overnight. After stirring, subsamples were taken with a micropipette, the internal standard was added and samples were brought to pH 9 and eluted with ethyl acetate (three times); organic phases were combined and evaporated to dryness under nitrogen flow. The dry residue was redissolved in 10% acetonitrile/90% 1mM ammonium acetate in water. Analysis with HPLC-MS.
The method of extraction was calibrated up to 5 mg/kg slurry in cow slurry. At 1 mg/kg slurry (dry matter content 2.9%) the recoveries for different sulphonamides and trimethoprim were 47-89% (pH 9; RSD 1-4%); in water 48-84% (pH 9, RSD 1-3%). Results are listed in table Table 36. Low concentrations of trimethoprim might be due to either rapid degradation or to irreversible sorption.

Table 36 Sulphonamide and trimethoprim residues in slurry grab samples [mg/kg$_{ww}$]

Compound	Mother pigs with farrows			Fattening pigs		Veal
	A	B	C	D	E	F
Sulfamethazine	8.7	5.5	3.3	0.23	0.13	3.2
N4-acetyl-sulfamethazine	2.6	0.59	0.15	Nd	<0.1	<0.1
Sulfathiazole	12.4	<0.1	Nd	0.10	0.17	Nd
Trimethoprim	<0.1	Nd	Nd	Nd	Nd	Nd
Dry matter % w/w	3.3	3.4	1.8	3.7	3.2	1.1

nd = not detected.

Sulphonamides
Several sulphonamides (sulfanilic acid, sulfanilamide, sulfaguanidine, sulfadiazine, sulfadimidine, and sulfametizole) were present in a groundwater plume underneath a landfill site containing waste materials from pharmaceutical industry. Within a distance of 115 metres, concentrations of sulphonamides would drop from values of up to 10 mg /L to below the LOD (20 µg/L). This attenuation cannot be explained by dilution or sorption, thus indicating anaerobic degradation in the zone characterised as methanogenic/sulphate-reducing [239]. Sulfanilic acid was found in the highest concentrations and the greatest distances. Sulfanilic acid was shown to be recalcitrant towards anaerobic degradation [240].

I-5 Laboratory degradation experiments with tetracyclines, sulphonamides and tylosin.

Tetracycline
An experimental system was investigated for the prediction of the environmental stability of tetracycline and its metabolite, 4-epi-tetracycline in water and liquid pig manure [241]. The experiment consisted of incubation systems for solutions with installations for stirring and ventilation. Four experiments were performed, each with an incubation system. Experiments

were performed at ambient temperature and samples were exposed to natural light conditions.
Experiment 1: Three vessels (vacuum desiccators) were filled with 1000 mL of Ringer's solution and autoclaved. In two vessels 20 mL was replaced with 20 mL of the tetracycline stock solutions. The third vessel served as a control.
Experiment 2: The same experiment as experiment 1, but in addition, the vessels were ventilated with forced, sterilised air.
Experiment 3: The same experiment as experiment 1, but using native liquid manure instead of Ringer's solution.
Experiment 4: The same experiment as experiment 1, but using liquid manure and the vessels were ventilated with forced, sterilised air.
Samples with tetracyclines were diluted with citric buffer. Then, samples were centrifuged and filtered. An aliquot of the filtrate was again centrifuged through a microspin centrifuge filter. This filtrate was directly used for HPLC. Recovery of tetracycline from HPLC was 102% from samples derived from Ringer's solution and 94% and samples derived form liquid manure. Recovery of 4-epi-tetracycline was 93% from samples derived form Ringer's solution and 97% from samples derived from liquid manure.

Table 37. Decrease of tetracycline concentrations (sum of tetracycline and its 4-epi tetracycline) in Ringer's solutions and liquid manure during an incubation for 8 days (TC) (mean of two replicates ± SD) and percentage of 4-epi-tetracycline on total tetracycline concentrations (4ET).

nr. exp.		Day 0		Day 1		Day 6		Day 8	
		TC [%]	4ET [%]	TC [%]	4ET [%]	TC [%]	4ET [%]	TC [%]	4ET [%]
1	Ringer's solution not ventilated	100	2.4 ± 0.2	78.6 ± 5.8	1.6 ± 2.2	70.4 ± 1.7	3.0 ± 1.3	64.1 ± 5.4	2.4 ± 0.3
2	Ringer's solution ventilated	100	0.5 ± 0.6	91.1 ± 2.1	1.0 ± 0.2	88.4 ± 6.5	2.7 ± 1.0	82.1 ± 2.8	3.3 ± 1.6
3	Liquid manure not ventilated	100	9.0 ± 2.8	73.9 ± 11.6	13.9 ± 0.6	61.2 ± 3.1	20.5 ± 1.6	57.6 ± 9.5	21.6 ± 0.2
4	Liquid manure ventilated	100	11.0 ± 0.2	57.6 ± 5.1	15.7 ± 0.6	39.1 ± 4.5	24.2 ± 0.5	30.3 ± 1.4	26.5 ± 0.6

According to the author the decrease of tetracycline was significant in exp. 1, 3, and 4. Supposing the decrease continued on the following days as calculated from the regression lines, the DT50 was 15 and 30 days in unventilated and ventilated Ringer's solutions, respectively. The DT50 for tetracycline in unventilated liquid manure was 9 days and it was 4.5 days when the slurry was ventilated. The recovery from the extraction method was not determined. It was not mentioned which statistical analysis was performed.

Table 38 Summary of the transformation study with tetracycline.

Substance	Water type	T [°C]	pH	Duration [d]	Transformation at end [%]	DT50 Hydrolysis, 20 °C [d]
tetracycline	Ringer's solution	room	6.2-6.4	8	36	15
tetracycline	Ringer's solution, ventilated	room	6.2-6.4	8	18	36
tetracycline	liquid manure	room	7.6-8.3	8	42	11
tetracycline	liquid manure, ventilated	room	7.7-8.7	8	70	5

The DT50 values have been recalculated with non-linear regression of first order exponential kinetics. The recalculated DT50 values are given in Table 38. Except for the last experiment, the DT50 values were extrapolated, as the tetracycline had not decreased below 50% in these experiments. The fits were not very well as the r^2 varied between 0.7 and 0.8. Apparently most dissipation occurred between day 0 and 1 indicating strong sorption behaviour.
However, stability testing of tetracycline in pig slurry (6.4% dry matter) was studied in Germany by Winckler and Grafe (2001) [242]. Testing was performed using 300 L tanks which were spiked with 20 and 100 mg/L tetracycline and stored at different temperatures (8°C and four ambient) for seven weeks in the lab and 70 days in the field. Tetracycline and 4-epi-tetracycline were detected with HPLC (see [241]). Recovery in slurry was 98-116% (at 0.6 – 20 mg/L) with RSD 4-15%. Tetracycline demonstrated a stability in slurry, which was not influenced by the initial concentration of the antibiotic, temperature of aeration through repeated stirring. Half-lives ranged between 55 and 105 days (all extrapolated) are recalculated using a non-linear first order exponential fit: 20 mg/L 8°C DT50 54 days r^2 0.99, 100 mg/L 8°C DT50 52 days, r^2 0.91; 20 mg/L outdoors 131 days, r^2 0.73.

Oxytetracycline and Tylosin
In studies on tylosin and oxytetracycline (OTC) with manure, the substances could not be detected within a few days [243,244]. It was not clear if sorption or degradation (either aerobic or anaerobic) was the major process for tylosin. Chemical degradation at 20°C was not found. The concentration of manure particles influenced the dissipation rate. The tests were performed with low concentrations of particles (0.1 to 6.4% manure). In the presence of manure metabolites tylosin B and D were found. In the experiments with oxytetracycline it was assumed that the compound was adsorbed quickly, as a constant low level of free OTC was observed over a period of time.

Tylosin
The biodegradability of tylosin, metronidazole and olaquindox in aerobic soil-manure slurries was investigated by Ingerslev and Halling-Sorensen (2001) [150]. Two soils (one loam and one loamy sand) were used with 0, 1 or 10% pig slurry (v/w). The mixtures were slurried in a mineral medium at 50 g/L and incubated at . Soils had been stored at 6°C for up to 6 months, which is rather long and microbial biomass might have been affected [245]. However, half-lives for tylosin were 3.3-8.1 days only with no relation to the amount of slurry added. Tylosin B and D, demycinosyltylosin and several other compounds were observed during the degradation test, but disappeared shortly after the parent compound from the test solutions. Olaquindox and metronidazole degraded with DT50 values of 5.8-8.7 days and 9.7-26.9 days, respectively. Test conditions are however very favourable for degradation: aerobic, higher temperature (presumably 20°C) than outdoors, mobile bacteria, and low amount of solids, high substrate concentrations.

Sulphonamides
A group of sulphonamides was investigated by Ingerslev and Halling-Sorensen [246]. Purpose of the study was to investigate whether biodegradation of different sulfanilamides varies significantly. In first instance the sulphonamides were tested in a biodegradation study using a respirometric screening test. The tested chemicals used were sulfacetamide, sulfabenzamide, sulfamethoxypyridazine, carbutamide, sulfadiazine, sulfapyridine, sulfadimidine, sulfadimethoxine, sulfanilamide, sulfamerazine, sulfameter and sulfadoxine (all purity >99%). Analine was used as a reference component. All tests were performed with activated sludge from the primary aeration tank at a pilot scale activated sludge sewage

treatment plant receiving municipal wastewater. Preconditioning (aeration) began within 1 h of collection and took 20 to 24 h at room temperature.
Toxicity tests were performed according to Guidelines in ISO 15522. The toxicity of sulfanilamide, sulfadazine, and sulfacetamide were tested at five different concentration levels (1-400 mg/L) in duplicate test flasks. 3,5-Dichlorophenol was tested as a reference substance. More than 20% bacterial inhibition was found for sulfadiazine (10 µg/L), whereas for sulfacetamide and sulfanilamide (up to 400 µg/L), very little inhibition was measured. EC50 values could not be calculated. Biodegradation experiments were not affected by bacteria toxicity using the toxicity test.
Screening tests were performed according to the Guidelines in ISO 9408. Sulfanilamide, sulfadiazine, sulfameter, and sulfabenzamide were not degraded within the test period of 28 d.
Activated sludge system. Undiluted, preconditioned sludge was added to aerated reactors (height 50 cm, diameter 5 cm). Sludge concentrations ranged from 3.2 to 3.5 g SS/L. Test substances were added in concentrations ranging from 250 to 1,000 µg/L. All 12 substances were tested at 20 ± 2°C. Sulfanilamide, sulfacetamide and sulfadiazine were tested at 6 ± 1°C. Three mixtures were tested in three sets of duplicate reactors in a first test series:
- sulfacetamide, sulfabenzamide, sulfamethoxypyridazine, and carbutamide
- sulfadiazine, sulfapyridine, sulfadimidine, and sulfadimethoxine
- sulfanilamide, sulfamerazine, sulfameter, and sulfadoxine

When the added compounds were degraded, the test compounds were respiked twice.
Two mixtures were tested in a second series of tests:
- sulfadiazine, sulfadimidine, and sulfadimethoxine
- carbutamide, sulfadoxine, sulfameter, and sulfanilamide

Two separate sets of duplicate reactors were used. In the first set sulphonamide was adapted with mixture 1, and after degradation mixture 2 was added. In the second set, the opposite took place: the reactor was adapted with mixture 2 and respiked with mixture 1.
The lag phase during the adaptation period was 7-10 days for all substances. Elimination after the lag phase occurred in 5 to 10 d. Half-lives ranged from 0.2 to 4.1 d after the first respiking. After the second respiking similar degradation rates (0.2-0.7 d) were obtained.
In the experiment where respiking was performed with a different set of sulphonamides than the set initially used for adaptation the half-lives ranged between 0.4 and 1.7 days. In the reversed order the half-lives were 0.9 days.
When the experiment were performed at 6°C the lag period was 34 to 47 days and degradation was completed in 12 to 30 d.
The authors made some remarks. Only the primary degradation of the test compounds was followed, complete mineralization of the metabolites cannot be proved. Biodegradation rate data obtained are based on experiments performed at concentrations much higher than could be expected in the STP. This means that the degradation rates found in the test may be unrealistically high degradation rates. Degradation studies were performed at a higher temperature than the average temperatures in Denmark. The authors concluded that sulphonamides are not readily biodegradable as they were not degraded in the screening test. As the data fit the logistic growth model the lag period was believed to reflect the time needed for specific degrading micro-organisms to grow to an extent where degradation was detectable. Bacteria had acquired general properties needed for degradation of several other sulphonamides. The rate of biodegradation may be overestimated for STPs as tests were performed at a higher temperature and at higher concentrations as generally occur in practice [247].

Oxytetracycline and Tylosin
The primary aerobic and anaerobic biodegradability at concentrations of 50-5000 µg/L of the antibiotics olaquindox, metronidazole, tylosin (TYL) and oxytetracycline (OTC) was studies in shake flask systems with water, water-sediment (1g/L) and water-sludge (3 g/L) at 15°C [248]. The test were carried out at non-toxic concentrations. In water the median DT50 for TYL were 17 and 40 days. With sediment these results were ≥65 days (extrapolated value) and 9.5 days. With sludge <23 and 15.5 days. Lag phases until degradation occurred were quite long: >23 days (experiment ended) and 31-40 days. In water the median DT50 for OTC was 46 days, with sediment and sludge 44 and 42 days, respectively. The rate of degradation of aniline is higher than for TYL and OTC. Under anaerobic conditions OTC DT50 were 31-175 days (again, no lag-phase). Degradation of TYL was preceded with longer lag phases and lower degradation rates.

Oxytetracycline
The hydrolysis rate of OTC in water at pH 4.6 is predicted by Vej-Hansen et al. (1978) to be 0.0056 h^{-1} resulting in a DT50 of 124 hours (5.2 days) at 25°C [249].
From the data presented by Soulides et al. (1962) an average DT50 in soil of 4 days (25°C) can be derived [3].

Chlortetracyclin
In one study by Gavalchin and Katz (1994), a standard solution of chlortetracyclin was mixed with chicken faeces to obtain a final concentration of 5.6 mg/kg soil [235]. After 30 days 44% remained at 30°C, 88% at 20°C, and no degradation occurred at 4°C.

Sulfachloropyridazine and Tylosin
Preliminary degradation rate experiments with SCP and TYL in soil were performed by Schmitt [250,251].

SCP. Samples of ca 1-2 gram of wet soil (unspecified) were taken in Eppendorf cups (without previous mixing of the soil) and stored at -20° C for max. 3 months. 1 g of soil was weighted into 5 ml glass cups (with black screw lids). Extraction was performed with 1 mL of 10 mM Na_3PO4 buffer (adjusted to pH 6 with H_3PO_4), and 1.5 mL Acetonitrile, added simultaneously. Samples were shaken overnight at room temperature on a lab shaker, centrifuged (2500 rpm, 10 min), and filtered with a glass syringe and Millipore GVWP filters (0.22 µm) into HPLC glass vials. For the determination of the extraction efficiency, samples of unexposed soil (same soil type, stored at 25°C together with the soils until extraction) were exposed to watery solutions of sulfachloropyridazine together with acetonitrile and phosphate buffer and shaken and treated as above. As control samples, a soil (another soil type, "mengbodem") contaminated with 300 mg/kg sulfachloropyridazine and manure, stored at 4°C until extraction, and the same soil, stored at –20°C, were used. A Supelco discovery C18 column (15 cm * 4.6 mm, 5 µm) was used (column No 13265-011). The mobile phase consisted of 70% 10mM Na_2HPO_4, adjusted to pH3 with H_3PO_4, and 30% acetonitrile, at a flow rate of 0.7 mL/min. SCP was detected with a UV detector at 260 nm. Retention time varied between 5.77 (average 24.8) and 5.92 (average 22.8).
Calibration was performed using watery samples of sulfachloropyridazine, prepared as dilutions from a MeOH standard of Na-sulfachloropyridazine (gift of producers) prepared the 21.8, and using the peak area. Calibration curves were prepared each day, and the concentrations were matched with calibration curves around the measured concentrations.

Table 39 Extraction efficiency of SCP in freshly spiked soils

mg/L	area	soil conc (SCP)	HPLC dil	extr vol [ml]	% recovery	mean	deviation
0.07	4.4	0.093	1	2.65	195.4		
0.05	2.8	0.093	1	2.65	152.7	174.1	30.22
0.32	31.8	0.93	1	2.65	92.4		
0.32	31.6	0.93	1	2.65	91.8		
1.03	102.1	2.8	1	2.65	98.4		
1.08	106.9	2.8	1	2.65	102.6		
9.22	993.1	27.9	1	2.65	87.7		
10.25	1105.7	27.9	1	2.65	97.5		
40.0	3754	278.5	3	2.65	114.3		
32.23	3499.0	278.5	3	2.65	92.0	97.1	8.4

Soil concentrations below 0.3 mg/kg could not be analysed due to too high recovery values.

Table 40 Variance of extractions (300 mg/kg SCP, soil at field humidity)

mg/L	area	soil extracted [g]	HPLC dil	Humidity [%]	extr vol [ml]	Soil concentr. [mg/kg wet soil]	Mean	deviation
35.1	3295	1.0000	2	20	2.67	62.5		
38.8	3640	1.0092	2	20	2.67	68.5		
35.7	3348	0.992	2	20	2.67	64.1		
35.2	3304	1.0143	2	20	2.67	61.8		
36.3	3406	1.0124	2	20	2.67	63.9	64.2	2.6
62.10	6750.8	1.0022	2		2.69	111.1		
60.95	6626.2	0.9946	2		2.69	109.9	110.5	0.86

The variance between multiple extractions was relatively low. While degradation occurs already at 4°C, it was still possible to extract the complete amount of sulfachloropyridazine from the soil stored at –20°C. Results of analysis were adjusted for the actual humidity and normalised to 18% field humidity. 97.1% was taken as mean recovery. The t ½ values calculated from a linear regression using the first three time points (0, 4 and 10 days) are in Table 41. Later, the degradation seems to proceed at a slower speed.

Table 41 Half-life times for SCP

Soil concentration [mg/kg]	T ½ (25°C) [d]
1000	35.7
300	9.6
100	9.7
30	5.7
10	5.0
3	5.4
1	7.8
0.3	11.3

TYL. Samples of ca. 1-2 gram of wet soil were taken in Eppendorf cups (without previous mixing of the soil) and stored at -20° C for max. 5 months (8.6. until 30.10.01). The humidity of the soil was controlled by regular additions of water and weighing of the total soil sample, and verified by drying 1-2 g of the samples overnight. 1 g of soil was weighted into 5 mL glass cups (with black screw lids). Extraction was performed with 4 mL of a 1:1 mixture of Methanol and 10 mM Na_3PO_4 buffer (buffer adjusted to pH 5 with H_3PO_4). Samples were shaken 30 min at room temperature on a lab shaker, centrifuged (2500 rpm, 25 min), and

filtered with a glass syringe and Millipore GVWP filters (0.22 µm) into HPLC glass vials. For the determination of the extraction efficiency, samples of unexposed soil (same soil type, stored at 25°C together with the soils until extraction) were exposed to fresh solutions of tylosin in H_2O:MeOH 1:1 and buffer (adding up to a total amount of 4 mL) and shaken and treated as above. As control samples, a soil (another soil type, 'mengbodem') contaminated with 312 mg/kg tylosin and manure, stored at 4°C until extraction, and the same soil, stored at –20°C, was used. A Supelco discovery C18 column (15 cm * 4.6 mm, 5 µm) was used (column No 13265-011). The mobile phase consisted of 60% 0.18 M $NaClO_4$ in water (pH=2.5 with trifluoroacetic acid) : 40% acetonitrile, at a flow rate of 0.7 mL/min and room temperature. Tylosin was detected with a UV detector at 290 nm. Calibration was performed using water : MeOH 1:1 samples of tylosin, freshly prepared and diluted the 30.10, using the peak area. Calibration curves were prepared each day, and the concentrations were matched with calibration curves around the measured concentrations.

Table 42 Extraction efficiency of TYL in freshly spiked soils

mg/L	area	soil conc (TYL)	HPLC dil	extr vol [ml]	% recovery	mean
0.80	21.6	3	1	4	106.4	
0.79	21.5	3	1	4	105.6	
0.76	20.7	3	1	4	101.8	104.6
2.69	73.6	10	1	4	107.6	
2.68	73.4	10	1	4	107.3	107.5
7.69	223.8	30	1	4	102.6	
7.69	223.8	30	1	4	102.6	102.6
25.73	780.5	100	1	4	102.9	
25.36	769.2	100	1	4	101.4	102.2
74.64	2290.6	300	1	4	99.5	
						103.4

Table 43 Variance of extractions (300 mg/kg TYL, soil at field humidity)

mg/L	area	soil extracted [g]	HPLC dil	Humidity [%]	extr vol [ml]	Soil concentr. [mg/kg wet soil]	Mean	deviation
69.30	2016.69	1.0478	1	24	4	84.8		
61.70	1906.62	0.9931	1	24	4	79.6		
66.14	2044.79	1.0464	1	24	4	81.0	81.8	2.7

The variance between multiple extractions was relatively low. While degradation occurs already at 4°C (analysis of 30-10: 1% remained after 5 months), it was still possible to extract tylosin from the soil stored at –20°C. Results of analysis were adjusted for the actual humidity and normalised to 18% field humidity. 97.1% was taken as mean recovery. The t ½ values calculated from a linear regression using the first three time points (0, 4 and 10 days) are in Table 44.

Table 44 Half-life times for TYL

Soil concentration	T ½ (25°C)
1000	4.7
300	4.4
100	6.5
30	5.8
10	5.6

I-6 Sorption of tetracyclines, sulphonamides and tylosin

Laboratory studies were conducted to characterise four different antibiotic compounds: metronidazole, olaquindox, tylosin and oxytetracycline with regard to sorption and mobility in soil [156]. Tylosin was used in the form tylosin tartrate consisting of two tylosin molecules. Analytical standards were used for all four antibiotic compounds. Solutions of olaquindox decomposed rapidly when exposed to daylight, thus all experimental procedures with olaquindox were carried out in darkness and sampling operations were done in dimmed light. Samples for analysis of all substances investigated were kept in amber glass. Four Danish agricultural soils were used in the experiments, two sandy loam soils, a loamy sand soil and a sand soil. All of the soils are typical agricultural soils according to their physical and chemical characteristics. All four soils were used in a sorption experiment. The soils were air-dried, gently crushed, sieved through a 2 mm sieve, and stored at approximately 4°C. Soil samples for sorption experiment were sterilised by radiation (10kGy). LC Detection limits: 3 µg/L for metronidazole and olaquindox, 9 µg/L for oxytetracycline and 7 µg/L for tylosin.

Sorption experiments (Table 45). The sorption experiments were performed in accordance with the OECD Test Guideline 106 (OECD, 1997). In a preliminary test sorption kinetics of each of the antibiotics have been studied, 24 h was found to be a sufficient equilibration time. Five different concentrations of the antibiotic were made in 0.01 M $CaCl_2$. The concentrations were: 1.25, 2.5, 5.0, 12.5 and 25.0 mg/L. Triplicate samples with 5 g of soil (only 2.5 g for oxytetracycline), 20 mL of $CaCl_2$ solution and 5 mL of antibiotic solution were made for each soil at each concentration to reach a final volume of 25 mL with antibiotic concentrations of 0.25, 0.5, 1.0, 2.5 and 5.0 mg/L in the test tubes. A blank for each soil and a control for each concentration were made. Blanks contained 25 mL of $CaCl_2$ solution and 5 g of each soil, and the control contained 20 mL of $CaCl_2$ solution and 5 mL antibiotic solution in a test tube without soil. Samples were analysed by LC with MS or fluorescence detection. The Kd values reported for tylosin in both sandy loam soils (not shown here) most likely contained a typing error and are a factor of 10 too high. The Kom values corresponding to the Kd are 447 and 321 L/kg for sand and loamy sand, respectively.

Table 45 Results from the sorption experiments.

Substance	Soil type	o.c.	pH	Clay	Ratio soil/water	Kd	KF	[1/n]	Koc	Kclay
		[%]	[$CaCl_2$]	[%]	[g/mL]	[L/kg]	[L/kg]		[L/kg]	[L/kg]
metronidazole	sandy loam 1	1.6	6.1	11.3	0.2		0.80	0.70	50	
metronidazole	sand	1.4	5.6	5.2	0.2		0.74	0.71	53	
metronidazole	sandy loam 2	1.1	5.6	16.9	0.2		0.79	0.75	72	
metronidazole	loamy sand	1.5	6.3	5.8	0.2		0.75	0.71	50	
olaquindox	sandy loam 1	1.6	6.1	11.3	0.2		1.20	0.73	75	
olaquindox	sand	1.4	5.6	5.2	0.2		1.06	0.79	76	
olaquindox	sandy loam 2	1.1	5.6	16.9	0.2		1.05	0.67	95	
olaquindox	loamy sand	1.5	6.3	5.8	0.2		0.84	0.80	56	
oxytetracycline	sandy loam 1	1.6	6.1	11.3	0.1	680			42500	6000
oxytetracycline	sand	1.4	5.6	5.2	0.1	670			47900	13000
oxytetracycline	sandy loam 2	1.1	5.6	16.9	0.1	1026			93300	6100
oxytetracycline	loamy sand	1.5	6.3	5.8	0.1	417			27800	7200
tylosin	sandy loam 1	1.6	6.1	11.3	0.2	*	7.0	0.88	438	
tylosin	sand	1.4	5.6	5.2	0.2	10.8	2.6	0.85	186	
tylosin	sandy loam 2	1.1	5.6	16.9	0.2	*	5.7	0.94	518	
tylosin	loamy sand	1.5	6.3	5.8	0.2	8.3	2.3	0.83	153	

* The Kd values reported for tylosin in both sandy loam soils (not shown here) most likely contained a typing error and are a factor of 10 too high. The Kom values corresponding to the Kd are 447 and 321 L/kg for sand and loamy sand, respectively.

Soil column leaching. Sandy loam and sand soils were used in the soil column leaching experiments. The experiments were conducted by the method described by the US EPA (1985). Three columns were used for each combination of antibiotic and soil. 1 mL antibiotic solution (500 µg/mL) and 1 mL KBr solution (4 mg/L) were applied to the centre of the soil column surface with 50 g of acid washed sand and a sintered glass filter on top. 1060 mL 0.01 M $CaCl_2$, equivalent to approximately 500 mm 'rain' was then lead through the column with a flow rate matching the maximum hydraulic capacity of the soil. The eluate was collected in fractions of approximately 25 mL and analysed for contents of the antibiotic and the bromide tracer, respectively. The soil columns were cut in twelve 2.5 cm fractions for extraction and analysis. The soil fractions were extracted three times with 100% methanol. Determination of antibiotic substances in the samples were performed using LC-MS. Standard solutions of each substance in three concentrations, 50, 500 and 5000 µg/L, were prepared in 0.01 M $CaCl_2$. For the analysis of soil extracts the standard solutions were diluted with methanol to the concentrations 25, 250 and 2500 µg/L.
Close to 100% of metronidazole and olaquindox was recovered from the leachate fractions. For both substances a slight delay of leaching was observed in the sandy loam compared to the sand. Oxytetracycline and tylosin were not detected in the leachate of the columns of both soil types. Average recovery (three samples) of tylosin from the sandy loam soil was 69% of the added tylosin and 68% for the sand soil. Oxytetracycline could not be extracted with methanol.
The authors state that storage of the columns at -20°C led to a significant decrease in the extraction efficiency. This was the result of an additional study (results not reported in this article) that was performed to determine the extraction efficiency. The reported physical and chemical properties of the loamy sand soil were for the soil layer of 5-15 cm and only top soil should have been used. The waterflux was much too high (>10 cm/day). The reported $K_{s/l}$ values could not be recalculated and are considered unreliable.

Tylosin
The sorption of tylosin, olaquindox and metronidazole to soil/slurry mixtures were investigated by Ingerslev and Halling-Sorensen (2001) [150]. Here, 5 g of a 10% v/w manure-soil mixture and 100 ml of minerals medium were used. Soil were sterilised with sodium azide. Six concentrations were used in triplicate. Water concentrations only were measured. Manure contained 422 g C/L; soil 1.4-1.6% o.c. w/w. The total organic carbon content is calculated as: 0.1*42.2% + 0.9*1.4 or 1.6% resulting in 5.5 and 5.7% for the loamy sand and loam soil, respectively. Metronidazole and olaquindox remained unsorbed in the experiments and proper sorption coefficients were not determined. Results for tylosin are given in Table 46. The 1/n value is rather low indicating a concentration dependent sorption behaviour. Only the result for the loam soil is comparable to other results [156]. The result for the loamy sand is also not accurate because only a 10% concentration decline in the liquid phase was reached [252].

Table 46 Freundlich parameters for tylosin for a soil-manure slurry.

Substance	10% pig manure and soil	o.c. [%]	Ratio soil/water [g/mL]	KF [L/kg]	[1/n]	Koc [L/kg]
tylosin	loamy sand	5.5	0.05	2.03±0.9	0.67±0.07	37
tylosin	loam	5.7	0.05	32.1±13.2	0.67±0.06	**563**

Annex II Model descriptions and input

II-1. PEARL

FOCUS_PEARL 1.1.1.
PEARL is dynamic, deterministic model starting with a dosage on the soil surface. A hydrological model (SWAP) describing water transport is combined with mathematical description of chemical behaviour is soil. Agricultural practice (tillage, irrigation, crops, daily information on meteorological conditions and soil properties variable with depth can be specified by the user or chosen from 9 standard FOCUS scenarios covering the counties of the EU. Adsorption behaviour can be entered in a flexible way: Kom, Kd, pH-dependent sorption and variation of sorption with depth can be defined. Standard output parameters are average annual concentration in the groundwater, but many hydrological and chemical output parameters can be obtained.

Endpoint:
FOCUS target concentration, PEC_{soil} at different depths, $PEC_{groundwater}$, many user defined output parameters.

Availability:
FOCUS website, free-ware, [75]

Compartments:
soil and groundwater

Input/processes:
Water flow in soil is described by Richard's equation including a range of possible lower boundary conditions (for instance groundwater levels that fluctuate in response to the rainfall input). Soil evaporation and plant transpiration is calculated via multiplying a reference evapotranspiration rate with soil and crop factors. For the FOCUS scenarios, crop growth is simulated with a simple growth model that assumes a fixed length of the growing season. In this growth model, both the leaf area index and the rooting depth are a function of the development stage of the crop. Heat flow in soil is described with Fourier's law. The thermal properties are a function of porosity and water content and are therefore a function of time and soil depth. PEARL is based on: (i) the convection/dispersion equation including diffusion in the gas phase with a temperature dependent Henry coefficient, (ii) a two-site Freundlich sorption model (one equilibrium site and one kinetic site), (iii) a transformation rate that depends on water content, temperature and depth in soil, (iv) a passive plant uptake rate. The model includes formation and behaviour of transformation products and describes also lateral pesticide discharge to drains (but drainage is switched off for the FOCUS scenarios). PEARL does not simulate preferential flow. Volatilisation from the soil surface is calculated assuming a laminar air layer at the soil surface. PEARL uses an explicit finite difference scheme that excludes numerical dispersion (the dispersion length was set to 5 cm).

Validation status:
The hydrological module SWAP has been validated extensively for water fluxes and nitrogen fluxes from manure in the Netherlands. Validation of the PEARL model is described

by [253-256] for several atrazine, bentazon, cadmium and zinc in the Netherlands. Validation on a European scale is ongoing in the APECOP project [257,258]

Advantages:
It is a state of the art leaching model, which is can use very specific input and produce very specific output. Daily output can be presented in graphs and spreadsheets.

II-2. PRZM

FOCUSPRZM
PRZM (Pesticide Root Zone Model) is a one-dimensional non-deterministic compartmental model for the prediction of chemical movement in unsaturated soils by vertical chromatographic leaching. The original PRZM version developed by Carsel, Smith, Mulkey, Dean and Jowise (1994) has been continuously improved. The actual PRZM3 is a standard model to be used in environmental risk and exposure assessments by the United States Environmental Protection Agency (U.S. EPA) and is included in the FIFRA list of recommended regulatory models for USA Pesticide Registration.

Endpoint: FOCUS target concentration, PEC gw at 1m, PEC_{gw} at core depth, m, mass balance at core depth, mass balance at 1m.

Availability:
FOCUS website, free-ware, [75]

Compartments:
soil and groundwater

Input/processes:
Summary of the processes in PRZM 3.20 (FOCUS release): (* process not used in FOCUS scenarios)

Process	Approach
water movement	capacity-based water flow (tipping bucket approach) using a daily time step for all hydrological processes, option for Richard's equation below the root zone. *Preferential flow, capillary rise and drainage are not considered
substance movement	convection dispersion equation based on a daily time step solved by an simplifying backward difference method which can produce artificially high numerical dispersion
crop simulation	changing root zone during growing season, changing foliage (both height and areal extent) during growing season, crop interception of water*, crop interception of substances*, foliar washoff*, foliar degradation*
degradation in soil	first order degradation rate with option for bi-phasic degradation, option for effects of soil temperature and moisture on degradation
substance sorption to soil	Kd, Koc, or normalised Freundlich equation for sorption; option for increasing sorption with time
substance volatilisation (from soil)	approach is a combination of results from previous research
runoff	Soil Conservation Service curve number technique
soil erosion*	Universal Soil Loss Equation
soil temperature	Approach is based on previous work by a number of researchers including Van Bavel and Hillel, Thibodeaux, Hanks, Gupta, and Wagenet and Hutson
plant uptake	simple model based on soil concentrations
substance applications	applications may be foliar sprays*, applied to the soil surface, or incorporated into the soil; for soil incorporated applications a variety of soil distributions can be specified
metabolism	up to two metabolites may be simulated simultaneously with the parent

Validation status;
The functional validation of PRZM has been described by several studies [259-261].

Advantages:
PRZM has advantages in a computer environment with limited hardware recourses.
Disadvantages:
FOCUS_PRZM is not capable of generating user-defined output parameters.

II-3. PELMO

PELMO is a one dimensional simulation model simulating the vertical movement of pesticides in soil by chromatographic leaching. The first version of PELMO was released in 1991 [67]. PELMO is based on the US-EPA's PRZM 1 model [69], but was improved with regard to the requirements of the German authorities responsible for the registration of pesticides. The actual version used for the FOCUS simulations is PELMO 3.2.

Endpoint:
FOCUS target concentration (PEC $_{gw}$) at 1 m depth and groundwater concentration at the bottom of the soil profile.

Availability:
FOCUS website, free-ware, [75]

Compartments:
Leachate and groundwater

Input/processes:
Summary of the processes in PELMO. [* = turned off for the FOCUS scenarios]

Process	Approach
water movement	capacity-based water flow (tipping bucket approach) using a daily time step for all hydrological processes, preferential flow and capillary rise are not considered
pesticide movement	convection dispersion equation based on a daily time step
crop simulation	changing root zone during growing season, changing foliage (areal extent) during growing season, crop interception of water*, crop interception of pesticides*, foliar washoff*, foliar degradation*
degradation in soil	first order degradation rate, correction of rate constant with depth, soil moisture and soil temperatures
pesticide sorption to soil	Kd, Koc, Freundlich equation for sorption option for increase of sorption with time option for automated pH-dependence*
pesticide volatilisation (from soil)	simple model using Fick's and Henry's law
runoff	Soil Conservation Service curve number technique
soil erosion*	Modified Universal Soil Loss Equation
soil temperature	an empirical model that uses air temperatures
plant uptake	simple model based on soil concentrations
pesticide applications	applications may be foliar sprays, applied to the soil surface, or incorporated into the soil; for soil incorporated applications a variety of soil distributions can be specified
metabolism	a sophisticated scheme with up to 8 metabolites (A -> B as well as A -> B <> C) may be simulated simultaneously with the parent

Validation status;
The functional validation of PELMO has been described in several studies [108,262].

Advantages:
Similar to PRZM, PELMO has advantages in a computer environment with limited hardware recourses. On standard PCs (e.g. Pentium III, 400 MHz, 64 MB) the typical computing time for a standard Tier 1 run with 9 scenarios is about 5 to 10 minutes. FOCUSPELMO allows efficient and user friendly calculations for compounds up to eight metabolites.
Disadvantages:
FOCUS_PELMO is not capable of generating user-defined output parameters.

II-4. Characteristics of FOCUS standard scenarios

Table 47 Major soil and meteorological characteristics of FOCUS standard scenarios.

Location	Annual temp. (°C)	Annual rainfall (mm)	Topsoil†	Organic matter (%)	Clay (%)
Châteaudun	11.3	648	silty clay loam	2.4	13
Hamburg	9	786	sandy loam	2.6	7.2
Jokioinen	4.1	638	loamy sand	7	3.6
Kremsmünster	8.6	900	loam/silt loam	3.6	14
Okehampton	10.2	1038	loam	3.8	18
Piacenza	13.2	857	loam	1.7	15
Porto	14.8	1150	loam	6.6	10
Sevilla	17.9	493	silt loam	1.6	14
Thiva	16.2	500	loam	1.3	25

Source:[73]

Figure 16 Map of FOCUS area

II-5. Substance properties

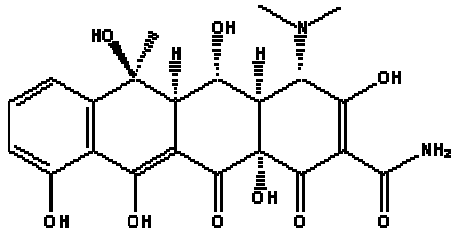


Figure 17 Structural formula of oxytetracycline

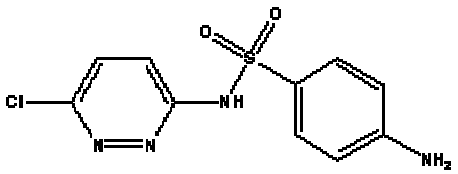


Figure 18 Structural formula of sulphachloropyridazine

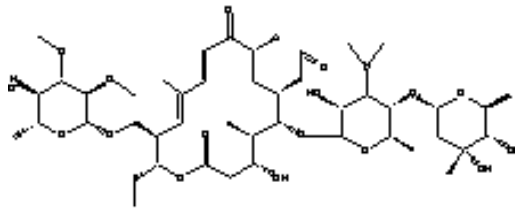


Figure 19 Structural formula of tylosin

Table 48 Physico chemical properties of the substances

Substance	Oxytetracycline			Sulphachloropyridazine	Tylosin		
Chemical name	4-(dimethylamino)-1, 4, 4a, 5, 5a, 6, 11, 12a-octahydro-3, 5,6, 10, 12, 12a-hexahydroxy-6-methyl-1, 11-dioxo-2-naphtacenecarboxamide						(2R,3R)-2,3-dihydroxybutanedioate
Common names	Oxytetracycline dihydrate	Oxytetracyclinehydrochloride	Oxytetracycline [263] calcium complex		Tylosin tartrate Tylosin A (90%), B, C en D	Tylosin phosphate	Tylosin tartrate
Trade name	Liquamycin, Terramycin		Tree Tech OTC		Tailexin	Tailexin	Tylan, Tylan Soluble
Application		Injection, orally			In drinking doses	Feed additive	
Emperical formula	$C_{22}H_{24}N_2O_9$	$C_{22}H_{25}ClN_2O_9$			$(C_{46}H_{77}NO_{17})_2 \cdot C_4H_6O2$	$(C_{46}H_{77}NO_{17}) \cdot PO_4$	$(C_{46}H_{77}NO_{17}) \cdot C_4H_6O_2$
Cas number	79-57-2	2058-46-0	79-57-2	80-32-0			1405-54-5, 74610-55-2
Molecular weight	498.5 [189], 460.4[1]	496.9		284.72	1982.3[1], 1998.31 [264]	1011.1[1] 1014.1 [264]	1066.2[265], 1002.2[1]
Solubility in water	1000 mg/L		100%	5000 mg/L at 25°C			5 g/L
Vapour pressure			0.13 mm Hg at 20 °C				
LogKow	-1.12 [266]					1.63-2.5 [266]	
pKa	3.3-3.5, 7.6, 9.2-9.5 [266]					7.7 7.1	

[1] Molecular weight calculator

Table 49 Overview adsorption experiments with tylosin

Substance	Matrix	Origin	pH	%OM	Clay	CEC	Soil:water ratio (g:ml)	Eq.time (hours)	Conc (mg/L)	Analysis	Kd	Kom	K_F	1/n	Ref.	Comment
Tylosin tartrate	Sandy loam	Askow, DK	6.1	2.8	11.3	100	5:20	24	0.25, 0.5,1.0, 2.5, 5	LC/MS	128		7	0.88	[267]	Probably an error occurred in the derivation of Kd in sandy loam, see inconsistency with the K_F. Irradiated soil OECD106
Tylosin tartrate	Sandy loam	Lundgaard, DK	5.6	2.4	5.2	67	5:20	24		LC/MS	10.8		2.6	0.85		
Tylosin tartrate	Sand	Flakkebjerg, DK	5.6	1.9	16.9	131	5:20	24		LC/MS	62.3		5.7	0.94		
Tylosin tartrate	Loamy sand	Borris, DK	6.3	2.6	5.8	353	5:20	24		LC/MS	8.3		2.3	0.83		
Tylosin tartrate 90% tylosin A	Fresh pig manure	Denmark, tank kept for 7 days, 10% d.m.	7.8	72	-	-	0.0001-0.002 kg/L 21 different manure concentrations	24	200	HPLC	240	331	-	-	[266]	
Tylosin tartrate 90% tylosin A	Sandy loam/pig manure (10%)	Denmark, Lundgaard	6.3	2.4	5.2	67	5:100	1,24, 48	0.5-25 (6 conc)	HPLC	n.r.	22	2.03	0.67	[268]	
Tylosin tartrate 90% tylosin A	Sandy Loam/pig manure (10%)	Denmark, Askov	6.8	2.7	11.3	100	5:100					327	32.1	0.67	[268]	
Tylosin	clay loam	UK, top	6.98	3.8	25.1							2247	85.4	1.13	[192]	Unpublised
Tylosin	clay loam	UK, sub	7.01	1.2	38.2								993	0.48		Unpublised
Tylosin	loamy sand	UK, top	6.82	2.2	10.4							310	6.83	0.68		Unpublised
Tylosin	loamy sand	UK, sub	6.19	0.35-0.86	9.9							3337-8200	28.7	0.74		Unpublised

Table 50 Overview of adsorption studies with sulphachloropyridazine

Substance	Matrix	Origin	pH	%OM	Clay	CEC	Soil:water ratio (g:ml)	Eq.time (hours)	Conc (mg/L)	Analysis	Kd	Kom	K_F	1/n	Ref.	Comment
Sulphachloropyridazine	clay loam	UK, top	6.6	3.8	25.1							54	2.04	0.97	[192]	Unpublised
Sulphachloropyridazine	clay loam	UK, sub	7.1	1.2	38.2							19	0.23	0.9		Unpublised
Sulphachloropyridazine	loamy sand	UK, top	6.9	2.2	10.4							48	1.06	0.91		Unpublised
Sulphachloropyridazine	loamy sand	UK, sub	7.5	0.35-0.86	9.9							63-154	0.54	0.85		Unpublised

Table 51 Overview of adsorption studies with oxytetracycline

Substance	Matrix	Origin	pH	%OM	Clay	CEC	Soil:water ratio (g:ml)	Eq.time (hours)	Conc (mg/L)	Analysis	Kd	Kom	K_F	1/n	Ref.	Comment
Oxytetracycline	Sandy loam	Askow, DK	6.1	2.8	11.3	100	5:20	24	0.25, 0.5,1.0, 2.5, 5	LC/MS	680	24650			[267]	Only two of five concentrations showed concentration >LOD, calculation of K_f not possible Irradiated soil OECD106
Oxytetracycline	Sandy loam	Lundgaard, DK	5.6	2.4	5.2	67	5:20	24		LC/MS	670	27757				
Oxytetracycline	Sand	Flakkebjerg, DK	5.6	1.9	16.9	131	5:20	24		LC/MS	1026	54098				
Oxytetracycline	Loamy sand	Borris, DK	6.3	2.6	5.8	353	5:20	24		LC/MS	417	16124				
Oxytetracycline hydrochloride	Fresh pig manure	Denmark, tank kept for 7 days, 10% d.m.	7.8	72	-	-	0.0001-0.002 kg/L 21 different manure concentrations	24	200	HPLC	78	107	-	-	[266]	
Oxytetracycline	Clay loam	UK top	7.4	3.8	25.1							47736	1814,	0.65	[192]	
Oxytetracycline	Clay loam	UK, sub	7.26	1.2	38.2							73916	887	0.8		
Oxytetracycline	Sandy loam	UK, top	7.47	2.2	10.4							30227	655	0.68		
Oxytetracycline	Sandy loam	UK, sub	6.87	0.35-0.86	9.9							70939-174286	610	0.79		

Table 52 Overview of degradation studies with tylosin

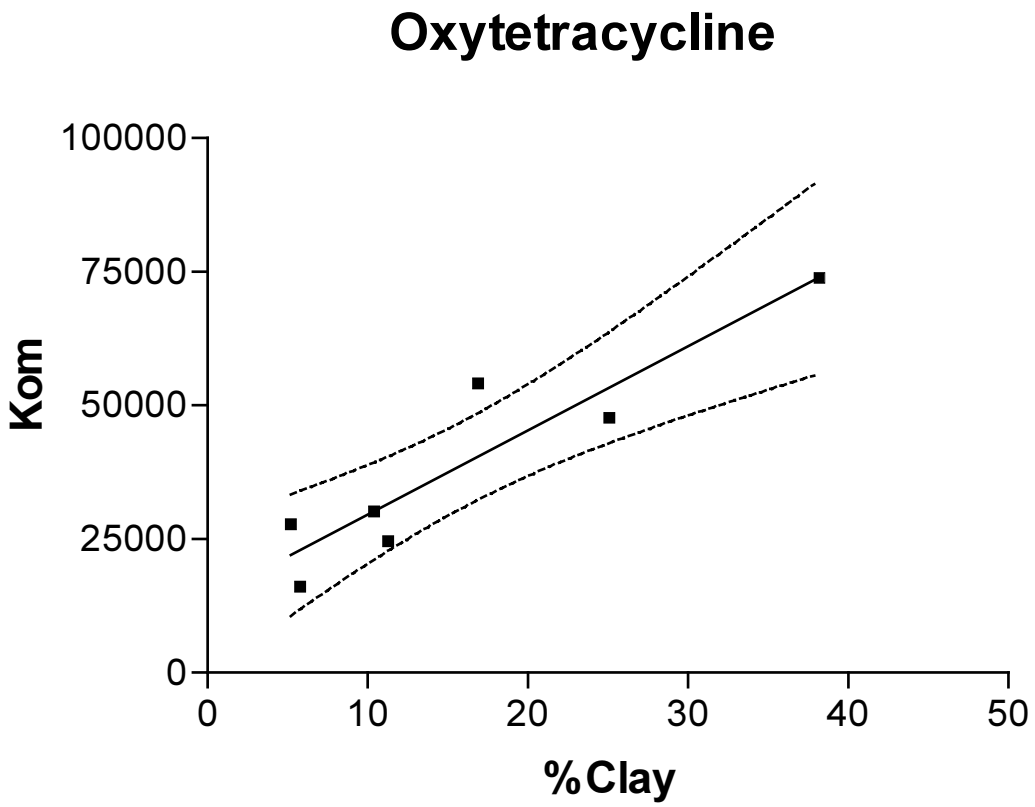
Substance	Matrix	Origin	PH	OM	%Clay	CEC meq/kg	Conc.	Incubation	Temp.	DT50 (days)	Analysis	Ref.	Comment
Tylosin tartrate	Soil									62			No details
Tylosin D	Soil									37			No details
Tylosin tartrate 90% tylosin A	Surface water	Fønstrup Bæk, Denmark (unpolluted small forest stream)					50-1000 µg/L	Aerobic	15	9.5-40	HPLC	[269]	ISO 14592 Test period too short
Tylosin tartrate 90% tylosin A	Surface water	Tude å, Denmark, (agricultural stream)					50-1000 µg/L	Aerobic	15	9.5-40	HPLC	[269]	ISO 14592 No concentration dependence Lag time
Tylosin tartrate 90% tylosin A	Soil slurry amended with 0, 1 or 10% pig manure	Denmark, Lundgaard	6.3	2.4	5.2	67	5000 µg/L	Aerobic	20	5.7 6.7-5.0 8.1-3.3	HPLC	[268]	ISO 11734 50 g soil in 1 L mineral medium,pH unknown
Tylosin tartrate 90% tylosin A	Soil slurry amended with 0, 1 or 10% pig manure	Denmark, Askov	6.8	2.7	11.3	100	5000 µg/L	Aerobic	20	4.1-4.2 4.1 N.D.	HPLC	[268]	
Tylosin	Field soil	Denmark, Lundgaard	5.6 $CaCl_2$	2.4	5.2	67	30-100 µg/kg	Field, May-October 2002		45, 52, 54	LC-ESI-MS/MS	[190]	Spiked manure of treated animals, no meteo, no application times
Tylosin	Field soil	Denmark, Askov	6.1 $CaCl_2$	2.7	11.3	100	30-100 µg/kg	Field, May-October 2002		58 ,76	LC-ESI-MS/MS	[190]	

Table 53 Overview of degradation studies with oxytetracycline

Substance	Matrix	Origin	PH	OM	%Clay	CEC meq/kg	Conc.	Incubation	Temp.	DT50 (days)	Analysis	Ref.	Comment
Oxytetracycline	Surface water	Fønstrup Bæk, Denmark (unpolluted small forest stream)			-	-	100-5000 µg/L	Anaerobic	15	31-175	HPLC	[269]	ISO 14592 No concentration dependence NolLag time
Oxytetracycline	Surface water	Tude å, Denmark, (agricultural stream)			-	-	50-1000 µg/L	Anaerobic	15	31-175	HPLC	[269]	ISO 14592 No lag time

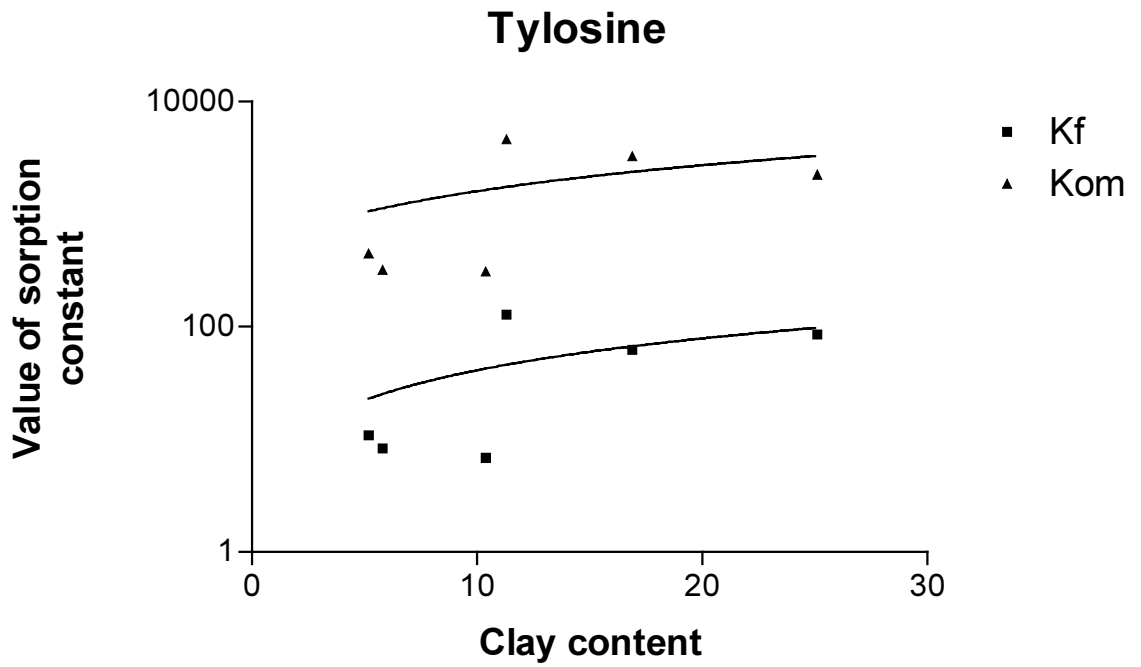
Annex II

Figure 20 Relation between Kom of oxytetracycline and clay content in the soil



	Data Set-A
Variables	
Slope	1575 ± 285.4
Y-intercept	13820 ± 5576
X-intercept	-8.773
1/slope	0.0006350
95% Confidence Intervals	
Slope	841.0 to 2309
Y-intercept	-519.3 to 28150
Goodness of Fit	
r^2	0.8589
Sy.x	8323
Is slope significantly non-zero?	
F	30.45
DFn, DFd	1.000, 5.000
P value	0.0027
Deviation from zero?	Significant
Data	
Number of X values	7
Maximum number of Y replicates	1
Total number of values	7
Number of missing values	0

Figure 21 Relation between sorption constants of tylosin and clay content in the soil



	Kf	Kom
Variables		
Slope	3.767 ± 2.764	112.3 ± 107.8
Y-intercept	3.370 ± 39.29	476.3 ± 1532
X-intercept	-0.8947	-4.240
1/slope	0.2655	0.008901
95% Confidence Intervals		
Slope	-3.906 to 11.44	-186.9 to 411.6
Y-intercept	-105.7 to 112.4	-3777 to 4730
Goodness of Fit		
r^2	0.3171	0.2135
Sy.x	46.43	1811
Is slope significantly non-zero?		
F	1.857	1.086
DFn, DFd	1.000, 4.000	1.000, 4.000
P value	0.2446	0.3562
Deviation from zero?	Not Significant	Not Significant
Data		
Number of X values	6	6
Maximum number of Y replicates	1	1
Total number of values	6	6
Number of missing values	0	0

II-6 PEARL input files for SCP in lysimeter

```
* INPUT FILE for Pearl version 1.1
* Generated by Pearl 1.2 sr-13 (November 2000) on 05/04/2003 14:41:16
*-------------------------------------------------------------------------------
* This file is intended to be used by expert users.
* Contact addresses:
* Aaldrik Tiktak                    Jos Boesten
* RIVM                              Alterra
* PO BOX 1                          PO BOX 47
* 3720 BA Bilthoven                 6700 AA Wageningen
* The Netherlands                   The Netherlands
* e-mail: a.tiktak@rivm.nl          j.j.t.i.boesten@alterra.wag-ur.nl
* (c) RIVM/Alterra
*-------------------------------------------------------------------------------
* Run characterization:
* RunID = 140
* ProjectID = 10
* Name = SCP initial hydro prerain step 1C-3 FOCUS substance input
* ChangeDetection = 0.426974042085931
* DisableResults = True
* IsRestrictedRun = False
* SelectedForExecution = Yes
* LocationCode = LYS_UK5
* CultivationSequenceCode = No
* SubstanceCode = SCP-L
* ApplicationSchemeCode = SCP_LYS
* IrrigationSchemeCode = LOCK1
* DepositionSchemeCode = No
* TillageSchemeCode = No
* TimStart = 22/09/2000
* TimEnd = 19/11/2000
* FocusResultsThere = False
* ResultsDetailed = NotThere
* ResultsSummary = NotThere
* DetailedOutput = True
* PrintCumulatives = True
* OptReportCode = No
* AmaSysEnd = 0
* OptDelTimEvt = NoRepeat
* RepeatHydrology = False
* DelTimPrn = 1
* DelTimSwaMin = 1E-7
* DelTimSwaMax = 0.2
* ThetaTol = 0.001
* OptDateFormatCode = DaysFromSta
* OptHyd = OnLine
* ZGrwLevSta = 1
* ZFoc = 1
* RealFormat = G12.4
* CreationDate = 03/04/2003
* ModificationDate = 04/04/2003
*-------------------------------------------------------------------------------
* Section 1: Control section
*-------------------------------------------------------------------------------
1  FocusGUIVersion
1  FocusDataBaseVersion
Yes               ScreenOutput
22-Sep-2000       TimStart
19-Nov-2000       TimEnd
0                 AmaSysEnd (kg.ha-1)
0.001             ThetaTol (m3.m-3)
1                 DelTimPrn  (d)
No                RepeatHydrology
OnLine            OptHyd
1E-7              DelTimSwaMin (d)
0.2               DelTimSwaMax (d)
No                OptDelOutput
Yes               PrintCumulatives
*-------------------------------------------------------------------------------
* Section 2: Soil section
*-------------------------------------------------------------------------------
UK_Sand5_Soil SoilTypeID
LYS_UK5 Location
```

```
table SoilProfile
ThiHor NumLay
(m)
0.3      12
0.2      8
end_table
table  horizon SoilProperties
Nr      FraSand    FraSilt    FraClay    CntOm         pH
        (kg.kg-1)  (kg.kg-1)  (kg.kg-1)  (kg.kg-1)     (-)
1       0.6917     0.2049     0.1034     0.0224        -99
2       0.7237     0.1769     0.0994     0.002         -99
end_table
table  horizon VanGenuchtenPar
Nr      ThetaSat    ThetaRes    Alpha      n       KSat      l
        (m3.m-3)    (m3.m-3)    (cm-1)     (-)     (m.d-1)   (-)
1       0.35        0.01        0.0152     1.412   0.1781    -0.213
2       0.25        0.02        0.0214     2.075   0.1556    0.039
end_table
Input              OptRho
table horizon      Rho             (kg.m-3)
1    1500
2    1500
end_table
0                  ZPndMax         (m)
1                  FacEvpSol       (-)
0.79               CofRedEvp       (cm1/2)
Table horizon LenDisLiq (m)
1  0.05
2  0.05
end_table
MillingtonQuirk    OptCofDifRel
2                  ExpDifLiqMilNom (-)
0.6667             ExpDifLiqMilDen (-)
2                  ExpDifGasMilNom (-)
0.6667             ExpDifGasMilDen (-)
*-------------------------------------------------------------------------------
* Section 3: Weather and irrigation section
*-------------------------------------------------------------------------------
LYS-UKP            MeteoStation
Input              OptEvp
52                 Lat
0                  Alt             (m)
10                 TemLboSta       (C)
Surface            OptIrr
LOCK1              IrrigationData
LOCK1 IrrigationScheme
*-------------------------------------------------------------------------------
* Section 4a: Lower boundary flux
*-------------------------------------------------------------------------------
1                  ZGrwLevSta (m)
Lysimeter          OptLbo
table   GrwLev (m)
01-Jan  1
31-Dec  1
end_table
-0.25              FlvLiqLboAvg (m.a-1)
0.1                FlvLiqLboAmp (m)
01-Oct             DayFlvLiqLboMax
Elliptic           OptShapeGrwLev
-1.1               HeaDraBase (m)
500                RstAqt (d)
-1.4               HeaAqfAvg (m)
0.2                HeaAqfAmp (m)
01-Apr             TimHeaAqfMax (d)
-0.0112            CofFncGrwLev (m.d-1)
-2.5               ExpFncGrwLev (m-1)
table   h (m)
01-Jan  -1
31-Dec  -1
end_table
*-------------------------------------------------------------------------------
* Section 4b: Drainage/infiltration section
*-------------------------------------------------------------------------------
0                  NumDraLev
*-------------------------------------------------------------------------------
* Section 5: Compound section
*-------------------------------------------------------------------------------
```

```
SCP-L SubstanceName
table compounds
SCP-L
end_table
284.7            MolMas_SCP-L (g.mol-1)
table FraPrtDau (mol.mol-1)
end_table
OptimumConditions OptCntLiqTraRef_SCP-L
3.3              DT50Ref_SCP-L (d)
20               TemRefTra_SCP-L (C)
0.7              ExpLiqTra_SCP-L (-)
1                CntLiqTraRef_SCP-L (kg.kg-1)
54               MolEntTra_SCP-L (kJ.mol-1)
table horizon FacZTra (-)
1      1
2      0.75
end_table
table horizon FacZSor (-)
1      1
2      0.51
end_table
pH-independent   OptCofFre_SCP-L
48               KomEql_SCP-L (L.kg-1)
1                ConLiqRef_SCP-L (mg.L-1)
1                ExpFre_SCP-L (-)
1E-5             PreVapRef_SCP-L (Pa)
20               TemRefVap_SCP-L (C)
5000             SlbWatRef_SCP-L (mg.L-1)
20               TemRefSlb_SCP-L (C)
27               MolEntSlb_SCP-L (kJ.mol-1)
95               MolEntVap_SCP-L (kJ.mol-1)
0                CofDesRat_SCP-L (d-1)
0                FacSorNeqEql_SCP-L (-)
0.5              FacUpt_SCP-L (-)
0.01             ThiAirBouLay (m)
Lumped           OptDspCrp
1000000          DT50DspCrp (d)
1000000          DT50PenCrp (d)
1000000          DT50VolCrp (d)
1000000          DT50TraCrp (d)
0.0001           FacWasCrp (m-1)
20               TemRefDif_SCP-L (C)
4.3E-5           CofDifWatRef_SCP-L (m2.d-1)
0.43             CofDifAirRef_SCP-L (m2.d-1)
*-------------------------------------------------------------------------------
* Section 6: Management section
*-------------------------------------------------------------------------------
SCP_LYS ApplicationScheme
1                ZFoc (m)
NoRepeat         DelTimEvt (a)
table Applications
21-Oct-2000  AppSolSur 1.18
end_table
table VerticalProfiles
end_table
table TillageDates
end_table
table interpolate CntSysEql (mg.kg-1)
0        0
50       0
end_table
table interpolate CntSysNeq (mg.kg-1)
0        0
50       0
end_table
No DepositionScheme
table    FlmDep   (kg.ha-1.d-1)
end_table
*-------------------------------------------------------------------------------
* Section 7: Crop section
*-------------------------------------------------------------------------------
No CropCalendar
Yes              RepeatCrops
Fixed            OptLenCrp
table  Crops
end_table
*-------------------------------------------------------------------------------
```

```
* End of Pearl input file
```

Meteorogical input file for lysimeter simulation

```
*************************************************************************
* Contents: SWAP  2.0 - Meteo data of 'LYS-UKP'     weather station
* 05/04/2003 12:52:37
*************************************************************************
*   Station    DD MM YYYY     RAD    Tmin    Tmax     HUM    WIND    RAIN    ETref
*              nr nr   nr    kJ/m2       C       C     kPa     m/s      mm       mm
'LYS-UKP'      22  9 2000         0      14    23.9    1.76       0      10        0
'LYS-UKP'      23  9 2000         0    12.7    17.4    1.27       0      10        0
'LYS-UKP'      24  9 2000         0     9.4    19.3    1.26       0      10        0
'LYS-UKP'      25  9 2000         0     9.4    19.8    1.35       0      10        0
'LYS-UKP'      26  9 2000         0    11.3    18.8    1.54       0      10        0
'LYS-UKP'      27  9 2000         0      11    16.9    1.43       0      10        0
'LYS-UKP'      28  9 2000         0    11.4    15.9    1.32       0      10        0
'LYS-UKP'      29  9 2000         0    11.3    15.9    1.25       0      10        0
'LYS-UKP'      30  9 2000         0      11    16.4    1.38       0      10        0
'LYS-UKP'       1 10 2000         0    12.5    15.9    1.25       0      10        0
'LYS-UKP'       2 10 2000         0    12.2    15.3    1.24       0      10        0
'LYS-UKP'       3 10 2000         0    12.4    15.3     1.2       0      10        0
'LYS-UKP'       4 10 2000         0     5.1    17.3    0.91       0      10        0
'LYS-UKP'       5 10 2000         0       5    22.2     1.1       0      10        0
'LYS-UKP'       6 10 2000         0       8    15.7    1.24       0      10        0
'LYS-UKP'       7 10 2000         0    10.2    12.8    1.12       0      10        0
'LYS-UKP'       8 10 2000         0     9.3    14.8    1.15       0      10        0
'LYS-UKP'       9 10 2000         0    10.7    13.5    1.19       0      10        0
'LYS-UKP'      10 10 2000         0     9.4    13.6    1.08       0      10        0
'LYS-UKP'      11 10 2000         0       9    13.9    1.26       0      10        0
'LYS-UKP'      12 10 2000         0    10.1    16.8    1.38       0      10        0
'LYS-UKP'      13 10 2000         0    11.6    16.4    1.34       0      10        0
'LYS-UKP'      14 10 2000         0     8.2    13.6    1.04       0      10        0
'LYS-UKP'      15 10 2000         0     6.7    14.4    0.99       0      10        0
'LYS-UKP'      16 10 2000         0     6.6    16.8     1.1       0      10        0
'LYS-UKP'      17 10 2000         0     5.5    18.4    1.06       0      10        0
'LYS-UKP'      18 10 2000         0     6.2    19.6    1.21       0      10        0
'LYS-UKP'      19 10 2000         0     8.6    18.5    1.38       0      10        0
'LYS-UKP'      20 10 2000         0    11.5    17.2    1.23       0      10        0
'LYS-UKP'      21 10 2000         0     5.2     8.6    1.42       0       0        0
'LYS-UKP'      22 10 2000         0     6.6    11.6    1.39       0       0        0
'LYS-UKP'      23 10 2000         0     8.5    11.9    1.49       0       0        0
'LYS-UKP'      24 10 2000         0     6.5     8.9    1.33       0       0        0
'LYS-UKP'      25 10 2000         0     5.8    11.4    1.29       0       0        0
'LYS-UKP'      26 10 2000         0     6.7    11.7    1.42       0       0        0
'LYS-UKP'      27 10 2000         0     6.8      12    1.24       0       0        0
'LYS-UKP'      28 10 2000         0     6.6     9.8    1.01       0       0        0
'LYS-UKP'      29 10 2000         0     5.7    10.3    1.11       0       0        0
'LYS-UKP'      30 10 2000         0     6.3    10.1    1.32       0       0        0
'LYS-UKP'      31 10 2000         0     5.9     9.9     1.4       0       0        0
'LYS-UKP'       1 11 2000         0     8.5    10.7    1.29       0       0        0
'LYS-UKP'       2 11 2000         0       8    12.6    1.26       0       0        0
'LYS-UKP'       3 11 2000         0     7.8    13.2    1.06       0       0        0
'LYS-UKP'       4 11 2000         0     6.1    12.1    0.98       0       0        0
'LYS-UKP'       5 11 2000         0     5.6    11.2    0.84       0       0        0
'LYS-UKP'       6 11 2000         0     5.1    16.3    0.83       0       0        0
'LYS-UKP'       7 11 2000         0     1.2    16.6    0.78       0       0        0
'LYS-UKP'       8 11 2000         0     3.7    10.7    1.02       0       0        0
'LYS-UKP'       9 11 2000         0     5.4    10.6    0.85       0       0        0
'LYS-UKP'      10 11 2000         0     4.6    10.2    1.04       0       0        0
'LYS-UKP'      11 11 2000         0     4.2      11    0.92       0       0        0
'LYS-UKP'      12 11 2000         0     3.9    11.1     0.9       0       0        0
'LYS-UKP'      13 11 2000         0     2.3    11.5    0.86       0       0        0
'LYS-UKP'      14 11 2000         0     1.7    12.9    0.84       0       0        0
'LYS-UKP'      15 11 2000         0     2.3    12.1    0.88       0       0        0
'LYS-UKP'      16 11 2000         0     2.6    10.6    0.71       0       0        0
'LYS-UKP'      17 11 2000         0     0.4    13.8     0.6       0       0        0
'LYS-UKP'      18 11 2000         0     1.9    12.9    0.69       0       0        0
'LYS-UKP'      19 11 2000         0     2.3    12.1    0.73       0       0        0
```

Irrigation input file for lysimeter 1 simulation

```
table IrrTab (mm)
21-Oct-2000     5
22-Oct-2000     10
23-Oct-2000     10
24-Oct-2000     10
25-Oct-2000     10
26-Oct-2000     10
27-Oct-2000     10
28-Oct-2000     10
29-Oct-2000     10
30-Oct-2000     10
31-Oct-2000     10
end_table
```

Annex II

Annex III UK soil tables and figures

III-1. Description of clay soil profile in UK field experiment

1. Field	
1.1 Study location	The Middle Field, Woodside Farm, Osgathorpe, Leicestershire, LE12 9ST, UK. Grid reference: OS SK 42667 20500.
1.2 Crop	Year 1: Winter oats, drilled on 26/10/00. Year 2: Winter wheat, drilled ca. 08/10/01.
1.3 Profile	See profile description. Tile drains at 65cm depth and 20m spacing, with a catchment area of 1.35 ha (total field area is 1.55 ha).
1.4 Cultivation	Year 1: Slurry applied to crop stubble on 19/10/00 and incorporated to 25cm 6 days later. Soil then disced to 10cm the following day, drilled and disced again. Year 2: Slurry applied to disced soil on 05/09/01, incorporated 2 weeks later, then disced and drilled on 08/10/01.
2. Application	
2.1 Rate	Sulphachloropyridazine applied at 1.18 kg/ha.
	Oxytetracycline applied at 0.87 kg/ha.
	Tylosin given to pigs continuously at 100g per tonne of feed and slurry stored for 0-3 months.
	Slurry applied at 45200 l/ha
3. Sampling	
3.1 Sampling times	Soil samples taken at: Pre-application, 0 DAT (days after treatment), 1, 7, 14, 21, 30, 60, 90, 120, 240 and 360 DAT in both years.
3.2 Sampling depth	50 cm soil cores taken, will be analysed in sections: 0-5, 5-10, 10-20, 20-30, 30-40 and 40-50cm.
4. Analysis	
4.1 Method	In progress
4.2 Stability	In progress
5. Meteorology	
5.1 Air temperature	See spreadsheet to follow with antibiotic concentrations
5.2 Rainfall	See spreadsheet to follow with antibiotic concentrations

PROFILE DESCRIPTION

Study Number:	JA6110Z
Project Number:	JA6110Z
Grid Ref:	SK 42667 20500
Profile No:	SK42/2705
Described by:	R C Palmer and P Kay
Date:	09/10/01
Weather:	Relatively wet summer, several periods of rain prior to sampling, showers on day of sampling, soil below field capacity
Locality:	The Middle Field, Woodside Farm, Osgathorpe, Leicestershire; Mr Sid Armett
Elevation:	Approx 90 m above OD
Regional relief:	Tributary valley of upper Soar catchment
Local relief	Middle valley slope
Slope:	2.5^{o}
Aspect:	South-south-west (200^{0})
Slope form:	Straight
Soil erosion:	None
Land use:	Winter wheat
Soil surface:	Harrowed and cultivated
Surface stoniness:	Slightly stony, small and medium rounded quartzites, flints and sandstones
Cultivation:	Ploughed, harrowed and cultivated
Soil series	Salop

Profile Number: SK/42/2705

Horizon depth (cm) and notation		**Bulk sample depths (cm)**	**Moisture release tins (cm)**
0-37	Ap	0-37	6-11
37-65	Btg 1	37-65	38-45
65-98	Btg 2	65-98	65-70
98-110	BCtg	98-110	98-103

Horizon Depth (cm) and Notation		Description of Horizons
0-37	Ap	Very dark greyish-brown (10YR 3/2) slightly stony, clay loam; no mottles; stones small and medium hard rounded quartzites, flints and sandstones; moist; moderately developed coarse and medium sub-angular fragments in top 15cm, below this, weakly developed coarse and very coarse angular fragments; low packing density; very porous; 1% medium to very fine macropores; moderately weak fragment and soil strength; moderately sticky, very plastic; common, very fine fibrous roots, ploughed in stubble towards base of horizon with a line at 35cm depth; non-calcareous; earthworms active; sharp, smooth boundary to;
37-65	Btg 1	Brown (7.5YR 5/3) slightly stony clay; common distinct very fine, strong brown (7.5YR 5/8) and many coarse to very fine pinkish-grey (7.5YR 6/2) clear mottles; stones medium to very small hard rounded and occasionally weathered sandstones, quartzites and flints; moist; moderately developed, coarse and medium angular blocky peds, ped faces often uniform pinkish-grey (7.5YR 6/2); high packing density; slightly porous; fine fissures between peds filled with topsoil material, 0.1% coarse and medium macropores; very firm soil and ped strength; moderately sticky, very plastic; few very fine fibrous roots concentrated along ped faces; non-calcareous; earthworms active; clear wavy boundary to;
65-98	Btg 2	Dark brown to brown (7.5YR 4/4) slightly stony clay; many prominent medium to very fine yellowish red (5YR 5/6) and pinkish-grey (5YR 6/2) sharp mottles; stones as above; moist; strongly developed coarse and medium prismatic peds, ped faces often coated with pinkish-grey (5YR 6/2); high packing density; slightly porous; 0.1% medium macropores (earthworms); moderately strong soil and ped strength; very sticky, moderately plastic; few very fine fibrous roots along ped faces; earthworms active; non-calcareous; clear, wavy boundary to;
98-110	BCtg	Reddish brown (5YR 4/3) slightly stony clay; common, prominent coarse (5BG 7/1), sharp and common, fine, sharp, strong brown (7.5YR 5/6) clear mottles; stones small, rounded, hard quartzites and very small, soft, limestone fragments; moist; structureless massive; high packing density; very slightly porous; <0.1% fine and very fine macropores; moderately strong soil strength; very sticky, moderately plastic; no roots; no earthworms; slightly calcareous.

Soil analysis

	Ap horizon	Btg 1 horizon
Sand (63µm – 2mm) %	42.63	34.17
Silt (2µm – 63 µm) %	32.26	27.68
Clay (<2µm) %	25.11	38.15
PH	6.8	7.3
CEC mEq/100g	22.4	25.2
OC %	2.2	0.7
Bulk density gcm^{-3}	1.3	1.56
Water holding capacity max (saturation) % w/ww	32.4	25.2
Water holding capacity max (saturation) % w/dw	40.2	
Water holding capacity 0.05 bar (field capacity) %	27.6	23.2
Water holding capacity 0.1 bar %	26.6	22.5
Water holding capacity 0.4 bar %	25.9	22.2
Water holding capacity 2.0 %	24.4	21.1
Water holding capacity 15.0 bar %	23.9	19.9

III-2. Description of sandy soil profile in UK field and lysimeter experiment

1. Field	
1.1 Study location	Long Field, Hall Farm, Lockington, Derby, DE74 2RH, UK. Grid reference: OS SK 4724 2767.
1.2 Crop	Year 1: Winter wheat, drilled on Sep/Oct/00. Year 2: Spring beans, drilled on 08/03/02.
1.3 Profile	See profile description.
1.4 Cultivation	Year 1: Ploughed and drilled in Sep/Oct/01, slurry applied 17/01/01, not incorporated. Year 2: Ploughed Oct/Nov/01, slurry applied 22/01/02, drilled 08/03/02.
2. Application	
2.1 Rate	Sulphachloropyridazine applied at 1.18 kg/ha.
	Oxytetracycline applied at 0.87 kg/ha.
	Tylosin given to pigs continuously at 100g per tonne of feed and slurry stored for 0-3 months.
	Slurry applied at 33333 l/ha
3. Sampling	
3.1 Sampling times	Year 1: no soil sampling. Year 2: 1, 6, 14, 21, 31, 59, 127 DAT.
3.2 Sampling depth	30cm cores taken, will be analysed 0-5, 5-10, 10-20 and 20-30 cm.
4. Analysis	
4.1 Method	In progress
4.2 Stability	In progress
5. Meteorology	
5.1 Air temperature	See spreadsheet to follow with antibiotic concentrations
5.2 Rainfall	See spreadsheet to follow with antibiotic concentrations

PROFILE DESCRIPTION

Study Number:	JA6110Z
Project Number:	JA6110Z
Grid Ref:	SK 47242767
Profile No:	SK 42/7276
Described by:	G R Beard
Date:	12/05/94
Weather:	Relatively dry winter, recent days of warm dry sunny weather.
Locality:	Long Field, Hall Farm, Lockington, Derby; Mr Charles Coaker
Elevation:	45 m above OD
Regional relief:	Gently undulating Triassic lowland with wide shallow valleys
Local relief	Valley side, lower slope (only a few metres above floodplain)
Slope:	1.5^{o}
Aspect:	North-north-west (333^{0})
Slope form:	Straight
Soil erosion:	Topsoil shows capping and in some wheel ruts small rills and fans of washed sand indicate soil erosion.
Land use:	Winter cereals
Soil surface:	Cultivated
Surface stoniness:	10%
Cultivation:	Ploughed and cultivated
Soil series	Arrow

Profile Number: SK/42/7276

Horizon depth (cm) and notation	**Bulk sample depths (cm)**	**Moisture release tins (cm)**
0-30 Ap	0-30	6-11
30-48 Bw(g)	30-48	40-45
48-82 Bg	48-82	
82-110 BCg	82-110	

Horizon Depth (cm) and Notation	Description of Horizons
0-30 Ap	Very dark greyish brown (10YR3/2); sandy loam; small and medium rounded quartzites; moist; moderate to weakly developed medium subangular blocky structure, thin finely laminated cap on soil surface; medium packing density; very porous, very fine and fine pores, occasional medium pore (est. 3%); moderately weak soil and ped strength; non-sticky, slightly plastic; no visible living roots; occasional earthworm; non-calcareous; smooth sharp boundary to:
30-48 Bw(g)	Brown to dark brown (7.5YR4/3); fine distinct and prominent clear dark yellowish brown (10YR3/4) and strong brown (7.5YR5/6) mottles; sandy loam (slightly higher clay than horizon above); stones as above; moist; weak coarse subangular blocky structure (vertical ped faces common); low packing density; very porous, common very fine and fine macro pores (est. 4%); moderately weak soil and ped strength; slightly sticky, slightly plastic; few very fine dead roots (see above); occasional earthworm channel prominently lined with topsoil; non-calcareous; few rounded ferri-manganiferous concentrations; thin discontinuous coats of clay appear to cover sand grains (maybe argillic); abrupt clear smooth boundary to:
48-82 Bg	Brown to dark brown (7.5YR4/4) (rubbed 7.5YR4/4) many medium and coarse distinct clear dark reddish brown (5YR3/2 and 3/4) mottles; few medium distinct clear brown (7.5YR5/4) mottles; sandy loam; stones as above; moist; weak medium subangular blocky structure; low packing density; very to extremely porous, fine pores (est. 5%); very weak to moderately weak soil strength, occasionally of a more cemented nature; slightly sticky, slightly plastic; few very fine dead roots; occasional earthworm channel lined with topsoil; non-calcareous; ferri-manganiferous concentrations; clear wavy boundary to:
82-110 BCg	Reddish brown (5YR4/4) (rubbed brown to dark brown 7.5YR4/4, poor match); few fine faint clear yellowish red (5YR4/6) and few coarse faint sharp brown (7.5YR5/4) mottles; loamy sand; small and medium rounded quartzite stones common, occasional small angular flint and large rounded soft weathered sandstone fragments; very moist/wet; weak coarse subangular to angular blocky structure with patches which are apedal single grain; low packing density; extremely porous, fine pores (est. 5%); very weak and loose soil strength; non-sticky, non-plastic; occasional fine fibrous root; occasional worm channel lined with topsoil; non-calcareous; many to very many small ferri-manganiferous concentrations (in places this cements soil particles together).

Soil analysis

	Ap horizon	Bw(g) horizon	Bg horizon	BCg horizon
Sand (63µm – 2mm) %	69.17	72.37	77.15	89.92
Silt (2µm – 63 µm) %	20.49	17.69	15.29	6.27
Clay (<2µm) %	10.35	9.94	7.55	3.82
PH	6.6	6.5	6.5	6.2
CEC mEq/100g				
OC %	1.3			
Bulk density gcm^{-3}	1.68			
Water holding capacity max (saturation) % w/ww	28.6			
Water holding capacity max (saturation) % w/dw	40.2			
Water holding capacity 0.05 bar (field capacity) %				
Water holding capacity 0.1 bar %				
Water holding capacity 0.4 bar %				
Water holding capacity 2.0 %				
Water holding capacity 15.0 bar %				

III-3. Sample collection

It is noted that the volumes collected on a number of occasions were quite large relative to the volumes applied, and that these occasions coincided with natural rainfall events. The experiment was designed to minimise the amount of rainfall entering the collection bottle: the lysimeters were sheltered; the collection bottles are situated under the pallets; the sides of the lysimeters and the exposed area of the gravel filled funnels are sealed with plastic; however there are a number of explanations for the increased

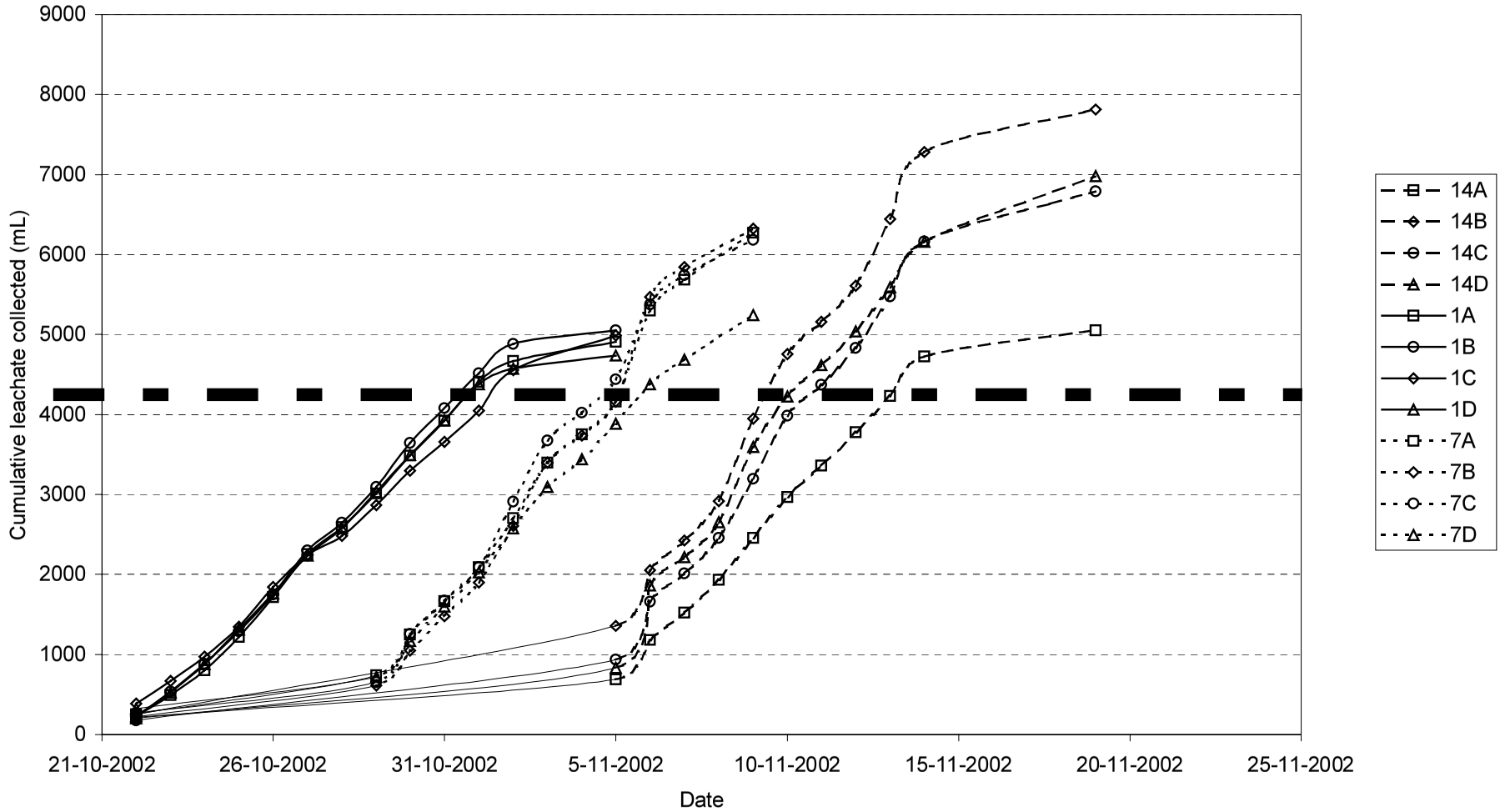
1. The samples are entirely leachate and the lysimeters are producing variable volumes and any correlation between rainfall and sample volume is coincidental
2. Rainfall is falling directly into the collection bottle funnels, hence the different volumes between lysimeters, given the difference in shelter between the bottles
3. Rainfall is running down the outside of the lysimeters and then down the outside of the gravel filled funnel and then into the collection bottle
4. Rainfall is running down the outside of the lysimeters and then through holes in the plastic seal and down through the gravel filled funnel and then into the collection bottle
5. The lysimeter caps are not waterproof allowing rainfall to fall directly onto the surface of the soil and this is adding to the applied volume.

Explanations 4 and 5 are discounted totally as thorough inspection of all lysimeters indicates that there are no holes in any of the caps or plastic seals. Explanation 2 would not account for the estimated 200-600-ml extra volume of sample per lysimeter given the small surface area of the funnel, the amount of natural rainfall and the fact that the bottles and funnels are well sheltered. The small funnels have a diameter of approximately 6-cm, therefore even if 10-mm of rainfall were fully collected by the funnels, this would only equate to around 30-ml volume.Explanation 1 will certainly be true with regard to lysimeters producing variable volume but it is thought that given the volumes from some lysimeters more than doubling when it is raining compared to days when it has not rained then explanation 3 is considered the most likely.However, it is noted that on 16 DAT, when sample volumes were larger for certain lysimeters, there was very little water in any of the collection bottles for lysimeters 1A to 1D which had not been irrigated since 10 DAT. It would be expected that these bottles would contain a few hundred mls of rainwater if explanation 3 is valid.In any event, the collection of rainfall will not affect the mass of compound in the collection bottles

Annex III

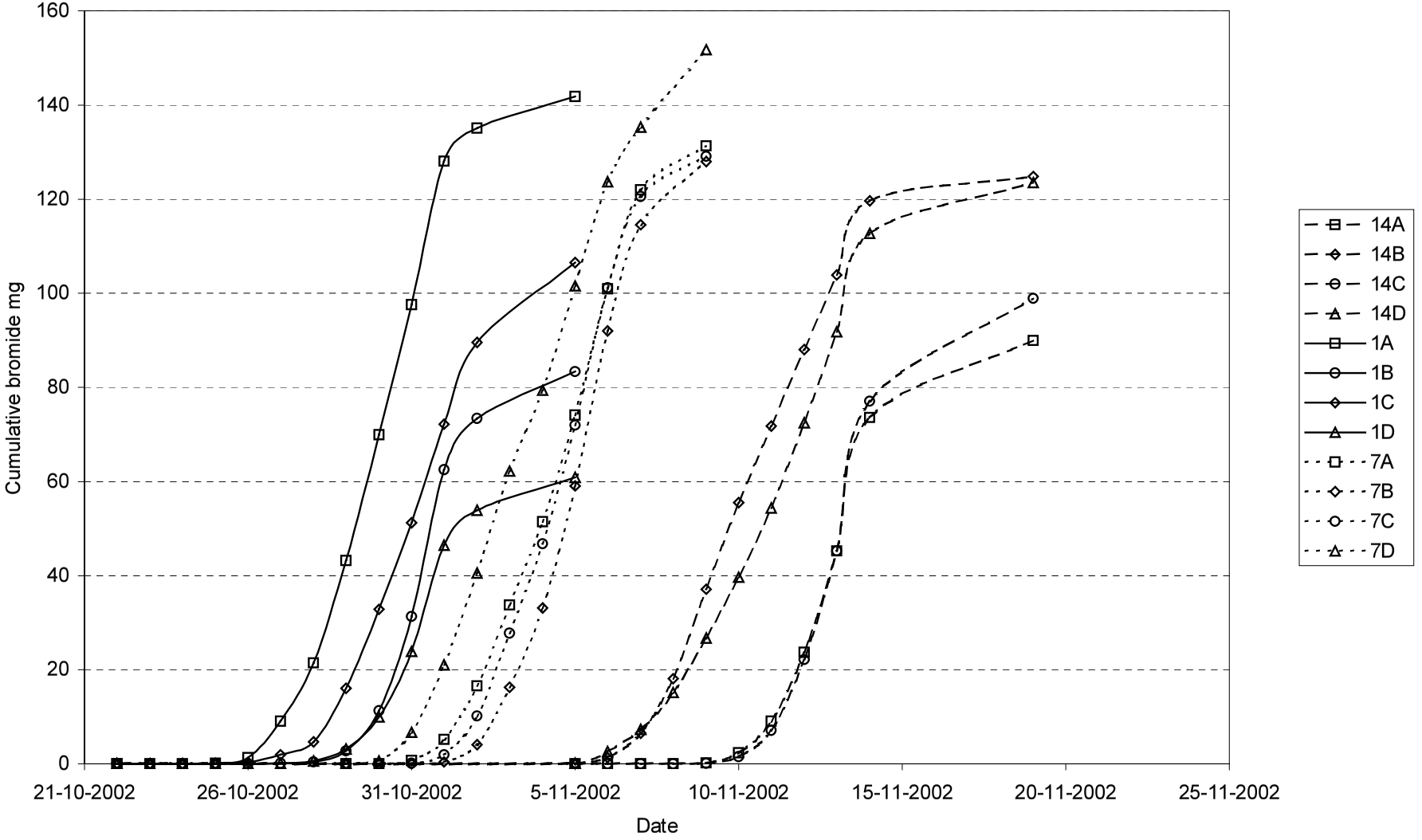
III-4. Figures lysimeter leachate

Figure-22 Cumulative leachate breakthrough (mL) in lysimeter 1A-D, 7A-D, 14A-D. Bold dotted line is total amount of irrigation applied on each lysimeter.



Annex III

Figure 23 Cumulative bromide breakthrough (mg) in lysimeter 1A-D, 7A-D, 14A-D



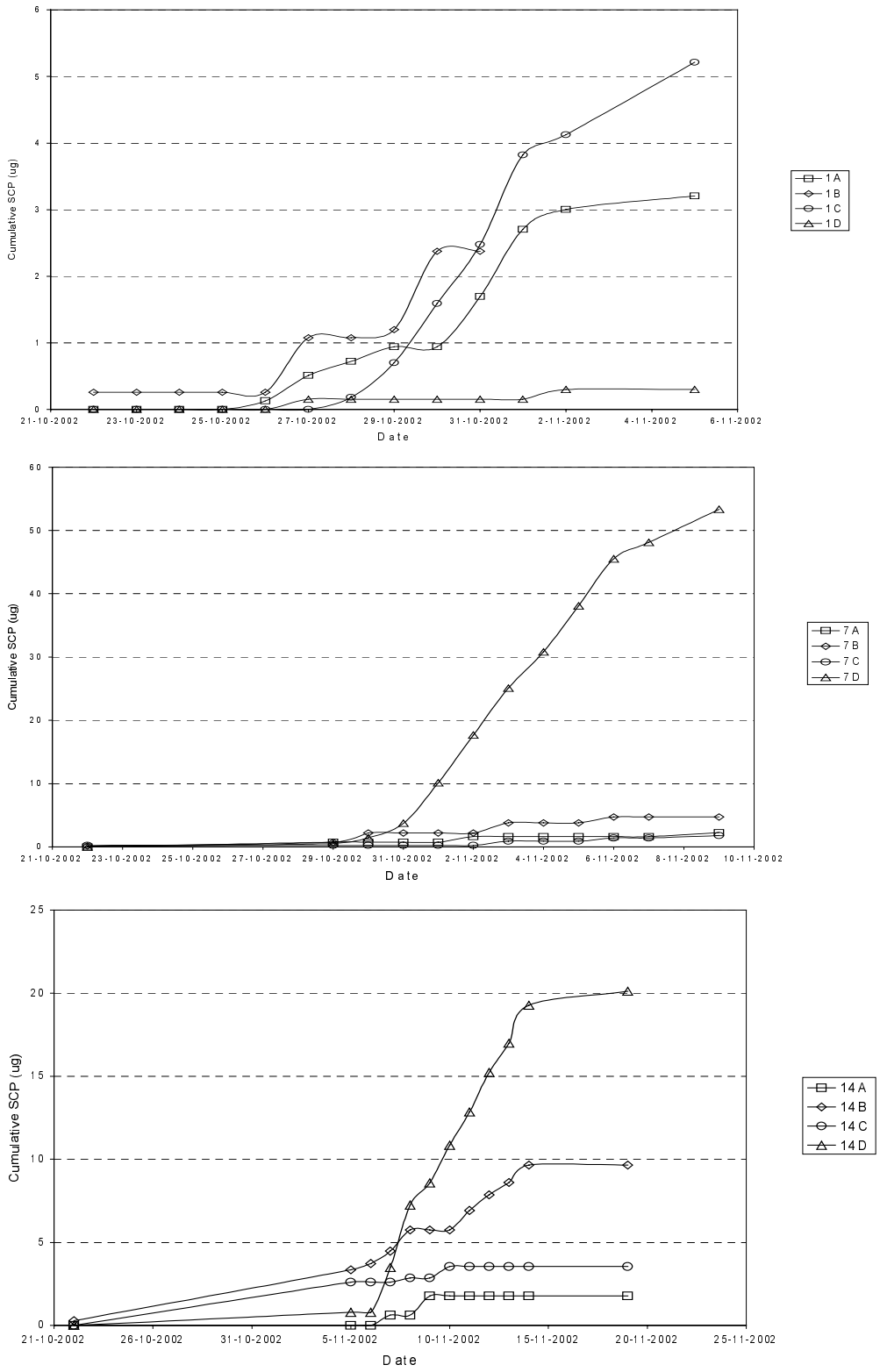


Figure 24 Cumulative breakthrough of sulphachloropyridazine in lysimeters 1A-D, 7A-D and 14A-D

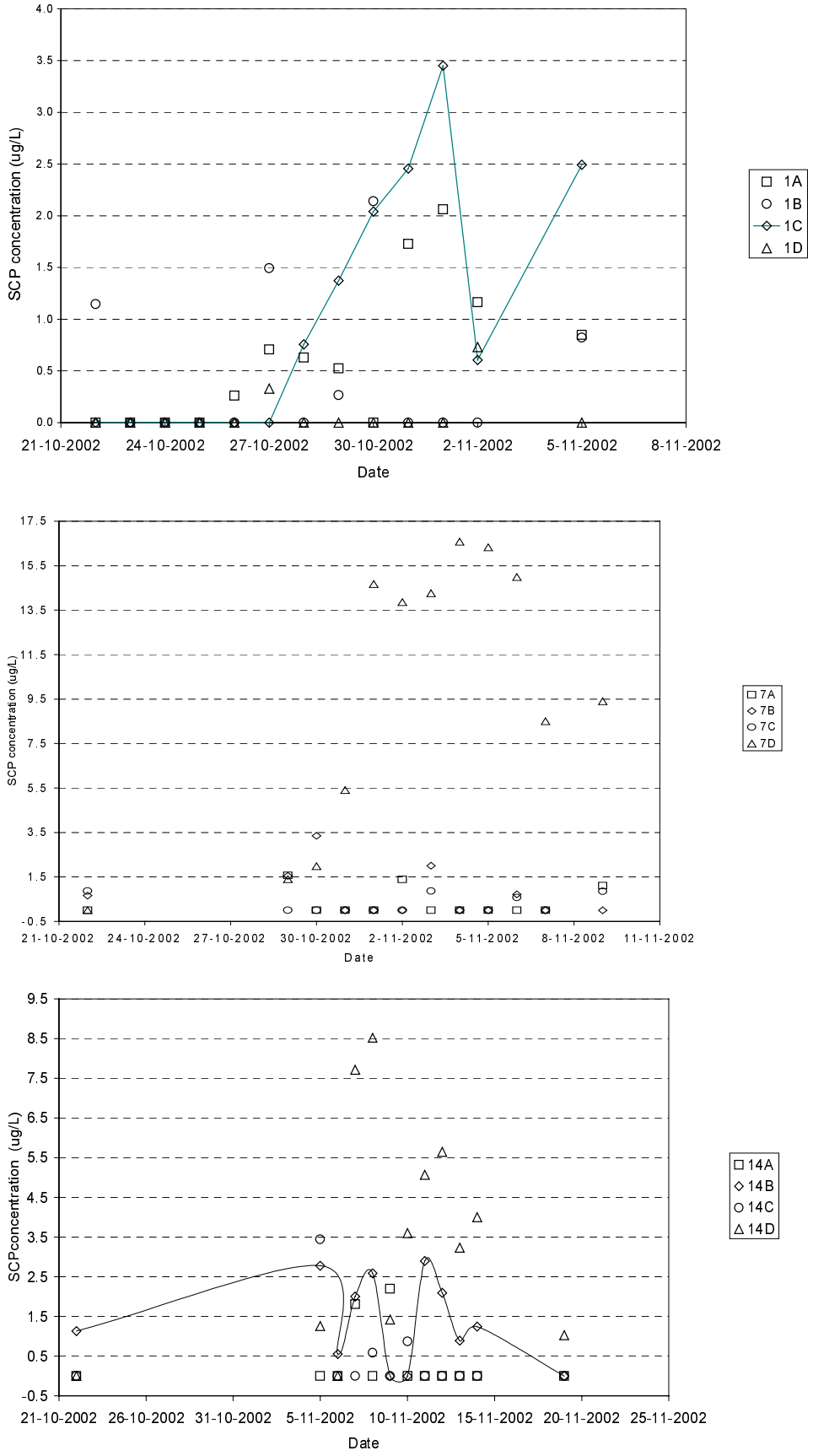


Figure 25 SCP Concentration in leachate (ug/L) in lysimeter 1A-D,7A-D and 14A-D

Annex III

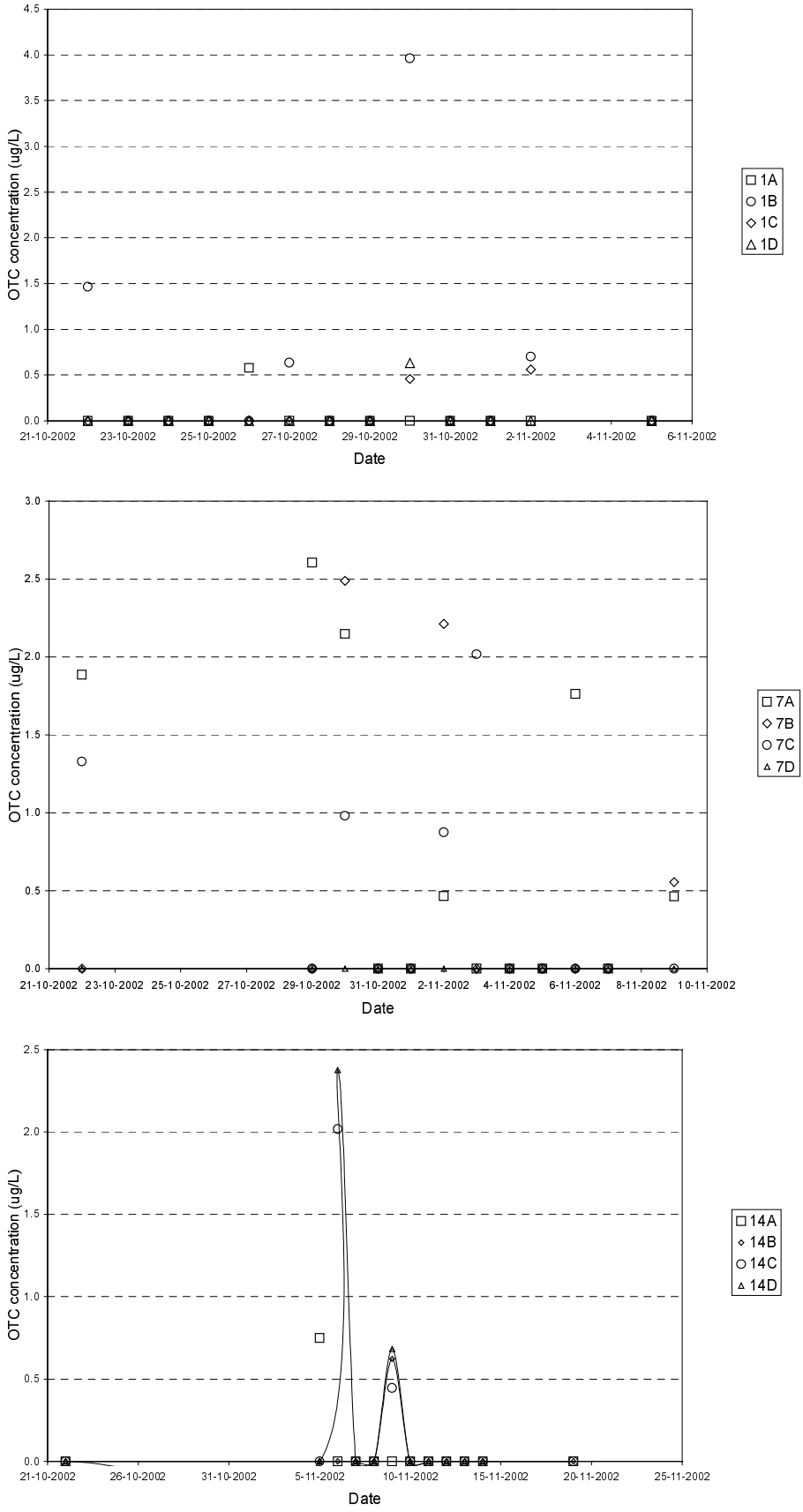


Figure 26 Oxytetracycline concentration (µg/L) in the leachate of lysimeter 1A-D,7A-D and 14A-D

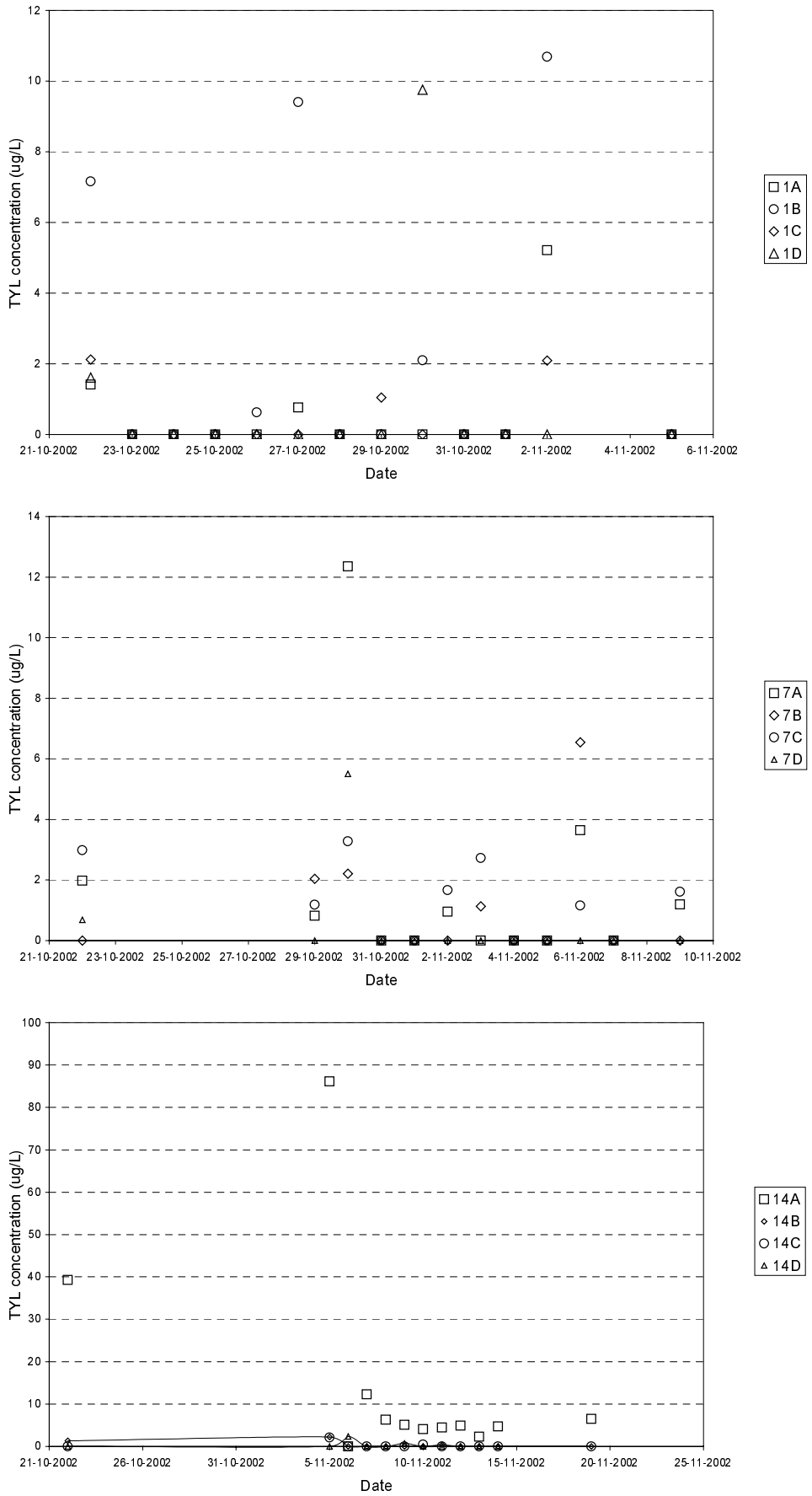
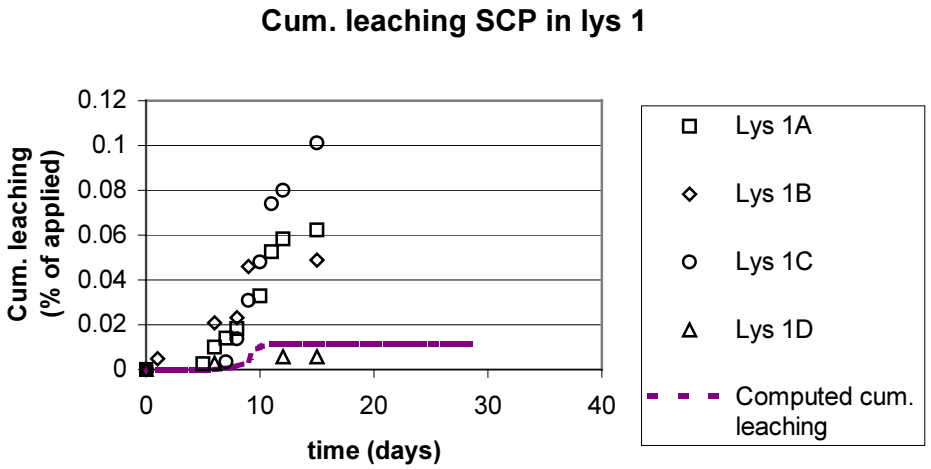
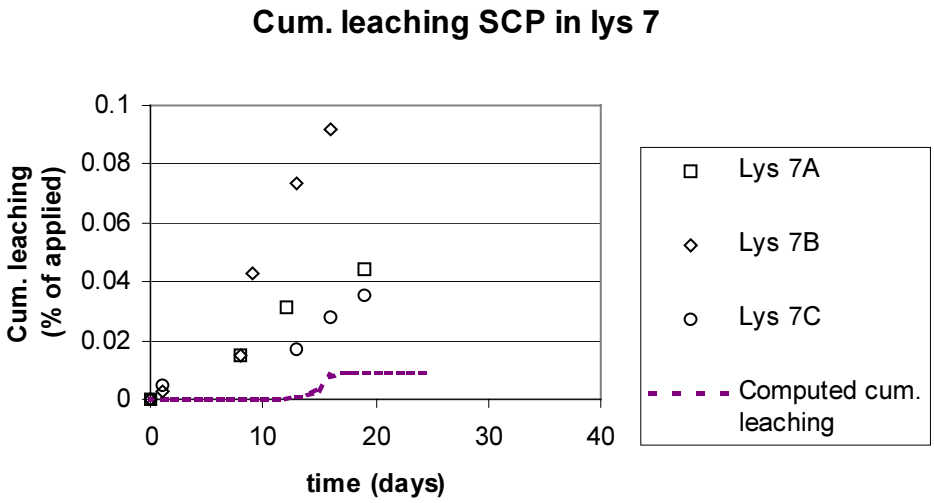


Figure 27 Concentration tylosin (ug/L) in the leachate of lysimeters 1A-D,7A-D and 14A-D.

Annex III





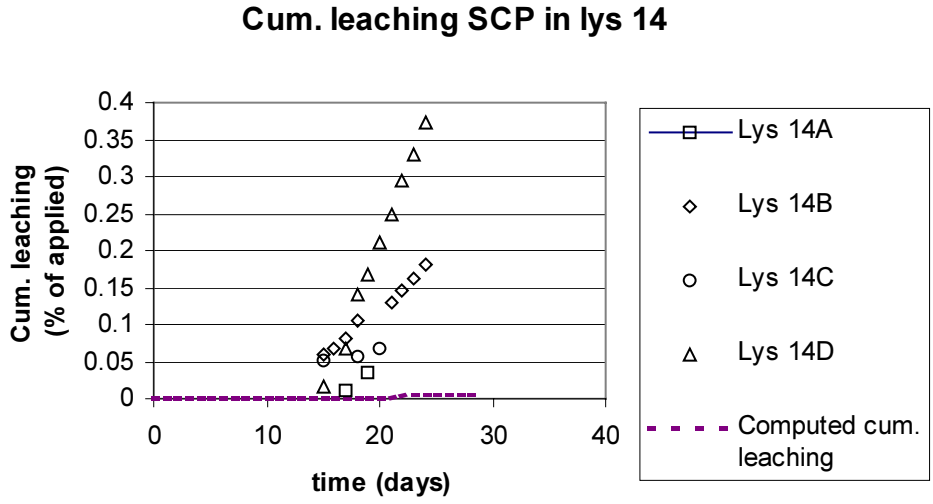


Figure 28 Computed versus measured cumulative leaching of sulphachloropyridazine

Annex III

Appendix 1 Mailing list

1 EC Research DG, Energy, Environment and Sustainable Development programme, K. Haavisto.
2 VROM, Directoraat Generaal Milieubeheer, Directie Bodem, Water en Landelijk Gebied, drs H.G. van Meijenfeldt
3 VROM, Directoraat Generaal Milieubeheer, Directie Bodem, Water en Landelijk Gebied, drs J. de Rijk
4 VROM, Directoraat Generaal Milieubeheer, Directie Bodem, Water en Landelijk Gebied, ir A. Roos
5 LNV, Directoraat Generaal Landbouw, Directie Voedings- en Veterinaire Aangelegenheden, drs M.E. Siemelink
6 LNV, Directoraat Generaal Landbouw, Directie Voedings- en Veterinaire Aangelegenheden, drs G.T.J.M. Theunissen
7 VROM, Directoraat Generaal Milieubeheer, Directie Stoffen, Afvalstoffen en Straling, dr J.A. van Zorge
8 VenW, Directoraat-Generaal Water Gebruik en Waterkwaliteit, dr P. G.-J. de Maagd
9 Rijksinstituut voor Integraal Zoetwaterbeheer en Afvalwaterbehandeling, dr G. Rijs
10 EMEA, Safety of Veterinary Medicines, London, UK, dr K. Grein
11 CVMP, Bureau Registratie Diergeneesmiddelen, Wageningen, dr J.P. Hoogland
12 Ministry of Health, National Chemical Bureau, Llubljana, Slovenia, dr S. Fajfar
13 AFSSA, ANMV, Fougeres, France, dr J.-C. Faucon
14 BMI, Safety of Veterinary Medicines, Brussels, Belgium, dr M. Goret
15 AUTH, Faculty of Veterinary Medicines, Thessalonike, Greece, dr A.P. Kamarianos
16 UBA, Berlin, Germany, dr J. Koschorrek
17 VMD, Pharmaceuticals and Feed additives, Addlestone, UK, dr C. Long
18 PKKZ, Medical Products Agency, Uppsala, Sweden, dr M. Malmqvist
19 Irish Medicines Board, Dublin, Ireland, dr D. Murphy
20 FEI, Chemicals Division, Helsinki, Finland, dr E. Nurmi
21 NPA, Direcção-Geral de Protecção das Culturas, Oeiras, Portugal, dr B. Oliveira
22 UBA, Berlin, Germany, dr I. Rönnefahrt
23 Danish Medicines Agency, Brønshøj, Denmark, dr C. Sommer
24 VMD, Pharmaceuticals and Feed additives, Addlestone, UK, dr A. Tait
25 UBA, Berlin, Germany, dr E. Thumm
26 IVCA, Department for Residue Research, Athens, Greece, dr A.E. Tyrpenou
27 FEI, Chemicals Division, Helsinki, Finland, dr V. Virtanen
28 IFAH-Europe, FIDIN, Den Haag, mr. M. van Assen
29 Danish Environmental Research Institute, Silkeborg, Denmark, dr J. Jensen
30 INIA, Laboratorio de Ecotoxicologica, Madrid, Spain, dr J.V. Tarazona Lafarga
31 Cranfield University, Centre for Ecochemistry, Derby, UK, dr A.B.A. Boxall
32 Danish University of Pharmaceutical Sciences, Section of Environmental Chemistry, Copenhagen, Denmark, dr B. Halling-Sorensen
33 Rostock University, Institut für Bodenkunde und Pflanzenernährung, Rostock, Germany, dr S. Thiele-Bruhn
34 Tierärztliche Hochschule Hannover, Germany, dr G. Hamscher

35 EAWAG Switzerland, Dübendorf, Switzerland, dr S. Müller
36 IRAS, Utrecht, drs T. van der Laak
37 ECT Oekotoxikologie GmbH, Flörsheim a.M., Germany, dr J. Moltmann
38 Henkel GmbH & KGaA, Düsseldorf, Germany, dr J. Tolls
39 TUT, Institute of Biotechnology and Environmental Engineering, Tampere, Finland, dr S. Kuusisto
40 Depot Nederlandse Publikaties en Nederlandse Bibliografie
41 Directie RIVM
42 Sectordirecteur Milieurisico's en Externe Veiligheid, dr ir R.D. Woittiez
43 Hoofd Stoffen Expertise Centrum, dr W.H. Könemann
44 Relatiebeheerder RIVM-MEV, drs J.H. Canton
45 dr R. Luttik, RIVM/SEC, projectleider Risicobeoordelingsmethodologie
46 dr J.A. de Knecht, RIVM/SEC
47 ir A.M.A. van der Linden, RIVM/LDL, projectleider Stofstromen
48 ir G.J. Schefferlie, RIVM/SIR, projectleider Beoordeling Diergeneesmiddelen
49 ing P.L.A. van Vlaardingen, RIVM/SEC, projectleider Beoordeling Diergeneesmiddelen
50 ir J.B.H.J. Linders, RIVM/SEC
51 ir P. van der Poel, RIVM/SEC
52 ir A.J. Verschoor, RIVM/SEC
53 Auteurs
54 SBC / Communicatie
55 Bureau Rapportenregistratie
56 Bibliotheek CSR
57 Bibliotheek RIVM
55-59 Bureau Rapportenbeheer RIVM