Validation of the EU environmental risk assessment for veterinary medicines

Montforts, M.H.M.M.

Mark Henricus Maria Marcellinus Montforts

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Promotores:	Prof. Dr. H.A. Udo de Haes
	Prof. Dr. N.M. van Straalen (Vrije Universiteit)
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	Dr. J.S.M. Boleij (College voor de Toelating van Bestrijdingsmiddelen)

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1. Introduction

1.1. Environmental concerns regarding veterinary medicines

In the late nineteenth century, the study of the relation between ecosystems and (micro) nutrients was an integral part of the science of life (Wells et al., 1930). The impact of man-made substances on ecosystems was however never considered[1], until the mid-1950's and 1960's when populations of birds of prey alarmingly declined (up to 91%) in Europe and the USA. These declines in reproduction success, as well as field observations of bird mortalities, were attributed to insecticides, substances for seed treatment, and rodent baits, applied in agriculture for crop protection (Cramp, 1963; Koeman et al., 1972; Mendenhall and Pank, 1980; Hill and Fleming, 1982). Regulatory responses to these particular findings involved, amongst others, the ban on certain pesticides, and the establishment of an environmental risk assessment scheme for the registration of pesticides (see e.g. EU Directive 79/117/EEC and Luttik (2003)).

In some incidents with bird mortalities, the substances involved had not been applied widespread as pesticides on crops. The organophosphate substances DDVP and famphur were applied as veterinary medicines to cattle, either by feed or by pour-on applications (Ludke and Locke, 1976; Hill and Mendenhall, 1980; Henny et al., 1985). Also the use of organophosphate sheep-dips in Scotland to combat scab and other parasites caused surface water contamination and fish mortality (McVeigh et al., 1997). These observations show that the medicinal use of pesticides has caused environmental damage comparable to that caused by their use in crop protection.

These examples suggest that measures taken for pesticides used as plant protection products[2] with respect to environmental risk, should also be taken for the pesticides and (related) substances used as veterinary medicines. There are, in fact, several classes of substances that are used both as veterinary medicines and as pesticides. Several insecticides (e.g. lindane, coumaphos, cypermethrin, avermectin, and imidacloprid) are used both in veterinary medicine and in crop protection. Moxidectin and milbemectin are both fermentation products from the soil actinomycete *Streptomyces sp.*. Moxidectin is used as an anthelminthic (a substance that expels or destroys intestinal worms) in animals, and milbemectin is used as an insecticide in crops. Thiabendazole is an anthelminthic in animals that is also approved for post-harvest treatment of citrus fruits against fungi. The fungicide trifluralin is applied both in crop protection and in shrimp cultivation. The antibiotic oxytetracyclin and streptomycin are applied as veterinary medicines in animals and as foliage

[1] Pollution associated with the use and mining of metals has occurred throughout history. Development of the chemical industry began in the second half of the nineteenth century. For example, Bayer began the production of dye-stuffs in 1863.

[2] A substance used for crop protection is generally denoted either a plant protection product or a pesticide. A pesticide used to fight pests in non-crop applications, such as buildings, industrial systems, and construction materials, is generally denoted a biocide. The authorisation of pesticides is regulated in the European Union under Directive 91/414/EEC, of

pesticides in crops. Some sulfonylurea substances are used to treat diabetes in pets, and other sulfonylurea substances are used as herbicides in crops. Copper is a micro-nutrient, and also a medicine for treatment of footrot and ringworm in animals. It is also a well-known fungicide in crops, and is used for wood preservation and in anti-fouling paint.

Paracelsus' theorem "*All substances are poisons: there is none that is not a poison. The right dose differentiates a poison and a remedy."* provides reason to extend the risk-based approach applied to pesticides to perhaps all veterinary medicines. Two illustrative examples of this concept are warfarin and paracetamol. Therapeutic medicinal use of warfarin prevents thromboembolism, while the same compound used as a rodenticide very effectively kills rats and mice. Paracetamol is a well-known pain reliever, that also effectively controls Brown Tree snakes when applied in baits (Johnston et al., 2002). Considering this theorem in combination with the fact that the environment contains countless organisms with different sensitivities leads to the hypothesis that veterinary medicines that are not pesticides may also pose a risk for the environment.

This hypothesis has already been substantiated. An alarming decline of vulture populations (up to 95%) occurred in Pakistan in the late 1990's. Recently, research has attributed this decline to the use of the anti-inflammatory drug diclofenac in cattle (Oaks et al., 2004). Incidental mortalities of bald eagles in the USA have been attributed to the use of the anaesthetic pentobarbital in pet animals (Krueger and Krueger, 2003). In general however, too little is known about effects of veterinary medicines and their metabolites (Boxall et al., 2003).

The reported environmental damages and the intrinsic pharmacological properties of veterinary medicines warrant an environmental risk assessment of the use of veterinary medicines.

The marketing of veterinary medicinal products is actively regulated in the European Union by Directive 2001/82/EC, amended by Directive 2004/28/EC, with the intent to protect the environment, next to animal health, consumers, and professional users[3]. An environmental risk assessment is to be performed at registration, and a clear policy and regulatory infrastructure exists to deal with this issue, as well as a number of regulatory guidance documents on the environmental risk assessment (EMEA, 1997; VICH, 2000; DG Enterprise, 2000).

Environmental risk assessment is a scientific discipline that investigates the possible damage that certain activities, such as the use of veterinary medicines, have for the

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biocides under Directive 98/8/EC. In the Netherlands, both are regulated by a single law (Bestrijdingsmiddelenwet, 1962) and are specified with the same noun ('bestrijdingsmiddel').

[3] The use of veterinary medicines (and other products containing chemicals) is also regulated by European environmental legislation. Typical examples are the Directives on water pollution 76/464/EEC, and on groundwater protection 80/86/EEC. This type of legislation operates from the starting point that all actions that may lead to pollution are forbidden unless a permit is granted by the national competent authority. The permit ought to regulate emission (e.g. by prescribing application or purification techniques) as well as the maximum permissible concentration of the substance in the environment (Van Rijswick, 2001). This legislation addresses different authorities than the Directive 2001/82/EC, and the implications for risk assessment in these frameworks are not explored in this thesis. They do provide, however, important preconditions for the methodology under Directive 2001/82/EC.

environment. It is a way of structuring and interpreting information on behaviour and effects of substances with the aim of creating a new type of information, namely estimations on the likelihood of the occurrence of effects (Rodricks, 1992). In general, environmental risk assessment addresses an overall level of protection, in most instances that of *no effect* (SSC, 2003). The aim of risk assessment in the registration process is to eliminate the no-risk situations from further regulatory actions. Risk assessment is most commonly used to elaborate on (which means to downsize) identified hazards in hierarchic levels, from screening level to advanced levels. If a risk in a lower level is deemed acceptable, no further assessment is made.

In the EU Directive 2004/28/EC, amending the Directive 2001/82/EC, Article 30 states that marketing authorisation is denied if the risk-benefit balance of the product is, under the authorised conditions of use, unfavourable. A risk/benefit balance is defined as: 'an evaluation of the positive therapeutic effects of the veterinary medicinal product in relation to the risks'. In Article 33, it is stipulated that a mutual recognition of a marketing authorisation can be denied if there are concerns for a potential serious risk to human or animal health or for the environment. Another response to an identified environmental risk is to mitigate the predicted risk to an acceptable level by addressing the user of the veterinary medicine through the information that accompanies the product (Koschorreck et al., 2002). This response has the intention of establishing a code of conduct that is reaching further than the Good Agricultural Practice taken as a starting point in the risk assessment. Risk mitigation through product labelling is held in high esteem, since it is explicitly worded in Article 12.3.j of the 2004/28/EC Directive and the recital. This option sets requirements towards the environmental risk assessment methodology, by which the effect of the precaution is to be demonstrated, and to the user of the product. One way or the other, the risk assessment methodology plays a crucial role both in the protection of the environment and in the sustainability of agricultural practice.

The focus in this thesis will be on the validation of the environmental risk assessment methodology[4] for the marketing authorisation of veterinary medicines in the European Union. Validation is a process of formulating and substantiating explicit claims about the applicability and accuracy of predictions, with reference to the intended purpose as well as the natural system that is represented (Dee, 1995). With respect to the regulatory objectives of the environmental risk assessment, validation contributes to a better understanding of the information generated in the risk assessment.

1.2. A definition of veterinary medicines

Before we begin an in depth look at the environmental risk assessment for veterinary medicines, we must define which compounds are considered veterinary medicines. Any substance or combination of substances presented for treating or preventing disease in

[4] This research is restricted to the methodology developed and implemented between 1994 and 2001.

animals is a veterinary medicine. Any substance or combination of substances, which may be administered to animals with a view to making a medical diagnosis or to restoring, correcting or modifying physiological functions in animals is likewise considered a veterinary medicinal product. These are the definitions given in the EU Directive 2001/82/EC on the marketing authorisation for veterinary medicines[5]. Substances are defined as any matter irrespective of the origin, which may be: e.g. blood and blood products, micro-organisms, parts of organs, whole animals, toxins, plants, extracts of plants, or chemicals, elements, naturally occurring chemicals or synthetic chemicals.

In short, veterinary medicines are substances for specific purposes, and are regulated and approved through special Community regulation. In a broader understanding, substances that are no longer authorised, or that are (or have been) used without authorisation on animals, are also named veterinary medicines.

This definition excludes certain substances in their applications, such as substances beneficial to, but not used on, or in, animals. Typical examples are disinfectants for animal housing and products to treat indoor surfaces against fleas or bacteria. The substance phenoxyethanol, when applied as a disinfectant on animals, is labelled as a veterinary medicine, and when applied on surfaces (e.g. floors, walls), it classifies as a biocide.

There are applications that can be considered either medicinal or biocidal. Some Member States register teat dips for dairy cows as biocides, others as medicines, provided a therapeutic claim is made (EC, 2002b; VMRF, 2003). Anti-parasitic substances used on animals appear to be on the borderline between pesticides and veterinary medicines[6], but in the European Union, the substances in these applications are defined as veterinary medicines.

Substances that are added to animal feed in order to increase animal production without preventing any specific illness are included with feed additives. Substances that are used for treating, diagnosing, or preventing disease, or restoring, correcting or modifying physiological functions, in man, are classified as human medicines. For these two product classes special regulation exists[7].

Comprehensive classification of veterinary medicines is determined primarily by their mode of action and also by their use category (CVMP, 2000). Factors upon which groups and categories are based include, amongst others:

- Origin: blood products, micro-organisms, chemicals

[5] The first EU Directive on medicines dates from 1965, and has been amended numerous times. Comprehensive reviews resulted in new Directives on veterinary medicines in 1981 (81/852/EEC), in 2001 (2001/82/EC), and recently in 2004 (2004/28/EC).

[6] In Australia these products are regulated by a single regulation. In New Zealand the definition used is set for an 'agricultural compound': a generic term for any substance or mixture of substances, or biological compounds, used or intended for use in the direct management of plants or animals or to be applied to the land or water on or in which the plants or animals are managed, for the purposes of managing pests, or plant or animal productivity, or diagnosing or preventing or treating the condition of animals, and includes any pesticide and veterinary medicine. The term pesticide includes fungicides, herbicides, insecticide, and chemicals which may be administered to animals for the control of ectoparasites (Vannoort, 2003).

[7] I.e. the Directive 2001/79/EC for feed additives and the Directive 2001/83/EC for human medicines. Although many substances (and emission routes) are shared with veterinary medicines, the environmental risks of the use of these products are not considered here.

- Route of application: topical (on the skin), oral, intra-ruminal (placed in the rumen), sub-cutane, intra-muscular or intra-venal by injection
- Type of treatment: prophylactic, curative, immune-stimulant (vaccine), homeopathic medicine, regular medicine
- Target species classes: mammals, fish, birds
- Target animal categories: companion animals, animals (not) destined for human consumption, major and minor species, major and minor use in major species, aquaculture, stabled animals, grazing animals
- Mode of action, or therapeutic class: antibacterial (antibiotics), antiprotozoal, antimyotic, anthelmintic, antiparasitic, anti-inflammatory, and agents acting on nervous systems, on reproductive systems, on the gastrointestinal system, and on the immune system
- Chemical classes.

The environmental fate and effects of such diverse substances included in the definition of veterinary medicines are, most likely, very different. Considering the difference between microorganisms and chemicals in this context is an illustrative example. Where most chemicals are expected to degrade in the environment, micro-organisms may multiply (EMEA, 1996; Montforts, 2000; Jones et al., 2003). Only chemicals will be considered further in these investigations. When referring to veterinary medicines, terms like pharmaceuticals, drug substances, drugs, and chemicals, compounds, and substances, are used interchangeably.

1.3. The extent of the consumption of veterinary medicines

By expressing the annual consumption of veterinary medicines in monetary value or weight, one gets an idea of the importance of these substances for society and the environment. Let us also consider the scale of animal husbandry operations, the consumption of human medicines, and the consumption of pesticides. The Dutch society of producers and importers of veterinary medicines (FIDIN) estimated the annual turnover reported by their members in 2001 at 165 million Euro, 6% of the European turnover (FIDIN, 2002). Table 1-1 shows the distribution over the therapeutic classes in 2001.

Table 1-1 Relative consumption of veterinary medicines by animal husbandry and companion animals in 2001 (FIDIN, 2002).

	animal husbandry [%]	companion animals [%]	total consumption [%]
Antibiotic	41	5	30
Vaccine	33	20	29
Other	13	18	17
Antiparasitic	12	31	17
Medicated feed	1	26	7

Table 1-2 Consumption of antibiotics in the Netherlands in 1999 and 2002 (MARAN, 2002).

Classes of antibiotics	kg (x 1000) in 2002 veterinary use	kg (x 1000) in 1999 veterinary use	kg (x 1000) in 1999 human use
Penicillines/cephalosporines	40	40	25
Tetracyclines	225	186	4
Macrolides	20	10	3
Fluoroquinolones	6	7	5
Trimethoprim/sulpha's	94	80	2
Other	21	27	1
Total	402	350	40

More specific consumption data on veterinary medicines are only available for antibiotics. Historical surveys have revealed that veterinary antibiotic consumption in the Netherlands has increased from 275 tonnes in 1990 to 402 tonnes in 2002. Only 2 tonnes were used for companion animals. Table 1-2 illustrates the relative importance of substance classes (MARAN, 2002).

In 2002, the animal population distribution in the Netherlands comprised of cattle (3.9 million), pigs (1.7 million), and poultry (101 million)[8]. A rough estimate of the total animal body weight amounts to 3 million tonnes in 2002. The average consumption of antibiotics by animals amounted to 150 mg per kilogram body weight in 2002.

The human consumption of non-immunological medicines is estimated at 400 tonnes in 1999 in the Netherlands (Tolls, 2001). For the antibiotics my estimate is 40 tonnes based on data from the SFK[9] and from (Janknegt et al., 2000) and (Baart and De Neeling, 2001).

The antibiotic consumption by man amounted to an approximate 50 mg per kilogram body weight per year in 1999. There is also a marked difference in the type of antibiotics used for veterinary and human treatment (Table 1-2).

The ratio between consumption of antibiotics for veterinary and human purposes in the Netherlands in 1999 was 9 : 1. In Denmark, another small country with intensive animal husbandry, the ratio was about 5 : 5, in the European Union the overall ratio is 3 : 7 (Halling-Sørensen et al., 1998; FEDESA, 2001).

In the Netherlands, pesticide consumption amounted to 8000 tonnes in 2002 (RIVM, 2003). Compared to this figure, the consumption of veterinary medicines, represented by the antibiotics, is rather small. Veterinary medicines are used in larger quantities than human medicines, especially the antibiotics. Potential ecological consequences or impacts depend,

[8] Aquaculture in the Netherlands is a small contributor. The production amounts to about 0.2% of the production of animal husbandry (Kamstra and Van der Heul, 1995; Luiten, 2002).

[9] Stichting Farmaceutische Kengetallen. Data on hospital use of antibiotics are from 1996; the same ratio between the use inside and outside hospitals was assumed for 1999.

however, on the typical use, the distribution and fate, and the toxicological profile of the substances classified as veterinary medicines.

1.4. Environmental risk assessment and management for veterinary medicines

Since it is impossible to assess the risks of all combinations of substances, uses, and environment, there is a well-established need to model the real world. Two levels of modelling are discerned in this thesis:

1. the exposure and effect assessment, and
2. the overall process of risk assessment at registration.

The objective of every individual exposure and effect model is to predict accurate exposure or effect concentrations. Exposure models describe transport, partitioning, and degradation processes, and enable us to predict concentrations in soil or water as a result of the use of a veterinary medicine. Effect models elucidate effects in model organisms or systems as a result of exposure to a veterinary medicine. These model results need to be translated to the situation of interest.

The objective of the overall risk model is to provide comprehensive information on all environmental risks related to the use of the veterinary medicines in order to optimise the risk-based decision (Di Fabio, 1994; Cranor, 1997). The level of the overall process includes all activities employed in the risk assessment procedure at registration. The integral collection of protection goals, exposure and effect models, and the conventions to apply the models and to harmonise their results, is by itself a model to assess the risk of the use of veterinary medicines. In Figure 1-1 the overall process of risk assessment is represented by the sub-levels hazard identification, exposure and effects assessment and risk characterisation. The rectangular boxes, from risk classification down to monitoring, describe the stages of risk management (Van Leeuwen, 1995).

Hazard identification is the stage at which possible effects (hazards) are characterised. In this stage questions are asked such as: Is the activity of concern (here, the use of veterinary medicines) sufficiently explored using available science?

Exposure assessment begins with the emission of the product from the source to the various compartments in the environment. It addresses all possible exposure and distribution routes, using emission and exposure models, as well as monitoring data. Underestimation of the exposure in a compartment can be avoided by making realistic worst-case assumptions. Research questions relating to exposure assessment focus on modelling approaches of distribution processes, the use of substance properties (sorption, degradation), the handling of variability and uncertainty in environmental parameters, and the definition of reference situations, to which model calculations can be compared (standardised).

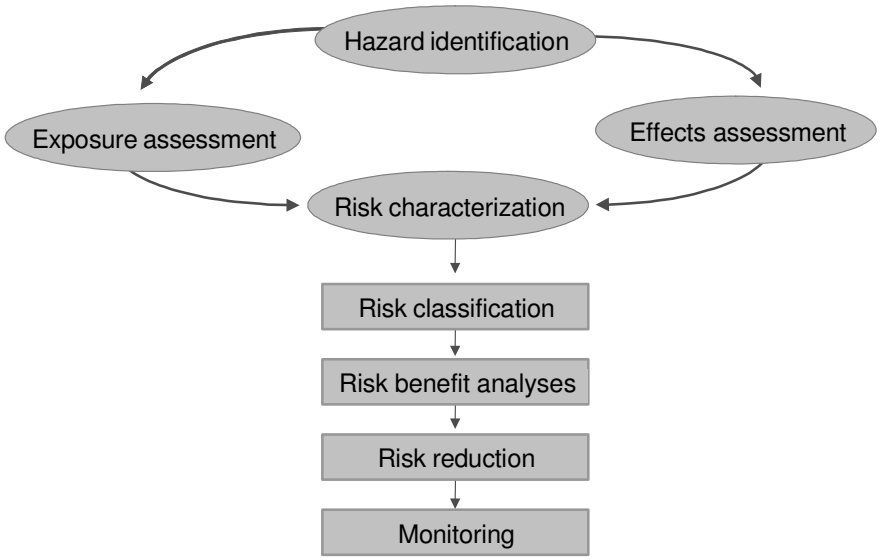

Figure 1-1 The basic framework of risk management. Hazard identification, exposure and effects assessment and risk characterisation are components of environmental risk assessment. Risk classification, risk-benefit analysis, risk reduction and monitoring are additional methods aiming at risk management.

Effect assessment or dose-response assessment is the estimation of the relationship between the level of exposure to a substance, and the incidence and severity of an effect. In environmental risk assessment (ERA) millions of species and processes may be exposed to contaminants by a variety of pathways. Effect assessment addresses all hazards identified, using dose-effect models and monitoring data, as well as the integration of the various effect model results, in e.g. predicted no effect concentrations (PNEC) or in probabilities (Posthuma et al., 2002). Research questions relating to effect assessment focus on the use of substance properties, the selection of relevant test species, test data and endpoints, the handling of uncertainty in these data, the justifications of extrapolation methods to unknown species, and harmonisation of endpoints to endpoints in other compartments.

Risk characterisation combines the information gathered, for example in a Risk Characterisation Ratio (RCR) that expresses the ratio of the predicted exposure concentrations (PEC) over predicted no-effect-concentrations (PNEC): PEC/PNEC. The modelling approach in the exposure assessment should relate to the mode of application of the veterinary medicine and should be harmonised with the effect assessment endpoint. Research questions in this stage relate to this harmonisation of data.

Risk classification is based on the total set of RCRs. Criteria that define the groups have to be agreed upon. Research questions relate to the choice of endpoints that are classified (for what compartments and hazards have RCR (not) been derived?) and the way these endpoints, and the criteria they are held against, are weighted and scaled.

Risk-benefit analysis. Decisions regarding classification or individual RCRs rely on regulatory choices that either dictate or exempt further assessment or risk mitigation measures. This choice will depend on uncertainty in the models as well as on the political or economic implications of mitigation measures (risk-benefit analysis) (Di Fabio, 1994). Risk-benefit analysis is not further addressed within the scope of this thesis.

Risk mitigation encompasses all the regulatory actions intended to diminish the environmental impact of the use of veterinary medicines. These measures may be directed to either the competent authorities that are responsible for the quality of soil, surface water, or drinking water, or to the users of the medicinal product. This research investigates which mitigation measures might be included in the methodology for the registration of veterinary medicines, as far as they are substantiated by the risk assessment.

Monitoring. This is the stage in risk management that aims to generate information on the accuracy of the risk assessment, and of the risk mitigation measures. Typically, for the authorisation of veterinary medicines and human medicines, a system of monitoring was created to respond adequately to unexpected effects of the use of medicines. According to Article 73 of Directive 2001/82/EC, member states are required 'to establish a veterinary pharmacovigilance system that takes into account any available information related to investigations on potential environmental problems'. This final stage in risk assessment is not further explored in this thesis.

The administrative process of the registration of medicines is mandated to the European Agency for the Evaluation of Medicinal Products (EMEA)[10]. The EMEA consists of a board, formed by two representatives from each member state, two from the European Commission, and two from the European Parliament (EP), and a staff. The EMEA functions among others as the secretariat to the scientific Committee for Veterinary Medicinal Products. The CVMP consists of independent scientists (two from each Member State) and formulates opinions on requests for registration with respect to quality, efficacy and safety (environment included) of the products. CVMP and EMEA also produce guidance documents on risk assessment. The first guidance document was released in 1997 and provided a comprehensive risk assessment methodology. After the release of the EMEA (1997) guidance, an international harmonisation of the guidance between the EU, USA and Japan was initiated by the International Co-operation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH)[11] to which both the European Commission and the EMEA are committed (DG Enterprise, 2000). The guidance document on Phase I was implemented by July 1st 2001 in the European Union and United States (VICH, 2000) and replaced the EMEA 1997 guidance on Phase I, the phase predominated by the exposure assessment. Currently (April 2004), the draft VICH guidance on Phase II, the

[10] Commonly referred to as the European Medicines Evaluation Agency. The name is changed by the Regulation (EC) 726/2004 to European Medicines Agency.

[11] Commonly referred to as the Veterinary International Conference on Harmonisation.

phase in which the risk assessment is conducted, is still engaged in the consultative process. For this reason this guidance document is not considered here.

In this thesis, the risk assessment methodology used in the registration framework of veterinary medicines is validated against the risk approach presented above. Validation is a word that is frequently misinterpreted. The word suggests a search for truth and through combinations of models it gives the impression of being all about creating the perfect model. Validation is not about creating models. Validation is a process of substantiating explicit claims on the applicability of predictions with reference to the intended purpose of the model. Validation is also concerned with the accuracy of these predictions for the system that is represented (Dee, 1995). All models are, by their nature, incomplete representations of the system they are intended to model, but, in spite of this limitation, models can be useful. Strictly speaking, a model cannot be validated in the sense that the validation proves that the model is true, only whether the model is well founded and applicable (Addiscott, 1998). Some models cannot be validated, but components or modules of the model can be validated on an individual basis. Dee (1995) has identified four major aspects associated with model validation, as follows:

1. Conceptual validation
2. Validation of algorithms
3. Validation of software code
4. Empirical validation of the functionality.

Conceptual validation contributes to a better understanding of the information generated in the risk assessment and to the transparency of the decision making process. Conceptual validation concerns the question of whether the model accurately represents the system under study. Was the simplification of the underlying process in model steps realistic; i.e. were the model assumptions credible? Usually, conceptual validation is largely qualitative, although experimental or observational data in support of the principles and assumptions can be integrated. Conceptual validation makes the consequences of the choices on what variables and relationships in the natural system are formalised in the model, explicit.

Algorithm validation concerns the translation of model concepts into mathematical formulae. Software code validation concerns the implementation of mathematical formulae in computer language. These aspects of validation are of marginal concern here.

Most validation studies do not refer to the way a model is assembled, but regard it as a black box: an input-output function, which might represent the system of interest. This approach, where empirical observations are compared to model predictions, is denoted functional or empirical validation.

Typical of risk assessment is utilisation of both scientific data and normative assumptions, and that both scientists and regulators determine the outcome of the risk assessment process. On one hand, regulators must indicate what should be assessed (hazard identification), what levels of protection should be taken as protection goals, and are simultaneously required to make risk-benefit decisions. The scientific expertise that

regulators are supposed to take into account must be objective and of high calibre[12]. On the other hand, scientists are required not only to provide information on the relevance of these hazards, but also to assess the fate and effects of the substances in a way that addresses the concerns, the standards, and the uncertainties[13].

The validation exercise performed here addresses the quality of the (modelling) science applied, including the use of this science in a regulatory context. Two levels of modelling were discerned in this thesis: the level of individual fate and effect models used in exposure and effect assessment, and the integral level of the assessment methodology for the environmental risk arising from the use of veterinary medicines. The validation is predominantly of a conceptual nature, but where possible, empirical validation of individual exposure models is performed. A profound research has recently been performed in a similar way on the uncertainty in environmental quality standards (Ragas, 2000).

A broader view on the strategic arena in which science is applied to develop guidance on environmental risk assessment and to execute assessments is necessary. Concerning the aspect of objectivity of science, there are potential controversies that require a carefully designated playing field, where science can be impartial and authoritative. One is at the demarcation line between science and regulation: who decides what should be investigated or protected? When is this protection goal achieved? The second is the choice of scientific disciplines: what science is allowed, whose scientists are selected? The third is the actual weight science is given in the process of decision making (Cranor, 1997; Joerges et al., 1997; Heyvaert, 1999a; Heyvaert, 1999b; Breyer and Heyvaert, 2000; Halffman, 2003).

The following research topics on model validation and on the interaction between science and regulation are addressed in this thesis.

1. **Harmonisation of protection goals and risk assessment methodology**
   - What relevant environmental protection goals can be considered?
   - Does the integral risk model address the protection goals?
2. **The conceptual and empirical validation of models and precautionary labelling**
   - Are screening level exposure models for surface water in aquaculture, for dung excreted by grazing animals, and for soil and water in intensive animal husbandry well founded and applicable?
   - Is the soil trigger value based on effect data functional and validated?
   - Can the efficacy of mitigation measures be demonstrated by the methodology used to predict the risk?
3. **The use of science in the registration framework**
   - Is science applied transparently and impartially in the development of risk assessment methodology and in the decision making for product registration?

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[12] Based on the rulings of the European Court of Justice (ECJ) in case C-41/93 PCP [1994] ECR I-1829 (Joerges et al., 1997 p. 319)

[13] According to the European Court of Justice (case C212-91 Angelopharm): "the Scientific Committee is the only party involved in the policy-making process that is competent to make those scientific and technical assessments on which the legal validity of the measures depends" (Heyvaert, 1999a).

1.5. Readers guide

The following chapters are publications submitted to or published in peer-reviewed journals or books. The publications cover one or more of the stages in the risk assessment process, and address one or more of the research questions outlined above. The relation between the contents of the chapter, the research questions and the other publications, is described in the short introductions below.

Methodological aspects concerning the environmental risk assessment for medicinal products: research challenges

This chapter takes the European legislation and guidance documents for the risk assessment, as a starting point in a conceptual validation exercise on the relation between protection goals, risk models and methodology. It provides a basis for this exercise, that will be continued, studied in depth, and repeated in the following chapters, by highlighting possible hazards and regulatory protection goals, and introducing concepts on risk assessment and harmonisation of models and effect endpoints.

The particular case of the human medicines is outside the scope of this thesis. However, the observations made are suitable as case studies for veterinary medicines as well.

The chapter focuses on remaining research needs for the environmental risk model of human and veterinary pharmaceuticals; in other words, on those items that may be considered to invalidate the risk model in the relation between protection goals, methodology and risk mitigation.

The exposure assessment of veterinary medicinal products

This chapter highlights a selection of exposure models for considering effects of veterinary medicines related to the following animal sources and receiving compartments: aquaculture and surface water and soil, grazing animals and dung, and stabled animals and slurry and soil. It investigates the selection of parameter values, such as number of applications, storage time and degradation rate. The implications of these findings are discussed in the light of the risk model set by the European Guidance document in 1997. Some other features in this risk model are discussed and considered for further research. It is a first step in the conceptual validation of the exposure assessment methodology that questions the high calibre of the science applied and the implications of choices made.

Validation of the exposure assessment of veterinary medicinal products

This chapter investigates the validity of exposure and distribution models for soil, groundwater and surface water, applied for veterinary medicines that reach the soil by contaminated slurry. The removal efficiency of substances in settling tanks, used in the previous paper, was verified with data from mushroom and flower bulb industries. Transport (mass transfer), concentration and impact of substances are influenced both by the environment and the substance. Environmental factors such as soil and climate are subject to a considerable spatial and temporal variation. Field measurements were used to functionally

validate the models: Do the models predict the field results? This represents a second step in the conceptual validation of the methodology: Is the methodology well founded, and what are the implications of choices made? The second objective of this chapter was to develop scenarios for the exposure assessment under different European conditions, incorporating information on agricultural and veterinary practice, land use, geomorphology and climate. Using the scenarios it will be possible to facilitate national, mutual, and central registration procedures, and European harmonisation of risk assessment methodology for chemicals.

Effect assessment at the base of an exposure trigger in soil – a critical appraisal

In the EU guidance documents a limited environmental risk assessment is foreseen for veterinary medicines with a presumed negligible exposure level in the soil compartment. The regulatory trigger value has been substantiated with a scientific assessment of ecotoxicological data. The science of ecotoxicology offers various tools to assess the presented data. This article focuses on the selection of tools and the scientific argumentation used, and will demonstrate that with the same information and tools, trigger values in a range of up to three orders of magnitude are justifiable.

European medicines and feed additives regulation are not in compliance with environmental legislation and policy

This chapter investigates transparent application of science in the drafting of environmental risk assessment methodology for veterinary products, and how science is used in the decision-making. The interactions between science and regulation in the drafting of the guidance document for the Phase I assessment are explored.

Legal constraints on special precautions in product labelling to mitigate the environmental risk of the use of veterinary medicines in the EU

This chapter concludes the validation of exposure models, of the use of science, and of the relation between science and regulation. It investigates what possibilities and obligations are created within the registration framework to bind authorities, applicants, and users to instructions and prohibitions. Effective risk mitigation measures could remove the need for refusal of product authorisation, or of risk-benefit analyses. This chapter analyses the contributing factors to effectiveness of mitigation measures. Risk mitigation is part of the risk management process, but as far as mitigation measures are (suggested to be) based on exposure or effect assessments, there is a direct relevance for the risk model.

Discussion

The findings from the presented research are summarised and discussed in coherence. First, the degree of harmonisation of protection goals and risk assessment methodology is discussed. Next, the implications of the conceptual and empirical validation of models and precautionary labelling are considered. Finally I present considerations on the use of science in this registration framework. In the light of recent developments in the risk assessment strategy in the registration framework, some suggestions on the way forward are given.

2. Methodological aspects concerning the environmental risk assessment for medicinal products; research challenges

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2.1. Introduction

The fate and behaviour of pharmaceuticals in the environment have been studied since several decades (Zondek and Sulman, 1943; Soulides et al., 1962; Tabak and Bunch, 1970), and the presence and effects of residues in the environment is a concern that has been identified not long after that (Berland and Maestrini, 1969; Manten, 1971; Blume et al., 1976; Rurainski et al., 1977; Patten et al., 1980). More recently several reviews on use, emission, fate, occurrences and effects of pharmaceuticals have been published and at national and supra-national regulatory levels the environmental risks of pharmaceuticals are on the agenda (Roij and De Vries, 1980; Römbke et al., 1996; Ternes, 1999; Jorgensen and Halling-Sørensen, 2000; Daughton and Jones-Lepp, 2001; Kümmerer, 2001; Halling-Sørensen et al., 2002; Dietrich, 2002; Boxall et al., 2004).

The environmental risk of the use of medicinal products is currently assessed at registration. The methodology has not been finalised yet (EMEA, 1997; EMEA, 2000; VICH, 2000) and suggestions for risk assessment methodology are given by several authors (Spaepen et al., 1997; Daughton and Jones-Lepp, 2001; Römbke et al., 2001a; Römbke et al., 2001b; Länge and Dietrich, 2002; Koschorreck et al., 2002; Schowanek and Webb, 2002). The proposed risk assessment procedure at registration of human medicines and veterinary medicines is discussed by several authors (Gärtner, 1998; De Knecht and Montforts, 2001; Montforts and De Knecht, 2002; Koschorreck et al., 2002; Long and Crane, 2003). Considerations on the assessment of pharmaceutical feed additives are given by Jorgensen et al. (1998).

This chapter focuses on research needs for the environmental risks of human and veterinary pharmaceuticals. National and European regulators are involved in managing environmental risks of pharmaceuticals from two perspectives. One is the regulation of pharmaceutical products, and the other is the management of a good environmental quality (Montforts and De Knecht, 2002). The chapter takes the registration assessment of medicinal products as a starting point in a validation exercise on the relation between protection goals, risk models and methodology, and will highlight research challenges.

The terms medicine, pharmaceutical, and drug will be used interchangeably here, but please note that registration has concerns for a product: a veterinary or human pharmaceutical, containing active ingredients (substances) and excipients, and that environmental quality policy deals with substances in compartments, and activities of legal persons concerning the emission of substances. A drug at registration is a product with a

certain intended use, whereas a drug in the environmental quality policy is a substance (be it a parent compound, pro-drug, or metabolite) emitted to, or present in, an environmental compartment.

2.2. Protection goals

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. As a general observation it is stated here that the primary goal of any environmental assessment should be risk mitigation and risk management. In order to mitigate or accept risks, a risk assessment has to be performed, both for products (e.g. drugs) and for activities (e.g. emission of drug residues). At registration it is possible to lay the burden of proof on the applicant (the principle that the polluter pays). The decision-making process and the risk models used should optimise (reduce) the costs to society in terms of environmental damage (due to false negatives implying registration of harmful products) and economic damage (due to false positives implying refusal of harmless products). Also the assessment process itself should neither hamper product development nor timely action (Cranor, 1997). Should the assessment remain inconclusive on the acceptability of the risk, further action depends on the cost-benefit analysis. Risk assessment is a key process in which both regulators and scientist determine the outcome (Joerges et al., 1997). On one hand, regulators have to indicate what should be assessed (hazard identification) and what level of protection should be taken as protection goals, and have to make risk-benefit decisions. On the other hand, scientists are required not only to provide information on the relevance of these hazards, but also to assess the fate and effects of the substances in a way that addresses the concerns, the standards, and provides suitable information for the risk-benefit analysis.

A risk assessment can only be performed, once the protection goals and the assessment methodology have been developed. The Directives 2001/82/EC and 2001/83/EC on the registration of pharmaceuticals do not contain explicit environmental protection goals, only procedural directions. Only the EU Directive 2001/82/EC on veterinary medicinal products contains some directions on the risk assessment model and decision making approach. It is stated that the assessment shall normally be conducted in two phases. In phase I, 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 phase II, 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. Commission Directive 93/67/EEC elaborates further on these protocols.

The decision making scheme (decision tree) has been fixed on a general level, and it is indicated that both fate and effects of drugs should be assessed. It is left to the scientific community to decide what information is to be generated and when the assessment is ended[14].

Important sources of information on protection goals are the legislation and policy documents concerning environmental quality (Heyvaert, 1999b). Focusing on the legislation for environmental quality, following the precautionary principle laid down in the Water Framework Directive (2000/60/EC), surface water and groundwater must be regarded as natural resources, which should be protected in their own rights. The EU included in the 6th Environmental Action Programme an outline for a future "thematic strategy on soil protection", which should lead to a European soil protection policy with adequate legislation in place. Most member states (if not all) have national legislation on soil quality. All this legislation operates from the starting point that all actions that may lead to pollution are forbidden unless a permit is granted by the competent authority. Thus, to emit or spread residues of medicines one needs a permit. The permit ought to regulate emission (e.g.

[14] On the European level this scientific community (CPMP and CVMP) consists of independent scientists appointed by the national authorities for registrations (Blasius and Cranz, 1998).

prescribing application techniques) as well as the maximum permissible concentration of the substance in the environment. The competent authorities should thus derive these quality standards for every substance of interest. Also, they have to develop action plans for the local resource management[15] (Van Rijswick, 2001).

It is very well possible that existing European directives on the environmental quality of water already contain standards for medicines, even though the product group 'medicines' is not named in the environmental directives 76/464/EC and 80/68/EC. 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 (Montforts and De Knecht, 2002). Once in the environment, the competent authority is not concerned with the intended use of the compound, but with 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, oxytetracyclin, 4-aminopyridine, paracetamol, warfarin, and cypermethrin.

The quality of drinking water is protected under the Directive 98/83/EC. This directive aims at protecting public health by setting quality criteria to drinking water. Within the Netherlands it has been environmental policy 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 (TK, 1989). To all substances that qualify as such, a numeric standard is already available for drinking water (for 'pesticides' 0.1 µg/L), and at least in the Netherlands, also for groundwater.

The registration process of products should thus primarily be concerned with the level of no effect (maximum permissible concentration) and the risk that this level will be exceeded. It applies to water (surface water and sediment, groundwater, drinking water) based on European legislation, and to soil based on national legislation. When a level of no effect (predicted no effect concentration PNEC), or an acceptable effect concentration, is reached is open for scientific and political debate. For example in the Netherlands, the level of no effect is considered to be eminent at the ecosystem level, and is defined at a level at which 95% of the species are protected at the no-observed effect concentration (NOEC). This analysis assumes a certain distribution of toxicity data representing the ecosystem sensitivity to the given substance (ECB, 1996; Crommentuijn et al., 2000; Forbes and Calow, 2002c). Ecosystem functionality and structure are thus protected when critical *concentrations* affecting population dynamics (growth, reproduction, and mortality) are not surpassed. Process parameters at population level such as C- or N-cycling, or resistance development, can be incorporated in deriving the critical concentration (Traas, 2001; Wösten et al., 2001).

[15] Medicines are acknowledged as a specific group of substances in the Netherlands' 4th Water Action Program (NW4, 1998).

2.3. Research challenges

As stated above, the risk assessment targets a desired level of quality. The assessment methodologies translate the protection goals in quantities: for example probabilities, concentrations, dosages, and risks. The protection goal (no effect) is generally pursued by assessing a reasonable 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 (e.g. nation, water catchment) and the impact itself are acceptably small. When this reasonable worst case exposure leads to concentrations below the maximum permissible concentrations, the risk that the level of no effect will be exceeded is considered acceptable.

Methodology, protection goals and decision making are strongly interconnected. The environment is at risk when a product reaches the environment. Transport (mass transfer), transformation, concentration, and impact of substances are influenced both by the environment, the substance, and the receptor (e.g. the species or populations). Environmental variables such as soil, climate, and receptors are subject to a considerable spatial and temporal variation. Because it is impossible to assess the risks of all combinations of substances and environment, 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.

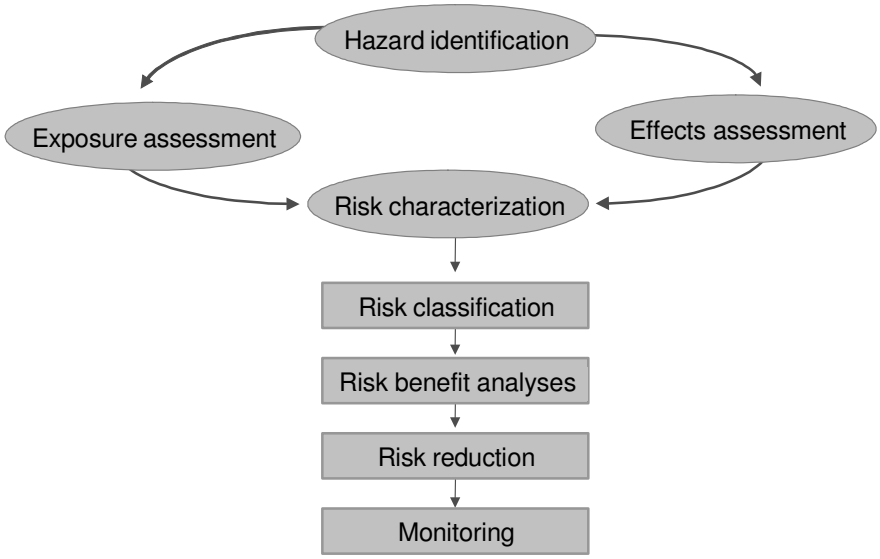

Figure 2-1 The basic framework of risk management. Hazard identification, exposure and effects assessment and risk characterisation are components of environmental risk assessment. Risk classification, risk-benefit analysis, risk reduction and monitoring are additional methods aiming at risk management.

Two levels of modelling are discerned: the level of the complete risk model covering the environmental risk assessment process, and the sub-level of the fate and effect models. The risk model includes all activities employed in the risk assessment process, including their harmonisation. It addresses the overall protection level: no effect. In Figure 2.1 the risk model is represented by the ovals containing hazard identification, exposure and effects assessment and risk characterisation.

Hazard identification is the stage at which possible effects (hazards) are characterised. Exposure assessment starts from the emission of the product to the different compartment and addresses all exposure routes, using emission and exposure models, and monitoring data. Effect assessment addresses all hazards identified, using dose-effect models and monitoring data, as well as the integration of the effect model results. Risk characterisation combines the information gathered. The risk model is as good as the weakest link in the model, be it an exposure model, an effect model, unidentified exposures or effect, the interpretation of effect data or the integration of exposure and effect. The rectangular boxes, from risk classification down to monitoring, belong the stage of risk management.

Ultimately, the quality of the assessment that can be achieved will depend upon the adequacy of available data as well as a suitable choice of model and modelling parameters (Dee, 1994; WRc-NSF, 2001). 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 (Brouwer et al., 1994; Resseler et al., 1997; Uffink and Van der Linden, 1998; Van der Linden and Van Beek, 1999). Modelling at levels of no concern requires a rigorous understanding of all relevant transport, fate and effect 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 (cf. Forbes and Calow (2002a) and Tarazona et al. (2002)). In an ideal situation, the assessment at registration functions as a tool in maintaining a good environmental quality.

Below the risk models for veterinary and human medicines are presented and regulatory needs and research challenges are indicated.

Veterinary medicines: protection goals and risk models

The European Agency for the Evaluation of Medicinal Products (EMEA)[16] has published guidance on the environmental risk assessment (ERA) of veterinary medicinal products (VMPs), and this assessment was implemented in 1997 (EMEA, 1997). 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, and 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

[16] Commonly referred to as the European Medicines Evaluation Agency.

Medicinal Products (VICH)[17] to which both the European Commission and the EMEA are committed (DG Enterprise, 2000). The guidance document on Phase I was implemented by July 1st 2001 in the European Union and United States (VICH, 2000) and replaced the EMEA 1997 guidance on Phase I. This guidance document is at this moment leading for the registration procedure.

Within the VICH guidance document a limited assessment is foreseen for substances with a generally accepted low hazard (e.g. vitamins, electrolytes), and with a presumed negligible emission and exposure level. The exposure level that is considered negligible for the total environment is quantified both for effluent and soil for some groups of compounds and several routes of emission: 1 µg/L and 100 µg/kg, respectively (Phase I), for residues reaching waste water through confined fish rearing facilities or reaching soil via manure application. These triggers are substantiated with an assessment of a dataset of toxicity values of several antibiotics, although the assessment to determine the value of the trigger is criticised from an ecotoxicological point of view (De Knecht and Montforts, 2001). It is crucial to note that the soil trigger is based on soil toxicity data, but also determines the eventual assessment of groundwater and surface water exposed through soil.

The triggers apply to a total residue, regardless of the actual substances in the residue (mixture of metabolites and active ingredients). Using this concept, the Phase I assessment addresses the entire product. However, a further use of substance related fate and effect data in exposure or effect assessment is questionable, because it is not defined what compound should be modelled.

Not just these exposure trigger values define the desired level of quality for soil and effluent. Should the Phase I triggers be breached, or should the product be applied to grazing animals or open water facilities, a further assessment in Phase II, as published by (EMEA, 1997), is risk based, and both exposure and effect are assessed. The VICH Phase I assessment does not seamlessly connect to the EMEA Phase II assessment. Phase II defines the substances and the environmental criteria that need to be assessed: substance persistency and bioaccumulation, and risks to soil, groundwater and surface water. Both intrinsic substance properties (insecticidal activity) and a risk quotient for earthworms define the extent of data requirements for grazing animals. Toxicity to grassland invertebrates and predators is also to be assessed. Whenever the soil is reached, persistency and sorption may trigger further standards and data requirements. Phase II makes use of several acceptability triggers:

- Specific risk ratios for taxonomic groups (plants, earthworms, micro-organisms)
- effect levels for single dose tests (arthropods and dung fauna)
- persistency levels for soil
- PEC/PNEC risk ratio for aquatic systems
- Expert judgement for bioaccumulation.

Breaching these acceptability triggers leads to a further refinement of the risk assessment on the trigger of concern.

[17] Commonly referred to as the Veterinary International Conference on Harmonisation.

The risk model challenges research on different aspects of the model. Some of these are addressed below. The risk model employed does not systematically address all environmental concerns identified above (i.e. groundwater), but leaves ample room for scientific input and assessment of e.g. persistency and bioaccumulation properties. The protection goal is addressed in several risk and hazard based endpoints, both for the terrestrial and aquatic compartment. The protection goals have not been characterised to an extent that boundary agreements for exposure and effect models have been set (e.g. time frame).

If the assessment aims to establish conditions under which an acceptable risk is present, model and data requirements may differ from those in a risk model that identifies the worst case. For example, the load of a residue in manure or slurry to soil is driven by the amount of manure applied. Under the Nitrate directive 91/676/EC vulnerable areas in river catchments are assigned, and in those areas immission standards for the nitrate in the slurry apply. A risk assessment for these areas establishes acceptable risks, but not worst case risks.

Degradation of the veterinary drug in the target animal and/or during storage of manure, and/or in soil are aspects of the environmental risk assessment that were mentioned in the Phase II guidance as information that may be considered in refining the PEC. The guidance does not provide the details on for example, standardisation of laboratory test results, repetitions in exposure, and time intervals, thus leaving these refinements to expert judgement. In Phase II all active ingredients and all metabolites formed >20% at metabolism or in environmental compartments are to be assessed. The guidance is unclear whether information on transformation (animal-slurry-soil-water) is compulsory or not after phase I.

Following a total residue approach a challenge lies in the assessment of the fate and effects of the residues through manure and soil. The total residue has no intrinsic properties (e.g. sorption, degradation) that can be determined and plugged into models that require this information. The different compounds in the residue probably cover a large range of properties: persistent to readily degradable, strongly adsorbing to weakly adsorbing, high impact to no effect. There are no directions how to determine the properties, or model compounds, that should be used to refine or advance the assessment of the total residue.

The impact on nitrification processes in soil is assessed at registration, but effects of some antibiotics on nitrification and decomposition in soil have been reviewed and the few studies available indicate effects at rather high concentrations only (Jensen, 2001; Thiele-Bruhn, 2003). Test duration and test type may play an important role however (Backhaus et al., 1997; Halling-Sørensen, 2001). The effects of antibiotics on the microbial community can range from simple parameters like a decrease in biomass, respiration rate or denitrification rate, to more complex parameters like the survival of genetically engineered micro-organisms (Landi et al., 1993; Badalucco et al., 1994; Da Gloria Britto De Oliveira et al., 1995). Therapeutic doses of chlortetracycline in cattle have been found to alter the rumen microflora, hence the possibility exist that it alters the nature and activities of the microflora participating in the decomposition process both in dung and in slurry (Elmund et al., 1971; Patten et al., 1980; Poels et al., 1984; Sommer and Bibby, 2002). Could other effect models and effect assessment approaches provide more relevant information (Van Beelen and Doelman, 1997; Salminen et al., 2001; Schmitt et al., 2004)?

The survival of adapted bacteria in absence of the compound that the bacteria have adapted to, is usually said to be limited, but the acquired functionality (e.g. resistance genes) remains present at low levels (Cooke, 1983; Stappen et al., 1989; Zuidema and Klein, 1993; Séveno et al., 2002; Park et al., 2003). The costs for resistance can however be compensated for (Björkman et al., 2000). An additional concern is hence found from the perspective of resistance development and transfer. This process is triggered at the Minimum Effect Concentration (MEC) at which growth is reduced (O'Reilly and Smith, 1999), which is tenfold below the Minimum Inhibitory Concentration (MIC), the endpoint used by EMEA to derive a safe exposure level in soil for antibiotics at phase I (AHI, 1997). This indicates that at and below the MIC level a selection pressure for resistance is present. Thus, even at concentrations below the Phase I trigger, resistance genes may be favoured, which can be transferred from manure to soil and groundwater (Chee-Sanford et al., 2001; Halling-Sørensen et al., 2002; Sengeløv et al., 2003). The management of resistance development in water and sediment face comparable challenges (Grabow et al., 1976; Cooke, 1983; Linton et al., 1988; Rodgers, 2001; O'Reilly and Smith, 2001). Should resistance development be identified as a hazard? And if so, how can it be used in decision making, knowing that it also applies to antimicrobial products used as pesticide and biocide (Séveno et al., 2002; Mcbain et al., 2002; Russel, 2002)?

Human medicines: protection goals and risk models

The EMEA has published a draft guidance on the environmental risk assessment of human medicines, but this guidance was not yet implemented in 2003 (EMEA, 2000). Emission to the environment is primarily foreseen through wastewater. In phase I a trigger of 10 ng/L in surface water was proposed to proceed to risk based assessment providing for a PEC/PNEC risk ratio for aquatic systems. Breaching this acceptability trigger leads to a further refinement of the risk assessment.

The predicted exposure concentration (PEC) is based on a simple dilution model, in which the total annual consumption is diluted over the total amount of wastewater produced. The concentration in wastewater is further diluted to surface water using a default dilution factor of 10. Retention in wastewater treatment plants (WWTPs) can be accounted for. The predicted no effect concentration is derived from a base set of aquatic toxicity data in accordance with Directive 67/548/EEC, and assessment factors according to the Technical Guidance Document for New and Existing Substances (TGD) (ECB, 1996).

The protection goal is narrowed down to, or represented by, the aquatic environment, which is exposed through wastewater. Before a risk assessment is performed, an exposure level has to surpass an action limit. The calculation of the exposure level is guided, not prescribed. It depends on the interpretation of the input data what outcome is generated, as indicated below:

- annual consumption: should seasonal and regional differences be taken into account (Abbas and Kratz, 2000; Cars et al., 2000; Baart and De Neeling, 2001)?

- how is a removal percentage in WWTPs determined: how can one translate the result of fate models (laboratory of field scale) to the representative exposure model (Tabak et al., 1981; Kümmerer et al., 2000; Balcioglu and Ötker, 2003).
- are WWTPs expected to be present in all urbanised areas (EC, 2001d)?;
- what is an appropriate dilution factor for effluent to surface water? In the Netherlands, 40% of all WWTPs (n=466) have a dilution factor of 1-10 within 100 metres. In the Netherlands, 40 out of 83 of the domestic WWTPs at tributaries (48%) and 48 out of 126 of the domestic WWTPs at polders (38%) have a dilution factor of 1-5 within 100 metres. About 60 of these domestic WWTPs (c. 20%) have a dilution factor of 2 within 100 metres; an extrapolated number of 15 (c. 5%) is expected to have a dilution factor of 1. In trench-like waters, owing to the low flow, only poorly developed turbulence is likely to occur from time to time. Hence, in polder waters and the like, it is to be expected that noticeable lengths of these channels be gradually filled with poorly diluted effluent. In these situations, at least about 20% of all domestic WWTPs in the Netherlands, a dilution factor of 1 is very well be applicable (De Greef and De Nijs, 1990).

If a risk assessment is performed, are the actual hazards investigated in an adequate way?

- are acute base set studies on algae, daphnids, and fish representative for continuous exposure (Berard and Benninghoff, 2001; Daughton and Jones-Lepp, 2001; Huggett et al., 2002; Ferrari et al., 2003)?
- are the common lethality, growth and reproduction endpoints representative (Hartmann et al., 1998; Chee-Sanford et al., 2001; Forbes and Calow, 2002b)?
- Are the effect models (model species and test designs) vulnerable to medicines (Fong, 1998; Thorpe et al., 2001; Länge and Dietrich, 2002; Brooks et al., 2003; Pro et al., 2003; Cleuvers, 2003)?

Is the risk model actually covering the environment?

- Given the hazard of groundwater and drinking water contamination, how should the risk be assessed? Are exposure triggers desirable, and how should exposure and effect be assessed (Webb, 2001a)?
- The human pharmaceuticals guidance focuses on surface water through waste water discharge, which in turn can connect to groundwater (Tröger, 1997; Heberer et al., 1998; Seiler et al., 1999; Kuch and Ballschmiter, 2001). Protection of surface water protects groundwater in this way, but are the quality standards the same (Notenboom, 2001)?
- The possibility of transfer of drug residues via sludge from sewage treatment plants to soil has been included in updates of the guidance in accordance with the TGD (ECB, 1996). In view of the total residue approach: are model calculations performed using the most relevant data?
- Literature indicates that transport out of the site to surface water, groundwater or drinking water wells may occur (Holm et al., 1995; Ahel and Jeličič, 2001), and that this general process can be modelled and assessed (Mills et al., 1999). Should the fate of drug residues in landfills be addressed?

2.4. Pharmaceuticals in drinking water: a comparison of human and environmental risk assessment

Groundwater, and as a derivative, drinking water pollution, are two hazards that are addressed both from a public health and an environmental point of view. The available public literature on pharmaceuticals in the environment was reviewed in 1996 by the German Ministry of Environment and in 2001 by the Dutch Institute for Inland Water Management and Waste Water Treatment (RIZA) (Römbke et al., 1996; Derksen et al., 2001; Jongbloed et al., 2001). The measured concentrations that were reported are summarised below (MC values in Table 2-1). In this section no attempt is made to be complete on all monitoring data in drinking water (Heberer, 2002; Sacher et al., 2003). Mostly maximum values are reported when ranges were available. It should be noted that information on negative samples, sampling strategy, and other compounds, is not used.

Based on the results obtained for the analysis of surface and groundwater in other European countries (Germany, Switzerland, Denmark, and United Kingdom) and the consumption of drugs, 13 pharmaceuticals were selected for drinking water analyses in the Netherlands by RIVM. Most of the 13 pharmaceuticals are medium polar and polar substances; therefore, liquid chromatography was the separation method of choice. As regards detection, the use of MS/MS will allow us to combine screening and confirmation in one procedure. Details of the analytical method are described in Stolker et al. (2004). The set of compounds included sulphamethoxazol, paracetamol, metoprolol, carbamazepine, diclofenac, bezafibrate, erythromycin, fenofibrate, acetylsalicylic acid, clofibric acid, ibuprofen, bisoprolol and chloramphenicol. With the described method, all compounds could be determined in surface water, ground- and drinking water with limits of detection ranging from 1-10 ng/l. The repeatability standard deviation ranged from 2-12% at the concentration of 100 ng/l (n=5). The within laboratory reproducibility (%RSD) at the same concentration level of 100 ng/l ranged from 4-29% (n=10). These results are very satisfactory for this type of analysis.

The identities of the compounds detected in real-life water samples were confirmed by using the EU draft guidelines for the identification of micro-contaminants, EU commission decision 2002/657/EC (EC, 2002a). Conform to these criteria all positive (screening) samples were re-injected and for the confirmation of the identity of the pharmaceutical compound two MS/MS ions were monitored and the ion ratios were checked against the reference ratio of standards or fortified samples.

The LC-MS/MS procedure has been used for the monitoring of surface, drinking- and groundwater within the Netherlands. In the spring of 2002 samples were collected at different spots all over the Netherlands. Finally the total amount of 15 groundwater, 29 surface water and 22 drinking water samples were screened for the pharmaceuticals. Table 2-1. shows the overview of number of samples found positive for the specific pharmaceutical compounds.

In all samples of water (acetyl)salicylic acid was detected. With the described method it was not possible to distinguish between salicylic acid and acetylsalicylic acid. Due to the fact that salicylic acid was detected in the real 'blank' samples of pure demi-water to a maximum of 50 ng/l, only those water samples containing concentrations of salicylic acid exceeding 50 ng/l were counted as real positive samples. In none of the samples fenofibrate, chloramphenicol, ibuprofen or paracetamol were detected.

From the results presented in Table 2-1. it can be concluded that for only a few samples of drinking water positive results were obtained. One sample of drinking water was positive for sulfamethoxazole and one sample was found positive for diclofenac, two samples were positive for (acetyl)salicylic acid and two for clofibric acid. A second conclusion is that all samples of groundwater contained one, two or three pharmaceutical compounds. Most frequently (acetyl)salicylic acid, carbamazepine and clofibric acid were detected. A third conclusion is that all surface water samples (not shown in Table 2-1) contained 2 to 8 different pharmaceutical compounds per sample (cf. Kolpin et al. (2002)). Two samples contained carbamazepine at the level of > 100 ng/l. Most frequently (acetyl)salicylic acid, carbamazepine, sulfamethoxazole and diclofenac were detected.

Toxicological limits of the 13 medicines plus ethinylestradiol in drinking water were determined. The limits are based on 10% of the ADI (acceptable daily intake) or the MRL (maximum residue limit) for milk determined for veterinary medicines, an average consumer bodyweight of 60 kg and a drinking water intake of 2 litres a day. If the medicine is not used as a veterinary medicine, the public databases are used to determine whether an ADI is determined for other purposes. If no ADI or MRL are available, a provisional ADI is determined from the lowest pharmacological effective dose and a safety factor of 100.

Table 2-1. Number of positive water samples in the Netherlands (2002) with LC-MS/MS analyses (Stolker et al., 2004), and measured concentrations (MC) in drinking water in Europe (Jongbloed et al., 2001).

	MC	drinking water (n=22) ng/L			groundwater (n=15) ng/L		
		<25	25-100	>100	<25	25-100	>100
(Acetyl) salicylic acid*	290	-	2	-	-	11	-
Bezafibrate	27	-	-	-	-	-	-
Bisoprolol		-	-	-	-	-	-
Carbamazepine		1	-	-	8	2	-
Chloroamphenicol		-	-	-	-	-	-
Clofibric acid	270	-	2	-	2	3	-
Dehydroerythromycine		-	-	-	1	-	-
Diclofenac	6	-	-	-	4	-	-
Fenofibrate	210	-	-	-	-	-	-
Ibuprofen	3	-	-	-	-	-	-
Metoprolol		-	-	-	-	-	-
Paracetamol		-	-	-	-	-	-
Sulphamethoxazole		2	-	-	3	-	-

*only qualitative analysis; concentrations >50 ng/l were counted as positive samples

From this provisional ADI, a provisional drinking water limit is derived. Although it is known thatsome medicines interact at pharmacological effective doses, no information is available on the interaction at the level of the proposed drinking water limits. Therefore, no attempt was made to determine drinking water limits for combinations of medicines.

Based on ecotoxicological data, predicted no-effect concentrations are derived by, or included as proposed in Webb (2001a) and Schowanek and Webb (2002).

Paracetamol (CAS No: 103-90-2). Use: antipyretic, analgesic and anti-inflammatory. Human oral dose minimum 5 mg/kg bw in children up to 4 times a day (=1.2 g per day). Paracetamol is included in Annex II of Council Regulation No 2377/90. An Acceptable Daily Intake (ADI) of 3 mg/person was determined based on a pharmacological Lowest Observed Effect Level (LOEL) of 5 mg/kg bw/day for an antipyretic effect in human infants and a safety factor of 100 (EMEA/MRL/551/99). Calculation: 3 mg/person * 10% / 2 L/person. Drinking-water limit: 150 µg/L. The proposed environmental PNEC is 9.2 µg/L (Schowanek and Webb, 2002).

Sulfamethoxazole (CAS No: 723-46-6) Use: antibiotic. Human oral dose minimum 2 gram per day. Sulfamethoxazole is also used as a veterinary medicine. Sulfamethoxazole is included in Annex I of Council Regulation No 2377/90 as part of the inclusion of the sulphonamides. No ADI was determined but a Maximal Residue Limit (MRL) of 100 µg parent drug/kg milk was proposed (EMEA/MRL/026/95). This MRL in milk is based on a consumption of 1.5 L per day. Calculation: 100 µg/kg milk * 1.5 kg milk/person / 2 L water/person. Drinking-water limit: 75 µg/L. For pathogenic bacteria the MIC_{50} was reported to be 0.002 - >256 mg/L. *Pseudomonas putida* gave a 16h IC50 of 256 mg/L (Al-Ahmad et al., 1999). The PNEC would be 0.2 µg/L based on the most vulnerable taxa.

Carbamazepine (CAS No: 298-46-4). Use: antiepileptic and psychotropic. Human oral dose: minimum 100 mg per day. A provisional ADI of 1 mg per person was derived from the lowest effective dose in humans. Calculation: 100 mg/person / 100 * 10% / 2 L/person. Provisional drinking-water limit: 50 µg/L. Ferrari et al. (2003) established a NOEC of 25 µg/L for *Cerodaphnia dubia*, the lowest value for four species. The PNEC would be set at 2.5 µg/L. Using the program ECOSAR a PNEC of 6 µg/L was calculated (Jones et al., 2002).

Metoprolol (CAS No: 54163-88-1; 37250-58-6). Use: cardio-selective betablocker. Human oral dose: minimum 100 mg per day. A provisional ADI of 1 mg per person was derived from the lowest effective dose in humans. Calculation: 100 mg/person / 100 * 10% / 2 L/person. Provisional drinking-water limit: 50 µg/L. Environmental PNEC 7.3 µg/L (Cleuvers, 2003).

Bisoprolol (CAS No: 66722-44-9). Use: cardio-selective betablocker. Human oral dose minimum 2.5 mg per day (Fuchs, 1997). A provisional ADI of 25 µg per person was derived from the lowest effective dose in humans. Calculation: 2.5 mg/person / 100 * 10% / 2 L/person. Provisional drinking-water limit: 1 µg/L. No PNEC can be derived, but some data on this group of beta-blockers are available.

Invertebrates (C. dubia, D. magna and H. azteca) were exposed to atenolol, metoprolol, nadolol and propranolol and average invertebrate 48h LC50 ranged from 0.85-29.8 mg/L. Reproduction of H. azteca after a 27 days exposure was impacted at sublethal levels of propranolol with a NOEC of 0.001 and a LOEC of 0.1 mg/L. C. dubia reproduction NOEC and LOEC were 0.125 and 0.250 mg/L (Huggett et al., 2002). A PNEC for propranolol of 1.87 µg/L was derived earlier in (Webb, 2001b), now a PNEC for all these beta-blockers might be established at 0.01 µg/L.

Diclofenac (CAS No: 15307-86-5). Use: anti-inflammatory drug. Human oral dose: minimum 0.25 mg/kg bw anti-pyretic effect in children (=15 mg per day) (Keinanen-Kiukaanniemi et al., 1980). A provisional ADI of 0.15 mg per person was derived from the lowest effective dose in humans. Calculation: 15 mg/person / 100 * 10% / 2 L/person. Provisional drinking-water limit: 7.5 µg/L. In

acute tests with *Daphnia*, *Desmodesmus* and *Lemna*, *Lemna* was the most sensitive species with an EC50 of 7.5 mg/L (Cleuvers, 2003). Ferrari et al. (2003) established a NOEC of 1 mg/L for *Cerodaphnia dubia*, the lowest value for four species. The PNEC would be set at 100 µg/L. However, in fish diclofenac concentrations of 1 µg/L significant differences in renal tissue compared to controls were observed, thus indicating potential adverse effects (Triebskorn et al., 2002). The PNEC is set at 1 µg/L.

Bezafibrate (CAS No: 41859-67-0). Use: lipid-regulating drug. Human oral dose minimum 67 mg per day (renal impairment). A provisional ADI of 0.67 mg per person was derived from the lowest effective dose in humans. Calculation: 67 mg/person / 100 * 10% / 2 L/person. Provisional drinking-water limit: 35 µg/L.

Fenofibrate (CAS No: 49562-28-9). Use: lipid-regulating drug. Human oral dose minimum 100 mg per day. A provisional ADI of 1 mg per person was derived from the lowest effective dose in humans. Calculation: 100 mg/person / 100 * 10% / 2 L/person. Provisional drinking-water limit: 50 µg/L.

Clofibric acid (CAS No: 882-09-7; 637-07-0 (clofibrate)). Use: lipid-regulating drug. Human oral dose minimum 20 mg/kg bw (= 1.2 g per day). A recent summary of the IARC is available (IARC, 1990). No NOELs were determined in this summary. The lowest LOEL was found in humans (Larsen et al., 1994). In this study, effects on serum cholesterol and triglycerides were found in patients with type III hyperlipoproteinemia treated with approximately 1 mg/kg bw/day for 8 weeks. Based on the effect level determined and a safety factor of 100, a provisional ADI of 0.6 mg/person can be calculated. Calculation: 60 mg/person / 100 * 10% / 2 L/person. Provisional drinking-water limit: 30 µg/L. An ambient water quality criterion (AWQC) of 220 µg/L was derived for clofibrate, and of 1930 for clofibric acid, by (Schulman et al., 2002).

Ferrari et al. (2003) established a NOEC of 246 µg/L for *Brachyonius calyciflorus*, the lowest value for four species. The PNEC would be set at 25 µg/L.

The environmental PNEC for clofibrate is calculated as NOEC/AF 10/50 = 0.2 µg/L (Schowanek and Webb, 2002) and might apply to the group of fibrate derivatives. In acute tests with *Daphnia*, *Desmodesmus* and *Lemna*, *Lemna* was the most sensitive species with an EC50 of 12.5 mg/L (Cleuvers, 2003), Ferrari et al. established a NOEC of 246 µg/L for *Brachyonius calyciflorus*, the lowest value for four species. The PNEC would be set at NOEC/AF 10/10 = 1 µg/L, due to the extended chronic dataset (Ferrari et al., 2003).

Erythromycin (CAS No: 114-07-8) Use: antibiotic. Humane oral dose minimum 1 g per day. Erythromycin is included in Annex I of Council Regulation (EEC) No 2377/90. A microbiological ADI was determined by the CVMP of 300 µg/person. An MRL of 40 µg parent drug/kg milk was proposed (EMEA/MRL/720/99). The total allowed uptake of erythromycin from veterinary use is 90% of the ADI. A limit for drinking water of 15 µg /L can be calculated based on 10% of the ADI and a water intake of 2 L per day. The total uptake based on these limits remains below the ADI because it is not assumable that someone drinks 1.5 L milk plus 2 L water per day.

Calculation: 0.3 mg/person * 10% / 2 L/person. Drinking-water limit: 15 µg /L. PNEC >74 µg/L (Webb, 2001b).

Acetylsalicylic acid (CAS No: 50-78-2). Use: anti-inflammatory drug. Human oral dose minimum 20 mg per day. Acetylsalicylic acid is included in Annex II of Council Regulation (EEC) No 2377/90. An ADI of 0.5 mg/person was determined by the CVMP based on a LOEL for effects on bleeding time and thromboxane B2 production in humans of 10 mg per person and a safety factor of 20 (EMEA/MRL/695/99). Calculation: 0.5 mg/person * 10% / 2 L/person. Drinking-water limit: 25 µg/L. An ambient water quality criterion (AWQC) of 480 µg/L was derived for acetylsalicylic acid, and of 190 µg/L for salicylic acid, by (Schulman et al., 2002). The environmental PNEC would be 168 µg/L (Schowanek and Webb, 2002).

Ibuprofen (CAS No: 15687-27-1). Use: anti-inflammatory drug. Human oral dose minimum children 5 mg/kg bw (= 0.3 g per day). A provisional ADI of 3 mg per person was derived from the lowest effective dose in humans. Calculation: 300 mg/person / 100 * 10% / 2 L/person. Provisional drinking-water limit: 150 µg/L. Based on a 48h EC50 for Daphnia magna of 9.06 mg/L the PNEC would be 9.1 µg/L (Webb, 2001a).

Chloramphenicol (CAS No: 56-75-7). Use: antibiotic (limited human use). Human oral dose minimum 25 mg/kg bw in neonates (= 1.5 g per day). Chloramphenicol is no longer allowed as a veterinary medicine in animals producing foodstuff for humans in the European Union and has only very limited use in humans. The CVMP or the WHO determined no ADIs or MRLs. According to the IARC, this substance should be regarded as carcinogenic to humans. Chloramphenicol is positive in in vitro mutagenicity tests and positive in some in vivo mutagenicity tests. Adequate carcinogenicity studies are not available. A limited carcinogenicity study in mice shows an increase in lymphoma's and livercell tumours. Many case reports have described an unusual succession of leukaemia following chloramphenicol-induced aplastic anaemia and bone marrow depression in humans. Additional evidence for the association between use of chloramphenicol and leukaemia has come from a single large case-control study in China, which demonstrated a relationship with duration of exposure (IARC, 1990). There is no clear dose effect relationship between exposure to chloramphenicol and the occurrence aplastic anaemia and it is considered that victims may have some genetic or biochemical predisposition. More recent epidemiological research indicates that an association between ocular chloramphenicol and aplastic anaemia cannot be excluded. The incidence among users was 0.36 cases per million weeks of treatment compared to 0.04 cases per million weeks in non-users. The adjusted odds ratio was 3.77 (95% confidence interval, 0.84-16.90) (Laporte et al., 1998). In a second epidemiological study, no evidence of an increased risk of developing adult acute leukaemia after topical chloramphenicol use was found (Smith et al., 2000). Also, it is unclear what dose of chloramphenicol is systemically available after ocular use (Walker et al., 1998b). Therefore, no NOEL or LOEL can be determined from the human data. The mouse study can not be used because it is a limited study only and it is unclear whether aplastic anaemia can be induced in mice by chloramphenicol. Subchronic exposure of mice to chloramphenicol induced a reversible anaemia but not a chronic bone marrow aplasia (Turton et al., 2000). Therefore, no limit in drinking water for chloramphenicol can be determined from the toxicological data. Seen the carcinogenicity, the concentration of chloramphenicol should be as low as possible. Therefore it is proposed to use the limit of quantification as the drinking-water limit. Environmental PNEC 305 µg/L (Webb, 2001b). In a 24h-bioluminescence test with *Vibrio fischeri* an EC10 of 0.0187 mg/L was found (Backhaus and Grimme, 1999). Based on these chronic data for a vulnerable species a PNEC of 1.9 µg/L is established.

Ethinylestradiol (CAS No: 57-63-6) Use: synthetic oestrogen. The minimum therapeutic dose is 0.010 mg per day (Webb, 2001a). A provisional ADI of 0.1 µg per person is derived from the lowest effective dose in humans. Calculation: 10 µg/person / 100 * 10% / 2 L/person. Provisional drinking-water limit: 5 ng/L. The PNEC is 0.1 ng/L (Schowanek and Webb, 2002). Concentrations of ethynilestradiol in drinking water ranged up to 22.5 ng/L (Rurainski et al., 1977; Kuch and Ballschmiter, 2001).

Depending on the data set and the effect assessment methodology different standards have been derived, as shown in the examples of acetylsalicylic acid and clofibric acid. The selected effect limits are presented in Table 2-2. For some substances the human effect limit is more stringent than the environmental limit, and environmentally acceptable concentrations in groundwater would surpass human drinking water limits if this groundwater were used untreated for human consumption.

Table 2-2. Effect limits [µg/L] for selected pharmaceuticals in drinking water. The Margin of Safety (MOS) is based on the most critical limit.

	Drinking water standard	Human limit	Environmental limit	Measured concentration	MOS
(Acetyl)salicylic acid*	0.1	25	168	0.290	0.35
Bisoprolol	0.1	1	0.01	-	-
Metoprolol	0.1	50	0.01	-	-
Carbamazepine	0.1	50	2.5	<0.025	>4
Chloroamphenicol	0.1	0.001 (LOD)	1.9	-	-
Bezafibrate	0.1	35	1	-	-
Clofibric acid	0.1	30	1	0.270	0.37
Fenofibrate	0.1	50	1	0.210	0.48
Dehydroerythromycine	0.1	15	74	-	-
Diclofenac	0.1	7.5	1	0.006	17
Ibuprofen	0.1	150	9.1	0.003	33
Paracetamol	0.1	150	9.2	-	-
Sulphamethoxazole	0.1	75	0.2	<0.025	>4
Ethinylestradiol	0.1	0.005	0.0001	0.0225	0.004

MOS is calculated as the [lowest limit]/[measured concentration in drinking water]

In 11 out of 14 substances the PNEC is more critical than the human drinking water limit, and it may from an environmental perspective not be acceptable to discharge this drinking water to surface water. The general numeric standard for pesticidal substances in the drinking water directive (0.1 µg/L) is not sufficiently protective for the environment for 3 out of 14 substances.

Four of the reported substances have been found in drinking water above the lowest limit, one concentration (ethinylestradiol) is above both the human and the environmental limit, although there should be a large margin of safety between daily intake and therapeutic dosage (Webb et al., 2003). It should be noted that measured concentrations in groundwater are often higher than in drinking water (Derksen et al., 2001).

A prediction of groundwater and drinking water exposure seems to have become very useful both from an environmental and a public health point of view.

2.5. Discussion

In regulatory frameworks, known modelling limitations, the applicable effect assessment approach, and acceptability standards should have been harmonised in the process. From the regulatory point of view three important aspects of risk 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).

Several of the challenges identified in the first edition of this book have been addressed in the recent regulatory guidance documents (Montforts, 2001). However more research challenges lie within these three aspects of risk modelling. They are centred on the coherence of the risk model components, the connection between risk model and user, and the development of methodology for hazards yet to be addressed, and include the following points:

- Harmonisation of protection goals. At this level policy makers and scientists should engage in a reconnaissance of regulatory goals (central marketing authorisation, national registration, protection goals, standards), assessment scales (e.g. landscape level vs. local), model approach (predictive or monitoring), and uncertainty and variability of data associated with the assessment.
- Within the risk model for the environmental risk assessment, emission and distribution routes, compartments, substance properties (persistency, bioaccumulation) and effect endpoints (ecosystem structures, population, processes, species) should be linked.
- The risk model of the environmental risk assessment process should produce predictions with reference to the quality levels that are pursued for the compartments. Exclusion of compartments (soil, surface water, groundwater, drinking water, and sediment) either because the risk is supposed to be covered through other submodels, or because models have yet to be developed, greatly impairs the risk characterisation, and thus the decision making. For different compartments different limits may apply. These limits should be harmonised.
- Also, it should be possible to feed risk mitigation measures back into the risk model. The targeted use (and waste) of the product should be explored. Repetitive use, season-related use or concurrent use over large areas should be considered, next to the timing and scale of emission to the environment (i.e. spreading of manure) and the restrictions set by the product registration (e.g. no comparative assessments). The guidance documents provided set out general directions and more directions on data selection, specifically on model parameterisation, are welcomed.
- The risk model should be able to handle other modelling approaches in a higher tier. How does one compare e.g. catchment level simulations to the first tier basic calculations (Di Guardo et al., 2001; Schowanek and Webb, 2002)? What kind of information is added by a mechanistic modelling of groundwater contamination at a depth of 1 metre, compared to an empirical model in which the porewater concentration is calculated using partitioning between the water and solid phase? The information to decide which of the two models represents the target groundwater better is not within the model, but with the definition of the protection goal.
- A prediction of groundwater and drinking water exposure seems to have become very relevant both from an environmental and a public health point of view. Exposure via water, soil and landfill waste should be taken into account. Harmonisation of environmental and public health concerns is needed to set an operative quality standard.

And finally, in case quality standards are not met, an indication of the actual impact or costs will be needed to come to a cost-benefit analysis. The registration process for medicines is the exponent of risk assessment (Di Fabio, 1994). Policy makers, scientist and other interested parties (ESC, 2001) should engage in a reconnaissance of expressing, scaling and weighing costs and benefits for society, including the environment.

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3. The exposure assessment for veterinary medicinal products

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Mark H.M.M. Montforts, Dennis F. Kalf, Peter L.A. van Vlaardingen and Jan B.H.J. Linders

3.1 Introduction

The EU has issued Directive 81/852/EEC (1981) in which is stated that with a request for registration of a veterinary medicinal product information is to be provided to enable an assessment of the safety for the environment. In 1997 the Committee for Veterinary Medicinal Products (CVMP) issued a note for guidance on the environmental risk assessment (EMEA, 1997)outlining procedures, trigger values and backgrounds to the proposed risk assessment methodology, in order to harmonise the assessment procedure in Europe. The proposed methodology is based on the hazard quotient approach, that is widely used in the environmental risk assessment frameworks of new and existing substances and of plant protection products and biocides (Anonymous, 1991a; ECB, 1996).

This article will shortly discuss the structure of the environmental assessment, and will elaborate on the exposure assessment with exposure models adapted to the Dutch agricultural situation.

Risk management

The basis for the 81/852/EEC directive is the precautionary principle[18]. The assessment is part of a risk management process consisting of two distinct phases: a risk assessment phase and a risk management phase (Van Leeuwen, 1995). Here only risk assessment will be considered. The first step in risk assessment is the hazard identification, for which is referred to Directive 81/852/EEC. The second step is the exposure assessment. Exposure assessment can either be done by measuring exposure concentrations or by predicting them with models. The latter involves determining the emissions, pathways and rates of movement of a substance and its transformation in order to obtain concentrations to which environmental compartments may be exposed (yielding PEC-values: Predicted Environmental Concentrations). Underestimation of the exposure in a compartment is avoided by making worst-case assumptions. Worst-case assumptions can be modified to realistic worst-case assumptions once reliable information is available.

Effect assessment or dose-response assessment is the estimation of the relationship between the level of exposure to a substance, and the incidence and severity of an effect. In

[18] This suggests that the precautionary principle was already operative within the EU at that time; it was not. A precautionary approach as a concept in public health policy had been advocated as early as 1854. In Germany the 'Vorsorgeprinzip' had been used in the Clean Air Act of 1974. On a trans-national level, the principle was first referred to in 1984 by the North Sea Ministerial Conference in the Bremen Declaration. At the EU level it finally made its first appearance in the Treaty of Maastricht in 1992. At the time of writing this paper, it was my intention to indicate that a scientific assessment of the risks connected to the use of a medicinal product before it is used, fits well into a precautionary approach (COM, 2000; Harremoës et al., 2001; De Sadeleer, 2002).

ecological or environmental risk assessment (ERA) millions of species and processes may be exposed by a variety of routes. Laboratory-established No-Observed-Effect-Concentrations (NOEC) are used to derive Predicted No Effect Concentrations (PNECs) for different environmental compartments: water, sediment, and soil, by applying "assessment factors" usually in the range of 10-10,000, or by extrapolation based on a statistical analysis. In this way the uncertainty that the effect value used underestimates the effect on species in the field is reduced.

The processes of risk assessment (how risky is the situation) and risk management (what shall we do about it) are influencing each other when it comes to deciding on endpoints, (un)acceptable effects, and uncertainty factors. Risk characterisation integrates the previous steps, for example in PEC/PNEC ratios, also called Risk Characterisation Ratios (RCR), as is often done in many international regulatory frameworks. We only know that the likelyhood of adverse effects increases when the RCR increases. Regulatory choices have to be made when deciding on the RCR that must lead to further assessment or risk mitigation measures. This choice will depend on the uncertainty in the models as well as on the political or economical implications of the mitigation measures.

What does the ERA for veterinary medicinal products based on the CVMP Guidance document look like (EMEA, 1997)? The amending Directive 92/18/EEC describes the ERA process as composed of two phases. The first phase (Phase I) shall assess the potential of exposure of the environment and is thus limited to product identification and exposure assessment. Several exemptions for further testing are given, such as trigger values for PECs: 100 µg/kg in slurry, 10 µg/kg in soil, 0.1 µg/l in groundwater, 10 µg/kg in dung; or trigger values for halflives: $DT50_{slurry}$ 30 days. These values are the result of the negotiations in the EMEA working group between all interested parties and have no scientific basis. Their primary function is to serve as management tools. When exemptions do not apply and trigger values are exceeded, one enters Phase II.

Phase II includes effect assessment and risk characterisation and here the notifier is facing considerable testing efforts. The possibility that the ERA may end at the exposure assessment is an 'escape route' that is welcomed by the pharmaceutical industry, as this could make the difference between an economically profitable product and a dead end. To the regulatory authorities this escape route increases the demand for a thorough exposure assessment. Considering that at the side of the effect assessment the uncertainties are relatively well known, and the concept of using 'assessment factors' is more and more refined, one has to realise that the exposure assessment has a relatively high level of uncertainty.

The risk assessment should cover the whole product, all its ingredients, and all relevant metabolites that are released into the environment. Identifying emission and distribution routes is an important stage in the process, as both the environment and the notifiers will be confronted with the consequences. A uniform and systematic analysis of possibilities guarantees a reliable assessment. Emission can take place at any step in the life cycle of the product. Dosage, route of application, type of target animals, excretion, route of entry into the environment, and agricultural practice, determine the point of emission:

- at application;
- at removal of waste material containing the product (manure, fish water);
- by excretion via faeces and urine;
- by contagion (immunological products);

The main categories of emission scenarios are:

- removal of waste containing the product (containers, manure, fish water, medicated drinking water);
- excretion via faeces and urine;
- spillage at external application or direct exposure/discharge outdoors

Based on product identity an exemption for further assessment is made for physiological substances such as vitamins, electrolytes, natural amino acids and herbs. Based on the use of the product and limited routes of emission, products for companion animals (excluding horses) are also exempted.

A third reason for exemption is the application to a small number of animals as opposed to mass treatment. A typical example is the treatment of a wounded cow with antibiotics, as a result of an accident. Should more than one animal be involved, it becomes evident that there is no clear borderline with mass treatment. In case of doubt, it is suggested to perform an exposure calculation, as there are more trigger values along this process.

One should be aware that some parameters used in exposure and distribution assessment are directly used in the risk characterisation process. The substance properties of sorption capacity (Koc) and transformation rate in soil and slurry (DT50) are used as triggers in the risk characterisation process because they are indicators of the outcomes of distribution routes and processes. This implies that attention should be paid to the reliability and usefulness of the studies investigating these parameters (Mensink et al., 1995).

Livestock breeding and rearing is an important industry in the Netherlands (Table 1). Considering the above and the diversity of target animals listed in table 1, it is worthwhile to put a major effort into elaborating the exposure assessment. To illustrate the consequences some exposure models based on the Dutch agricultural practice are given (section 2). The results of these models are discussed in section 3.

Table 1. An overview of animal husbandry in the Netherlands.

Category	number of animal places (x 1000)	number of farms
dairy cows	1,675	36,000
cattle	4,550	54,400
pigs	14,400	21,250
horses and ponies	107	20,000
sheep	1,625	21,000
broilers	44,000	1200
laying hens	39,500	2700
turkeys	1,250	140
fish	2500 tonnes	50

Sources: (Kamstra and Van der Heul, 1995; CBS, 1996)

3.2 Exposure assessment: agricultural practice, emission modelling, and trigger values

A realistic worst-case exposure assessment can be performed if the daily agricultural practice is taken into account. The Dutch situation is characterised by a number of distinct features: bio-industry; manure surplus; restricted application time (March-September) of manure in certain regions; slurry injection (5 cm depth) or direct tillage (20 cm depth); large areas with grassland and cattle, and indoor fish cultivation. Three exposure scenarios are discussed: excretion by grazing cattle (section 2a); manuring of land (arable and grassland; section 2b)); and fish farm wastewater discharge (section 2c). In section 2d the use of the trigger values is discussed.

2a. Exposure assessment: excretion by grazing cattle

In the Netherlands beef cattle and heifers, suckler cows and dairy cows are grazed. A common treatment before animals are grazed is an anthelminthic treatment by injection, oral dosage, or intra-ruminal bolus. Residues of these substances are secreted into urine and faeces and are excreted onto the land or into the water. To determine the concentration in these compartments, one needs to know how much of the substance is excreted, and the rate of excretion. In case these distribution parameters are not available, the worst-case concentration in faeces can be calculated with the following model and defaults (table 2). Cattle defaecate 10-11 times per day (Marsh and Campling, 1970). This model suggests that the entire administered dose is excreted in one defaecation event.

Model for the calculation of the maximum concentration in dung if PEC_{dung} is not measured:

$$PECdung = \frac{Q_{product} \cdot C_c \cdot m_{animal} \cdot T_{treatment} \cdot F_{\max .excreted.dung} \cdot Nexcretion}{Pdung_{animal}}$$

input		
$Q_{product}$	dosage of product used	[$kg.kg_{bw}^{-1}.d^{-1}$]
C_c	concentration of chemical (c) in product	[$mg_c.kg^{-1}$]
m_{animal}	(averaged) body weight	[$kg_{bw}.animal^{-1}$]
$T_{treatment}$	duration of treatment	[d]
$F_{max.\ excreted\ dung}$	highest fraction excreted in dung in one day	[-]
$Pdung_{animal}$	dung production animal in field	[$kg_{wwt}.animal^{-1}.d^{-1}$]
Nexcretion	number of dung excretion events per day	[d^{-1}]
output		
PECdung	predicted environmental concentration in dung	[$mg_c.kg_{wwt}^{-1}$]

Table 2. Default settings for the module for the calculation of the maximum concentration in dung.

parameter	symbol	unit	value
duration of treatment	$T_{treatment}$	[d]	1
highest fraction excreted in dung in one day	$F_{max.\ excreted\ dung}$	[-]	1
number of dung excretion events per day	Nexcretion	[d^{-1}]	10.5

Table 3. Body weight, therapeutic dosage, and dung production of selected target species.

animal	body weight m_{animal} [$kg_{bw}.animal^{-1}$]	production dung P_{dung} [$kg_{wwt}.animal^{-1}.d^{-1}$]	dosage $Q_{product} \cdot C_c$ [$mg.kg_{bw}^{-1}$]
dairy cows	600	37	0.2
beef cattle	330	17	0.2
horses	600	23	0.2

In the assessment process the trigger is 10 µg/kg_{wwt}, below which further assessment is not necessary. Information on the excretion pattern is therefore very useful. Ivermectin is used here as an example. Ivermectin (CAS 70288-86-7) is an anti-parasitic substance used as anthelmintic in animal husbandry. Ivermectin is hardly excreted in urine (<2%), and c. 90% of a dosage is excreted via faeces in the 7 to 14 days following administration, depending on the route of administration (Campbell, 1989; Halley et al., 1989). After oral application of labelled ivermectin to cattle, 60% of the label is excreted within two days, the remaining fraction is excreted in the following four days. On the second day after application 93-94% of the measured radioactivity was found to be ivermectin, whereas after 6 days only 44-52% of the measured radioactivity was found to be ivermectin (personal communication Dr. H. Rogiers, Merial NV/SA, Belgium, 1997). For reasons of convenience, the excretion of the labelled ivermectin is described in two steps, with a fast initial phase of two days releasing 60% of the label, and a second phase releasing 39% in the following four days. It is further

Table 4. The excretion of labelled ivermectin by cattle after oral dosage.

days after application of the dose	**phase**	**fraction excreted r.a.**	**fraction ivermectin**	**excreted ivermectin $F_{excreted\ dung}$**	**PECdung beef cattle [$mg.kg_{wwt}^{-1}$]**	**PECdung dairy cow [$mg.kg_{wwt}^{-1}$]**
directly				0.88	36	30
1	1	0.37	0.97	0.359	1.39	1.16
2	1	0.23	0.94	0.216	0.84	0.70
3	2	0.23	0.84	0.193	0.75	0.63
4	2	0.11	0.72	0.079	0.31	0.26
5	2	0.04	0.61	0.025	0.10	0.08
6	2	0.01	0.51	0.006	0.02	0.02
7	2	0.008	0.43	0.004	0.02	0.01
11	2	<0.001	<0.3	<0.0001	<0.0004	<0.0003

Table 5. Measured ivermectin concentrations in dung.

animal and body weight	dose and route of administration [mg/kg$_{bw}$]	highest concentration in dung		source
		as reported	recalculated to [mg/kg$_{wwt}$]	
cattle 276 kg	0.2 (s.c.)	0.42 mg/kg$_{wwt}$ after 5 days	0.42	Lumaret et al., 1993[1]
cattle c. 300 kg	0.5 (p.)	9.0 mg/kg$_{dwt}$ after 1 day	1.35	Sommer and Steffansen, 1993[2]
cattle c. 300 kg	0.2 (s.c.)	3.9 mg/kg$_{dwt}$ after 2 days	0.58	
cattle 278 kg	12 mg/d (i.r.)	0.66 mg/kg$_{wwt}$	0.66	Strong et al., 1996[3]
horse	0.2 (o.)	8.5 mg/kg$_{dwt}$ after 1 day	1.70	Herd, 1995[4]

i.r. = intra-ruminal; s.c. = subcutaneous; o. = oral; p. = pour-on; dwt = dry weight; wwt = wet weight; bw = body weight

[1] After 12 days no ivermectin detected. Limit of detection was 0.02 mg/kg.

[2] After 14 days no ivermectin detected (pour-on); or 0.3 mg/kg dwt (subcutaneous). Limit of detection was 0.05 mg/kg.

[3] Determined with bioassay with the fly *Neomyia cornicia*.

[4] After four days no ivermectin detected. Limit of detection was 0.05 mg/kg.

assumed that the decrease of unchanged ivermectin is also a two step process: from 100 to 93.5% in two days and from 93.5 to 44% in the following four days. The calculation of exposure concentrations in dung requires a dosage, animal body weights and dung production data (table 3). With the assumption that the processes in every step follow first-order kinetics, the course of the excretion and concentration is given in table 4.

One can see in table 4 that the calculated concentrations using excretion data (maximum 1.39 mg/kg) are over 25 times lower than calculated with the worst-case scenario (36 mg/kg). Table 5 shows measured concentrations ivermectin in dung reported in scientific literature. The concentrations based on wet weight in the fourth column are recalculated using a moisture content of 85% for cattle dung and 80% for horse dung. Unfortunately, the results with cattle were obtained from subcutaneous or pour-on treated animals, and show that the excretion period following these types of treatment is longer than is expected after oral treatment (ca. one week). The results from the intra-ruminal sustained-release bolus can be compared to the model calculations. If the model beef cattle of 330 kg was treated with this sustained-release bolus (Strong et al., 1996) the calculated concentration in dung would be 0.62 mg/kg, which is equivalent to the 0.66 mg/kg measured. Assuming that the excretion pattern for cattle is representative for horses as well, the concentration in horse dung after 1 day can be calculated to 1.9 mg/kg. This value is also very close to the observed concentration of 1.7 mg/kg.

These data show that the worst-case calculations are overestimating the concentrations, and that an exposure assessment based on concentrations derived with adequate excretion data, leads to more accurate results.

2b Exposure assessment: manuring of land.

Calculation models for the concentration in soil are presented by Spaepen et al. (1997) introducing the key to the concentration in soil: the phosphate and nitrogen immission standards for manure. The basic calculations (here based on phosphate immission) are given in formula set A.

How do these calculations, based on yearly manure production figures and yearly number of cycles, relate to agricultural manuring practice? A limited inquiry showed that manure is hardly ever stored for over half a year, and that the manuring regime on grassland might be quite different from the regime on arable land planted with corn (*Zea mais*). In certain regions in the Netherlands farmers have to deal with restricted spreading times (spreading of slurry is only allowed between March and September). The following scenarios for arable land and grassland were developed, using the specific situation to improve the worst-case exposure calculations to realistic worst-case calculations (Montforts, 1997a).

Manuring of land: $PIEC_{soil}$ calculations for arable land.

The only time the farmer can manure the arable land is before the crop is growing. Taking into account the immission standards the farmer will spread the manure that has been stored during the winter (October-March: 152 days) in one event. The possible concentration of excreted residues in the slurry now depends on the number of treatments during this storage time and the time lapse between the excretion of the residues and the moment the slurry is spread. In a worst-case scenario all residues are excreted the day before the slurry is spread. The best case scenario will allow the residues to break down during the maximum available time. With the assumptions that every animal cycle is treated once, and all cycles are treated at the same life-stage (i.e. at their averaged body weight), the following formulas calculate the concentration in slurry taking degradation into account over the averaged storage time for the residues.

Formula set A.

$$Q_{excreted} = Q_{product} \cdot C_c \cdot T_{treatment} \cdot F_{excreted} \cdot m_{animal} \cdot Ncyclus_{animal}$$

$$C_{P2O5} = \frac{Q_{excreted}}{P_P} \cdot e^{-k\deg_{slurry} \cdot T_{rest}}$$

$$PIECsoil = \frac{C_{P2O5} \cdot Q_{P2O5}}{RHOsoil \cdot CONV_{area\ field} \cdot DEPTHfield}$$

input		
$Q_{product}$	dosage product used	[$kg.kg_{bw}^{-1}.d^{-1}$]
C_c	concentration chemical (c) in product	[$mg_c.kg^{-1}$]
$T_{treatment}$	duration of treatment	[d]
m_{animal}	(averaged) body weight	[$kg_{bw}.animal^{-1}$]
$F_{excreted}$	fraction excreted in faeces and urine	[-]
$Ncyclus_{animal}$	number of cycli per year	[$animal.place^{-1}.yr^{-1}$]
P_P	phosphate production animal in stable in one year	[$kg_{P2O5}.place^{-1}.y^{-1}$]
$T_{storage}$	average storage time slurry grassland/arable land	365 [d]
T_{rest}	maximum duration of storage after last treatment	[d]
$kdeg_{slurry}$	reaction constant transformation in manure	[d^{-1}]
Q_{P2O5}	phosphate immission standard	[kg_{P2O5}. $ha^{-1}.yr^{-1}$]
RHOsoil	bulk density of soil	[$kg.m^{-3}$]
DEPTHfield	mixing depth with soil	[m]
$CONV_{area\ field}$	conversion factor for the area of the agricultural field	[$m^2.ha^{-1}$]
intermediate results		
$Q_{excreted}$	amount substance excreted	[$mg_c.place^{-1}.yr^{-1}$]
C_{P2O5}	concentration in phosphate	[$mg_c.kg_{P2O5}^{-1}$]
output		
PIECsoil	predicted initial environmental concentration in the soil	[$mg_c.kg_{soil}^{-1}$]

The second term in the first formula of formula set B (with $Q_{excreted}$) calculates the initial concentration in the slurry (phosphate) after one treatment. The third term (with 1-(F_{rsl})^$N_{application}$) corrects for multiple treatments during the storage period, and the degradation during the time between these treatments. When there is only one treatment, this term is equal to 1. When the degradation rate is unknown, this term equals Napplication. The fourth term corrects for degradation in the slurry during the time left after the last treatment during the storage period. The maximum time left after the last treatment (T_{rest}) is calculated with the third formula, and is averaged with the minimum time (zero days) by dividing with a factor two. When there is only one treatment, T_{rest} equals $T_{storage}$.

To illustrate the difference with the basic formulas of Spaepen et al. (1997) some calculations are made (table 7) using the information shown in table 6 and the two sets of formulas: set A based on the year-based approach; set B based on the Dutch agricultural practice. For matters of convenience, the calculation of T_{rest} is from the same formula for both sets.

Formula set B.

$$C_{P2O5} = \frac{Q_{excreted}}{T_{storage} \cdot P_{P2O5}} \cdot \frac{1 - F_{rsl}^{Napplication}}{1 - F_{rsl}} \cdot e^{-k\deg_{slurry} \cdot T_{rest}/2}$$

$$F_{rsl} = e^{-kdeg_{slurry} \cdot Tcyclus_{animal}}$$

$$T_{rest} = T_{storage} - (Napplication - 1) \cdot T_{cyclus}$$

$$PIECsoil = \frac{C_{P2O5} \cdot Q_{P2O5}}{RHOsoil \cdot CONV_{area\ field} \cdot DEPTHfield}$$

input		
$Q_{excreted}$	amount substance excreted	[$mg_c.place^{-1}.yr^{-1}$]
P_{P2O5}	phosphate production of animal in stable	[$kg_{P2O5}.place^{-1}.d^{-1}$]
$DT50deg_{slurry}$	halflife time in slurry	[d]
$T_{cyclus,\ animal}$	duration of cyclus	[d]
$T_{storage}$	average storage time slurry grassland/arable land	[d]
Napplication	number of applications per storage period	[-]
$kdeg_{slurry}$	reaction constant transformation in manure	[d^{-1}]
Q_{P2O5}	phosphate immission standard	[$kg_{P2O5}.\ ha^{-1}.yr^{-1}$]
RHOsoil	bulk density of soil	[$kg.m^{-3}$]
DEPTHfield	mixing depth with soil	[m]
$CONV_{area\ field}$	conversion factor for the area of the agricultural field	[$m^2.ha^{-1}$]
intermediate results		
$kdeg_{slurry}$	reaction constant transformation in manure	[d^{-1}]
F_{rsl}	fraction of the concentration remaining in slurry after time $T_{cyclus,\ animal}$	[-]
C_{P2O5}	concentration in phosphate	[$mg_c.kg_{P2O5}^{-1}$]
T_{rest}	maximum duration of storage after last treatment	[d]
output		
PIECsoil	predicted initial environmental concentration in the soil	[$mg_c.kg_{soil}^{-1}$]

The example is performed with data for oxytetracycline hydrochloride (oxytetracycline HCl) (CAS 2058-46-0), oxytetracycline (CAS 79-57-2) and chlortetracycline (CAS 57-62-5), used for sows and turkeys in rearing.

Oxytetracycline HCl is dosed with 40 mg/kg bw for 5 days. It was found that wethers (castrate ram) 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 (Roij and De Vries, 1980). The $F_{excreted}$ used here is 0.75. Chlortetracycline is found to degrade in cattle manure with a DT50 of approx. 1 week at 37°C, increasing to a DT50 >20 days when decreasing the temperature to 28°C. Using the Arrhenius-equation to recalculate the DT50 from 37°C to 20°C the DT50 (20°C) amounts to 30 days. From the data presented by Soulides et al. (1962) an average DT50 in soil of 4 days (25°C) can be derived, and recalculated to a DT50 (20 °C) of 6 days[19]. To illustrate the relative weight of the DT50 in slurry and soil, calculations with DT50 of 100 or 15 days are also presented.

The results in table 7 show that exposure concentrations differ by a factor thirty, depending on the substance properties.

[19] Recent investigations have resulted in half-life values in soil up to 175 days (see Chapter 4).

Table 6. Agricultural parameters for arable land in the Netherlands.

Symbol	Parameter	value	unit
$m_{animal, sow}$	(averaged) body weight sow	240	[$kg_{bw}.animal^{-1}$]
$m_{animal, turkey}$	(averaged) body weight turkey	2	[$kg_{bw}.animal^{-1}$]
$P_{P2O5\ sow}$	phosphate production sow in stable sow	0.0556	[$kg_{P2O5}.place^{-1}.d^{-1}$]
$P_{P2O5\ turkey}$	phosphate production turkey in stable turkey	0.00071	[$kg_{P2O5}.place^{-1}.d^{-1}$]
$T_{cyclus, sow}$	duration of cyclus sow	365	[d]
$T_{cyclus, turkey}$	duration of cyclus turkey	49	[d]
$T_{storage, set A}$	average storage time slurry arable land	365	[d]
$T_{storage, set B}$	average storage time slurry arable land	152	[d]
Napplication,A,sow	applications per storage period to sow set A	1	[-]
Napplication,A,turkey	applications per storage period to turkey set A	7.4	[-]
Napplication,B,sow	applications per storage period to sow set B	1	[-]
Napplication,B,turkey	applications per storage period to turkey set B	4	[-]
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]
$CONV_{area\ field}$	conversion factor for the area of the field	10,000	[$m^2.ha^{-1}$]

Table 7. Results of $PIEC_{soil}$ calculations (µg/kg dw) in arable land for two distribution scenarios (A and B), two animal species, and various DT50 values in slurry.

animal	**sow**		**turkey in rearing 0-6 weeks**	
formula set	A	B	A	B
no DT50 slurry	64	155	315	410
DT50 slurry 100 days	18	92	275	260
DT50 slurry 30 days	1	28	200	140

Manuring of land: PIECsoil calculations for grassland.

The farmer can manure the grassland during the whole season. The farmer will fill the immission standard in more events, in order not to harm the grass. The dosage of the product to the land depends on the number of treatments during the storage time and the time lapse between the excretion of the residues and the moment the slurry is spread. The concentration in the soil after the last batch is spread depends on the degradation rate in the soil. The Dutch model assumes that within the period March-September the phosphate immission standard is filled in four events: one at the start of the season, one at the very end, and two at equal distances inbetween. The slurry spread at the start of the season has been stored for 152 days, the other batches for 71 days. The formula set for sows is almost equal to set B, with one animal treatment per year only, but now only one of the batches contains contaminated manure. The formula set for turkeys is different, because the period between the moment of animal treatment and the moment of manure spreading changes with every cycle. In case of seven animal cycles per year, two treatments may take place during the winter storage period and five during the three summer storage periods. Every storage period results in a dosage that is spread onto land; C_A after the first period, C_B after the second period, and so on.

Table 8. Agricultural parameters for grassland in the Netherlands.

Symbol	Parameter	value	unit
$m_{animal, sow}$	(averaged) body weight sow	240	[$kg_{bw}.animal^{-1}$]
$m_{animal, turkey}$	(averaged) body weight turkey	1	[$kg_{bw}.animal^{-1}$]
$P_{P2O5\ sow}$	phosphate production sow in stable	0.0556	[$kg_{P2O5}.place^{-1}.d^{-1}$]
$P_{P2O5\ turkey}$	phosphate production turkey in stable	0.00071	[$kg_{P2O5}.place^{-1}.d^{-1}$]
$T_{cyclus, sow}$	duration of cyclus	365	[d]
$T_{cyclus, turkey}$	duration of cyclus	49	[d]
$T_{storage, set\ A}$	average storage time slurry grassland, set A	365	[d]
Q_{P2O5}	phosphate immission standard	135	[$kg_{P2O5}.\ ha^{-1}.yr^{-1}$]
RHOsoil	bulk density of soil	1500	[$kg.m^{-3}$]
DEPTHfield	mixing depth with soil	0.05	[m]
$CONV_{area\ field}$	conversion factor for the area of the agricultural field	10000	[$m^2.ha^{-1}$]

The concentration in the soil directly after the last treatment is calculated while taking degradation in the soil before the last treatment into account. The most realistic period that degradation can take place in slurry and in soil cannot be determined with a rule of thumb. Given the number of cycles per year, and assuming that the time between the applications in the cycles is constant, one can search for the worst-case and best case combinations of slurry storage time and soil residence time. However, as these combinations largely depend on the (unknown and individual) substance properties, selected values are given for every livestock category of interest.

The formula set for sows (C) is given by:

Formula set C:

$$C_{P2O5} = \frac{Q_{excreted}}{T_{storage} \cdot P_{P2O5}} \cdot e^{-k\deg_{slurry} \cdot T_{rest}/2}$$

$$T_{rest} = T_{storage}$$

$$PIECsoil = \frac{0.25 \cdot C_{P2O5} \cdot Q_{P2O5}}{RHOsoil \cdot CONV_{area\ field} \cdot DEPTHfield}$$

The formula set for turkeys (D) is given by:

Formula set D:

$$C_{P2O5\,A} = \frac{Q_{excreted}(e^{-k\,deg_{slurry}\cdot 104} + e^{-k\,deg_{slurry}\cdot 52})}{152 \cdot P_{P2O5}}$$

$$C_{P2O5\,B} = \frac{Q_{excreted}(e^{-k\,deg_{slurry}\cdot 71} + e^{-k\,deg_{slurry}\cdot 19})}{71 \cdot P_{P2O5}}$$

$$C_{P2O5\,C} = \frac{Q_{excreted}}{71 \cdot P_{P2O5}} \cdot e^{-k\,deg_{slurry}\cdot 38}$$

$$C_{P2O5\,D} = \frac{Q_{excreted}(e^{-k\,deg_{slurry}\cdot 57} + e^{-k\,deg_{slurry}\cdot 5})}{71 \cdot P_{P2O5}}$$

$$PIECsoil = \frac{0.25 Q_{P2O5}(C_{P2O5\,A} \cdot e^{-k\,deg_{soil}\cdot 213} + C_{P2O5\,B} \cdot e^{-k\,deg_{soil}\cdot 142} + C_{P2O5\,C} \cdot e^{-k\,deg_{soil}\cdot 71} + C_{P2O5\,D})}{RHOsoil \cdot CONV_{area\ field} \cdot DEPTHfield}$$

To illustrate the difference with the basic formulas of Spaepen et al. (1997) the same calculations as for arable land (table 9) are performed using the information shown in table 8 and the two sets of formulas: set A based on the year-based approach; set C & D based on the Dutch agricultural practice.

The results show that exposure concentrations differ by a factor of fourty, depending on the model and the substance properties. The availability of a $DT50_{soil}$ can lower the $PIEC_{soil}$ for grassland substantially.

These calculations show the difference between the models for grassland an arable land. In table 10 the differences in the results of the grassland and arable land scenarios are shown.

Table 9. Results of $PIEC_{soil}$ calculations (µg/kg dw) in grassland for two distribution scenarios (A and C/D), two animal species and various DT50 values in slurry and soil.

animal		**sow**		**turkey in rearing 0-6 weeks**	
formula set		**A**	**C**	**A**	**D**
DT50 slurry	**DT50 soil**				
none	none	315	400	1550	1710
100	50	90	315	1350	580
100	15	90	315	1350	440
30	15	4	180	980	290
30	6	4	180	980	280

Table 10. Results of $PIEC_{soil}$ calculations (oxytetracycline, in µg/kg dw) for arable and grassland (distribution scenarios B and C/D), two animals, and various DT50 values (days) in slurry and soil.

animal species		sow		turkey in rearing 0-6 weeks	
target land		arable	grass	arable	grass
DT50 slurry	DT50 soil				
none	none	156	400	410	1710
30	6	28	180	140	280

Using experimental data on degradation in slurry and soil, PIECs are lowered by factors 2 - 6 compared to worst-case calculations. With the new models presented here PIEC in grassland will always be higher than in arable land, but given the differences in soil depth and phosphate immission standards, the incorporation of a $DT50_{soil}$ can lower the $PIEC_{soil}$ substantially. The new models give higher estimations for animals with long life cycles compared to the basic year-averaged model, whereas for animals with short life cycles (young turkeys, broilers) estimations can be lower, depending on the substance properties. These calculations show also that the choice of the trigger values ($DT50_{slurry}$ 30 days, $PIEC_{soil}$ 10 µg/kg), as made by the EMEA, are not harmonised. For example: with a DT50 of <30 days further assessment is not necessary, but $PIEC_{soil}$ may still be >10 µg/kg.

2c: Exposure assessment: fish medicines.

The scale of fish cultivation for commercial purposes is limited in the Netherlands (Kamstra and Van der Heul, 1995). In 1994 in total 26 and 10 companies were involved in cultivating eel and catfish, respectively. Most nurseries use water recirculation systems, in which the water is recycled after a water treatment by filtration. Catfish nurseries discharge on the municipal Sewage Treatment Plants (STP), but 40% of the eel nurseries discharge directly on surface water. The exposure scenarios presented here are based on a fish farm that breeds 50 tonnes of eel a year, the median production in The Netherlands. Two scenarios are distinguished, based on the incidence of administration of the product, and the type of wastewater treatment:

- continuous medication; without recirculation/filtration, followed by a settlement tank;
- occasional medication (≤4 times a year), without recirculation/filtration before discharge on the settlement tank.

On a yearly basis the nursery discharges 250 m^3 water per tonne of fish, resulting in a turnover rate of 35 $m^3.d^{-1}$. It is assumed the total water volume of the nursery is 70 m^3. After the settlement tank the water fraction is either dicharged on surface water or into the STP. This STP module is not described here and the reader is referred to (Struijs et al., 1991; RIVM, 1994; Linders and Jager, 1997) for further reading.

Table 11. Pick list for the default settings of the fraction of retention in sludge, treatment time and volume of wastewater from fish nurseries.

type of treatment	type of water treatment before STP	F_{ret} [-]	Vwaste water [l]	$DILUTION_{fish}$ [-]
continuous	filtration and settlement tank	0.75	35000	5
occasional	settlement tank	0.5	70000	3

Due to the settlement tank the total amount of substance emitted is equally spread out over 25 days. The surface water concentration in case of continuous treatment is therefore constant.

The fish farm and the STP discharge on a small waterway that dilutes the effluent by a factor 5 or 3, depending on the water volume discharged. The recirculation/filtration system and the settlement tank both have an estimated removal efficiency of 50% of the dose of organic substances from the water (table 11).

The use of several medicinal products in fish nurseries is listed in table 12 (Kamstra and Van der Heul, 1995).

Model for the calculation of the emission to surface water.

$$Q_{emitted} = Q_{product} \cdot C_c \cdot Vwaste\ water$$

$$Elocal_{water} = \frac{Q_{emitted} \cdot (1 - F_{ret})}{Temission_{stp}}$$

$$PIECsw_{fish} = \frac{Elocal_{water}}{DILUTION_{fish} \cdot Vwaste\ water}$$

input		
$Q_{product}$	dosage product used	[$kg.l^{-1}$]
C_c	concentration chemical in product	[$mg_c.kg^{-1}$]
Vwaste water	volume of waste water discharged	[l]
F_{ret}	fraction of retention in sludge	[-]
$Temission_{stp}$	emission period for discharge to STP	[d]
intermediate results		
$Q_{emitted}$	amount of substance emitted	[$mg_c.d^{-1}$]
$Elocal_{water}$	emission to waste water during episode	[$mg_c.d^{-1}$]
output		
$PIECsw_{fish}$	highest initial concentration in surface water	[$mg_c.l^{-1}$]

Table 12. Summary of medicinal products used in fish nurseries and $PIEC_{sw}$ calculated without STP.

Substance	max. conc. in water basin (mg/l)	PIECsw continuous treatment (mg/l)	PIECsw occasional treatment (mg/l)
mebendazole	5	0.010	0.067
oxytetracycline	75	0.15	1.005
malachite green	0.3	0.0006	0.004
flumequine	11	0.022	0.147

It is interesting to see what the effect of the STP module would have on the concentrations in surface water (table 13). The $Elocal_{water}$ is the input for the STP module. A logKow is needed to calculate the partition between water and sludge. Using the structure-activity calculation method of Mackay et al. (1980) a logKow of 0.02 is calculated for oxytetracycline, a value that is consistent with the weak adsorption found for this substance in soil (Soulides et al., 1962; Roij and De Vries, 1980). The effect of the degradability of a substance is also modelled in table 13.

In this example sorption to sludge removed only 0.01% of the daily load. Differences in the results between the scenarios 'no STP'and 'STP without degradation' are a result of different water volumes before (input from fish nursery) and after the model STP. Reliable data on sorption to sludge (or removal rates) can lower the PIECsw further.

2d: Exposure assessment: trigger values.

Decisions for further assessment are made by comparison of predicted exposure concentrations to trigger values. Some remarks can be made concerning these triggers given in the EMEA (1997) document. The exposure trigger level for further testing when the environment is exposed by dung from grazing animals is 10 $\mu g/kg_{wwt}$. This trigger will have no practical consequences for the pharmaceutical industry. Even substances with relative low dosages (like ivermectine) will exceed this trigger 100 times. It is even more remarkable that ivermectin at concentrations of 0.5 $\mu g/kg_{wwt}$ still induces abberrations in wings of the dung fly *Scatophaga stercoraria* (Strong and James, 1992). In this case the trigger is not even protective enough.

Table 13. Predicted concentrations in surface water of oxytetracycline with and without STP.

oxytetracycline	concentration in water basin (mg/l)	PIECsw (mg/l) no STP	PIECsw (mg/l) with STP, no degradation	PIECsw (mg/l) with STP, ready biodegradable
continuous treatment	75	0.15	0.07	0.003
occasional treatment	75	1.00	0.49	0.017

Calculations with STP based on USES1.0 (RIVM, 1994)

The duration of exposure is not taken into account in Phase I. 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 (Wardhaugh and Rodriguez-Menendez, 1988), whereas after oral treatment all ivermectin residues are excreted after one week (see table 4). The Phase II assessment will eventually have to answer to the question whether field populations of insects will be reduced due to the use of the medicine. In view of this population-dynamic approach in Phase II (e.g. field testing), the Phase I assessment should at least take the expected distribution in space and time into account.

The exposure trigger for the concentration in slurry (housed animals) is 100 $\mu g/kg_{wwt}$, and for the concentration in soil 10 $\mu g/kg_{dwt}$. Without going into calculations here, it is stated that these two triggers are independent of each other. As demonstrated in previous paragraphs, the triggers for the DT50 in manure and the PIEC in soil are irreconcilable. For instance, a DT50 does not take the amount of substance into account. A rapid degradation may still result in high concentrations. The value for the DT50 in manure (30 days) is not recognisably related to manure storage times, and further investigations into this storage practice, in relation to manuring regimes, and into manure degradation studies are desirable. Attention should be paid to conditions concerning pH, redox potential, moisture content and the temperature in manure storage facilities.

Environmental exposures of fish medicines will always lead to a Phase II assessment as there are no trigger values. The lack of a trigger for surface water (and sediment) is not consistent with the fact that there are triggers for soil and dung. However, is there a reasonable trigger value for the aquatic ecosystem? Because various existing chemicals are known to cause harmful effects at low concentrations, the trigger value would equal zero, i.e. only no exposure of surface water is acceptable. For example, tributyltin compounds cause imposex in gastropods at 1 ng/l in the sea along major ship routes (Bryan et al., 1986) and the EC50 of ivermectin for daphnids is 25 ng/l (Halley et al., 1989). Besides this in many countries fish are cultivated in open water (e.g. salmonids) and medicines are administered directly to the water in therapeutic, thus effective concentrations. As a consequence, there can be no safe trigger value for exposure to fish medicines.

One model not discussed here is the calculation of the concentration in groundwater. The trigger for a Phase II assessment is 0.1 µg/l. The EMEA document refers to a certain partition model that calculates the concentration in soil porewater (ECB, 1996). Degradation, climate data and hydrological information are not taken into account. The parameters that influence the result are therefore the concentration in soil and the sorption coefficient of the substance. Suppose the concentration in soil is just below the soil trigger of 10 $\mu g/kg_{dwt}$, the sorption coefficient (Koc) has to be >5600 l/kg not to exceed the ground water trigger of 0.1 µg/l. This high sorption coefficient does not apply for hydrophylic substances like most antibiotics. Therefore, unless the DT50 in slurry is <30 days, every antibiotic is bound to enter a Phase II assessment.

3.3. Discussion

Given the fact that at the stage of the exposure assessment already decisions are made on the acceptability of the environmental risk of a product, regulatory authorities have to be careful in choosing exposure scenarios. A simple worst-case exposure approach might satisfy the safety demands with respect to the environment, but the implications with respect to the benefits of a product do not allow a too rigid cut-off procedure. The exposure and distribution models should lead to practical risk management.

Four models were presented for three routes of emission and distribution of veterinary medicinal products that reflect realistic agricultural practice in the Netherlands. It was shown that simple general models, considering common agricultural practice and useful substance properties can change predicted exposure concentrations by a factor 2 - 40. It is not an imaginary situation that the registration of a product is hampered by the choice of a too restricted exposure model, or serious environmental risks are overlooked. It is obvious that arbitrarily chosen values concerning the percentage of the herd treated, and misinterpretations of phosphate immission standards or phosphate contents of slurry, will lead to further differences in outcomes of calculations. A clear presentation of parameter values for every country of the EU is needed.

With the models presented here the PEC in grassland after manuring the land will always be higher than in arable land. It might therefore be worthwhile to limit the phase I calculation to grassland only. A reason to maintain this distinction is that EMEA has required studies on plants for arable land risk assessment (to protect crop production), and not for grassland risk assessment. There might also be reasons why slurry will be disposed on one type of land only.

The results shown in tables 12 and 13 indicate that substantial concentrations of fish medicine (up to 1 mg/l) might be present in surface water bodies near discharge points of fish nurseries for a period of time after treatment (25 days). STP treatment should be able to reduce these concentrations; for example steroids were found to be eliminated for 58-91 % in sewage treatment (Stumpf et al., 1996). Depending on the degradation rate in the STP, the calculated concentration in surface water can differ up to 29 times. Even with these elimination factors, the environmental burden of fish water treatment will be considerable. Monitoring of surface water for antibiotics and anthelminthics used in fish nurseries is needed to validate this assessment module.

Because of the diversity in trigger values, and the absence of any logic in their appearance in the decision scheme, they rather contribute to the uncertainty of the exposure assessment, than to a margin of safety. However, the current values were agreed upon in the European working group and are at the moment one of few broadly accepted management tools in the EU. Furthermore, the possibility to overrule the trigger value and continue the assessment is left open.

The compartments that are exposed determine what effects should be assessed. Effect values are the product of harmonised and validated experimental procedures based on recognised guidelines, with known uncertainty ranges and a broad acceptance. The CVMP

note for guidance has identified several endpoints (species and functions) and triggers that are well known from pesticide and new chemicals assessment. Effect parameters used are NOEC, LC50, EC50, MIC, %effect, and PNEC. The RCRs are compared to trigger values of 0.01, 0.1, and 1. This means that decisions are based on no-effect situations (PNEC, NOEC) or on maximum effect situations (MIC: the minimum concentration that inhibits growth completely), and RCR are compared to numerous triggers, clearly incorporating assessment factors. A clear approach, using only PNEC values and one trigger value (1) is more appropriate. Amongst others, this approach leaves the opportunity to the notifier to provide data on more species in order to refine the PNEC.

There can be great differences in agricultural practice and environmental conditions from one country to another, while at the same time on the European scale there is a need for harmonised exposure models and realistic trigger values. An interdisciplinary approach using knowledge of veterinary and agricultural practice, risk management, and environmental toxicology and chemistry, is needed to discriminate between relevant exposure scenarios and far-fetched worst-case calculations. These dilemmas call for an ongoing harmonisation process, preferably guided by a European technical guidance document on the environmental assessment of veterinary medicinal products.

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4. Validation of the exposure assessment for veterinary medicinal products

Submitted to Science of the Total Environment

4.1 Introduction

The marketing of veterinary medicinal products is actively regulated in the European Union by Directive 2001/82/EC, amended by Directive 2004/28/EC, in order to protect the environment, next to animal health, consumers, and professional users. An environmental risk assessment is to be performed at registration, and there is a clear policy and regulatory infrastructure to deal with this issue, as well as a number of regulatory guidance documents on the environmental risk assessment (EMEA, 1997; VICH, 2000; DG Enterprise, 2000; VICH, 2003).

Given the nature of risk assessment for new applications, there is a need to model exposure concentrations and associated risks. Addiscot et al. (1995) stated that some form of critical evaluation procedure is essential both to maintain the integrity of modelling and to ensure that the use of models by regulators does not result in the propagation of misleading information. The validation of screening models contributes to a better understanding of the information generated in the risk assessment and thus to the quality of the decision making process (Addiscot and Wagenet, 1985; Addiscot et al., 1995; Dee, 1995). Validation is used here in the meaning of establishing whether the model is ‘well founded and applicable’.

The first objective of this paper is to validate existing screening level exposure models for the risk assessment of veterinary medicines spread in manure, that have been presented previously in this journal by Montforts et al. (1999).

A second objective is to develop scenarios for the risk assessment under different European conditions, incorporating information on realistic agricultural and veterinarian practice, land use, geomorphology, and climate. The objective of the scenarios is to make the models applicable for European registration procedures for veterinary medicines.

A third objective is the verification of the 50% removal factor, the dilution factor and the emission pattern, assumed for the exposure assessment of the use of medicines in fish nurseries, by Montforts et al. (1999).

4.2 Screening model validation

Screening level models do not intend to represent reality accurately, but to provide rapid predictions of the potential environmental fate of a compound (Tarazona et al., 2003). To validate this claim, the available screening models were compared to field data published for soil, surface water and groundwater (Hamscher et al., 2000; Winckler and Grafe, 2001b;

Haller et al., 2002; Hamscher et al., 2002; Boxall et al., 2002; De Liguoro et al., 2003; Aga et al., 2003; Schlüsener et al., 2003).

4.2.1 Empirical validation of the soil exposure models

Soil exposure screening models for veterinary medicines have been proposed and discussed in literature (Spaepen et al., 1997; Boxall et al., 1997; Jorgensen et al., 1998; Montforts et al., 1999; WRc-NSF, 2001; Kelly et al., 2003). The deterministic and functional modelling of local exposure concentrations was also applied for other environmental contaminants such as heavy metals, pathogens and biocides (Breimer and Smilde, 1986; Montfoort et al., 1996; Walker and Stedinger, 1999; Van der Poel and Bakker, 2002). The modelling of regional distributions as applied for nutrients or pathogens in manure, was generally not used for registration of chemicals (Walker, 1997; Walker et al., 1998a; Walker and Stedinger, 1999; Vega et al., 2001; Menzi et al., 2002). The exception to the rule was the VetPec model used in the UK for veterinary medicines (WRc-NSF, 2001).

The soil exposure models at the screening level describe the concentration of the excreted residue in the slurry, and the concentration of the residue in soil after application of the slurry. The elementary deterministic soil concentration model used for veterinary medicines is described by:

$$Q_{excreted} = Q_{product} \cdot C_c \cdot T_{treatment} \cdot F_{excreted} \cdot m_{animal} \cdot Ncyclus_{animal}$$

$$C_{slurry} = \frac{Q_{excreted}}{P_{slurry}} \cdot e^{-k\deg_{slurry} \cdot T_{storage}}$$

$$PECsoil = \frac{C_{slurry} \cdot Q_{slurry}}{\rho_{soil} \cdot CONV_{area\ field} \cdot DEPTHfield}$$

with the following explanation of symbols (Table 4-1).

Empirical data on slurry concentrations and soil concentrations obtained in controlled studies are most useful for the empirical validation of this model. The data for the veterinary antibiotic sulfachloropyridazine (SCP) obtained in a field experiment in the United Kingdom are used to verify the soil concentration function (Boxall et al., 2002). This field experiment was performed with spiked manure spread over a sand soil and a clay soil, and all terms in the model were controlled. The sand soil was amended with slurry containing SCP at a concentration that would result in a nominal concentration in our standard models of 440 µg/kg. Corrected for the actual soil bulk density of the sand soil the nominal soil concentration amounts to 393 µg/kg dw. The initial measured concentrations (averaged over 20 cm depth) range from 232 to 669 µg/kg dw and were overall in close agreement to the

Table 4-1 Explanation of symbols for the soil concentration model.

input		
$Q_{product}$	dosage product used	[$kg.kg_{bw}^{-1}.d^{-1}$]
C_c	concentration chemical (c) in product	[$mg_c.kg^{-1}$]
$T_{treatment}$	duration of treatment	[d]
m_{animal}	(averaged) body weight	[$kg_{bw}.animal^{-1}$]
$F_{excreted}$	fraction excreted in faeces and urine	[-]
$Ncyclus_{animal}$	number of cycli per year	[$animal.place^{-1}.yr^{-1}$]
P_{slurry}	slurry production animal in stable in one year	[$kg.place^{-1}.y^{-1}$]
$T_{storage}$	duration of storage	[d]
$kdeg_{slurry}$	reaction constant transformation in slurry	[d^{-1}]
Qslurry	slurry immission standard	[kg. ha-1.yr-1]
ρ_{soil}	bulk density of soil	[$kg.m^{-3}$]
DEPTHfield	mixing depth with soil	[m]
$CONV_{area\ field}$	conversion factor for the area of the agricultural field	[$m^2.ha^{-1}$]
intermediate results		
$Q_{excreted}$	amount substance excreted	[$mg_c.place^{-1}.yr^{-1}$]
C_{slurry}	concentration in slurry	[$mg_c.kg^{-1}$]
output		
PECsoil	Concentration of the chemical in the soil	[$mg_c.kg_{soil}^{-1}$]

predictions. Thus the model performed satisfactorily as a screening tool. The clay soil was amended with slurry containing SCP at a concentration that would result in a concentration, corrected for the actual soil bulk density, of 500 µg/kg dw. The range of SCP concentrations (averaged over 20 cm depth) was between the limit of detection and 120 µg/kg.

Assuming a normal distribution for these measurements, regression analysis indicated that 90 percent of the distribution of soil concentrations would be within a range of 8 - 145 µg/kg dw soil. The modelled concentration of 500 µg/kg corresponds to the 0.11 percentile of the distribution. The probability of a concentration taking a value of 500 µg/kg or greater is only 0.11%, which makes it very unlikely that the theoretical concentration actually had been present anywhere in the field. Spatial heterogeneity may have accounted partly for these deviations (Vischetti et al., 1997). Thus the screening model did not predict the measured field concentrations in clay very well, or alternatively, about 90% of the SCP was lost between spiking of manure and analysis of clay soil samples.

Another paper contained empirical data on both soil and slurry concentrations of a veterinary medicine. Soil concentrations of 15 µg sulfadimidine per kg soil were found in Eastern Westphalia (Germany) in January 2002, in soil where 7 months earlier pig slurry had been applied (Christian et al., 2003). Pig slurry from the same location as used on the soil, although sampled some four months after this soil amendment, contained sulfadimidine at a concentration of 1.1 mg/kg ww. Assuming incorporation to 30 cm in a soil with a dry bulk density of 1740 $kg.m^{-3}$, the authors argued that the soil concentration was explained by the slurry concentration. However, since it concerns different batches of slurry, this argumentation is highly speculative.

The UK field study cited above was only concerned with the soil compartment, not with the slurry compartment. Using the data reported by Hamscher et al (2000; 2002) on tetracyclines (tetracyclin (TC), chlortetracyclin (CTC), oxytetracyclin (OTC)), a comparison was made between model calculations for one animal type (sows) and one dosage, and measurements in both slurry and soil. Two screening models were used: the Spaepen model (Spaepen et al., 1997) and the RIVM model (Montforts, 1999). In the model calculations, per place only one sow per year was supposed to be present, dosed with tetracyclines at 40 mg/kg bw for 5 days. The excretion factor was set to 1. DT50 for tetracyclines as a group may vary between 4 and 175 days, without correction for matrix, aeration or temperature (Jagnow, 1977; Kühne et al., 2000). (Soulides et al., 1962; Vej-Hansen et al., 1978; Gavalchin and Katz, 1994; Ingerslev et al., 2001; Winckler and Grafe, 2001a; Lumaret and Errouissi, 2002). In the calculations the degradation was assumed negligible. With the Spaepen model, soil concentrations of 75 µg/kg dw were expected. See Figure 4-1. The model calculations presented by Spaepen et al. (1997) were underestimating both the mean initial and maximum concentrations for tetracyclines (TC, OTC, and CTC). In the RIVM model the upper limit for the predicted soil concentrations was 207 µg/kg dw. Measurements reported were below or just above the RIVM prediction. The RIVM model provided for the more protective results, thus performing better as a screening tool.

The authors noted however that the measured soil concentrations were a factor 2-4 higher than expected based on the corresponding measured slurry concentrations. The authors

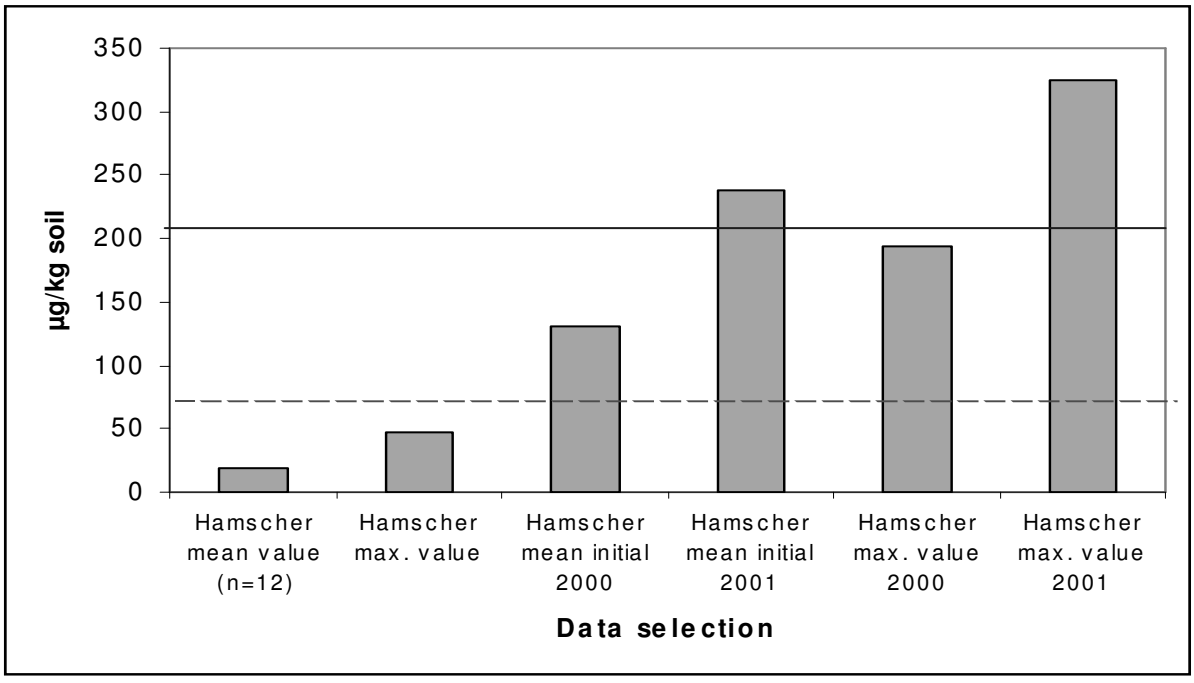


Figure 4-1 Graphical presentation of mean and maximum measurements of oxytetracycline (OTC) in soil in Germany (1999-2001). Straight line: initial PEC according to Montforts (1999) ; dotted line: initial PEC according to Spaepen et al. (1997) for fattening pigs dosed at 40 mg OTC/kg for 5 days.

considered that this difference indicated the presence of residues in the soil, additional release of bound residues between the sampling in the years 2000 and 2001, or higher application volumes than stated (Hamscher et al., 2002). Alternatively, due to incomplete mixing of the slurry matrix before the slurry sample is taken, strongly sorbed substances may have concentrated in the solid fraction in deeper layers. Measured concentrations in the liquid fraction may hence have lead to underestimation of the total substance load present. The RIVM model predicted a maximum concentration of 52 mg/L in slurry at a dosage of 40 mg/kg for 5 days (fattening pigs), which resulted in the predicted 207 µg/kg in soil.

Winckler and Grafe (2001c) found in German pig breeding facilities mean tetracycline concentrations of 11.6 mg/L and a maximum concentration of 66 mg/L in slurry. Compared to these data, the measured concentration in the 'controlled' slurry samples of only 4 mg/L reported in (Hamscher et al., 2002) was indeed rather low.

The soil sub-routine in the RIVM model tends to be conservative in predicted exposure levels, which satisfies the need to err on the safe side in the screening phase. In particular the prediction of the slurry concentration is challenged by uncertainties concerning dilution, mixing, and dissipation of residues.

4.2.2 Functional validation for surface water

Three screening level models were available for surface water. The EMEA and RIVM model contained a transport subroutine (soil-to-water transport rate) and a catchment subroutine (distribution and concentration in surface water) (EMEA, 1997; Montforts, 1999). The models were conceived around one algorithm (an export coefficient depending on sorption properties and a static water volume). The RIVM model assumes a dilution factor of 10, and describes the transfer as follows:

$$PECsw_{leaching} = \frac{PECporewater}{DILUTION_{leaching}}$$

$$PECporewater = \frac{PECsoil \cdot \rho_{soil}}{K_{soil-water} \cdot 1000}$$

$$K_{soil-water} = Fwater_{soil} + Fsolid_{soil} \cdot \frac{Kp_{soil}}{1000} \cdot \rho_{solid}$$

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

The EMEA model assumes a dilution factor of 3.3 and describes the partitioning function as follows. For both models the explanation of symbols is in Table 4-2.

$$PECporewater = \frac{PECsoil}{Foc_{soil} \cdot Koc}$$

Table 4-2 Explanation of symbols for the surface water exposure functions.

input		
$DILUTION_{leaching}$	dilution factor for leaching	[-]
PECsoil	concentration in the soil	[$mg_c.kg_{soil}^{-1}$]
ρ_{soil}	fresh bulk density of soil	[$kg.m^{-3}$]
ρ_{solid}	density of soil solids	[$kg.m^{-3}$]
$Fair_{soil}$	fraction air in soil	[$m^3.m^{-3}$]
$Fwater_{soil}$	fraction water in soil	[$m^3.m^{-3}$]
$Fsolid_{soil}$	fraction solids in soil	[$m^3.m^{-3}$]
Foc_{soil}	fraction organic carbon in soil (w/dw)	[$kg.kg^{-1}$]
Koc	partition coefficient organic carbon - water	[$dm^3.kg^{-1}$]
intermediate results		
$K_{soil\text{-}water}$	partition coefficient solids and water in soil (v/v)	[$m^3.m^{-3}$]
Kp_{soil}	partition coefficient solids and water in soil (v/w)	[$dm^3.kg^{-1}$]
PECporewater	predicted concentration in pore water	[$mg_c.l^{-1}$]
output		
$PECsw_{leaching}$	predicted concentration in surface water	[$mg_c.l^{-1}$]

In this methodology, the concentration in surface water depends on the concentration in soil as a result of spreading of slurry. The degree of surface water contamination in these lower level exposure models is not related to the actual transport processes (erosion, run-off, and drainage), the ratio between treated soil and receiving surface water, nor to the distance to the surface water. The screening models consider that the soil to water transfer is linked exclusively to the soil pore water concentration, which is estimated from equilibrium processes based on the Koc or related parameters, followed by a dilution factor between soil porewater and surface water. The distance to the surface water is not modelled.

The VetPec model is more complex, consisting of a run-off model and an aquifer model that accompany the soil concentration model (WRc-NSF, 2001). Distribution of chemicals between porewater and solids are calculated using fugacity equations. 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 time step, substance degradation, inflow and outflow are calculated, after which a new equilibrium is obtained (MacKay et al., 1986). The surface water predictions by VetPec have been validated with a selection of data on the pesticide isoproturon. The hydrology in the models was however not validated. No validation on less mobile compounds has been performed.

These models were subjected to an empirical validation using data from a field experiment with the veterinary antibiotic SCP performed in the United Kingdom (Boxall et al., 2002). In the sand soil, soil porewater concentrations of SCP had been measured, and in the clay soil, drain water concentrations had been measured. The fate in the receiving water

compartments was however not assessed. The validation of the fugacity models in VetPec was hampered by this approach, since the transfer rates were to be validated and not the equilibrium concentrations. This validation exercise generated highly variable and unsatisfying results, as shown in Table 4-3. These model approaches did not perform satisfactorily as a screening tool.

Few data on surface water contamination by medicines and hormones via land are found in literature. A selection is briefly discussed below to add further considerations to the validity of screening level exposure models.

Ivermectin has a very high sorption coefficient K_{om} of 4500-5500 L/kg, and is rather persistent in soil with degradation half-lives (determined at 22°C under laboratory conditions) of 93 to 240 days (mean 187 days, n=4) (Halley et al., 1989). Transport of ivermectin by drainage would be considered negligible, since this route is not assessed in the EMEA model at K_{om} >500 L/kg. In a study on run-off from cattle feedlots in the United States, only trace amounts (up to 2 ng/L) of ivermectin were indeed detected occasionally (Nessel et al., 1989).

Oxytetracycline shows strong adsorption behaviour in soils with logKoc of 4 - 5 (Rabølle and Spliid, 2000). In an Italian study, no oxytetracycline was detected in drainage ditches adjacent to treated fields (LOD 1 µg/L) (De Liguoro et al., 2003), which is consistent with the EMEA model trigger that surface water is not exposed if Koc is >500 L/kg (EMEA, 1997). In an US investigation, 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 (Campagnolo et al., 2002). In an UK field study, mass losses from soil into drainage water accounted for not more than 0.5% of the dose applied to the soil. Peak concentrations of oxytetracycline were 36 µg/L. For sulfachloropyridazine, the peak concentration was 613 µg/L (Kay et al., 2004). Of sarafloxacin and related fluoroquinolones the logKoc is reported to be 5 – 6 (AHI, 1997; Nowara et al., 1997). However, the concentrations of tetracyclines and sarafloxacin found occasionally (1-36 µg/L and 4 µg/L, respectively) do not support the above-mentioned EMEA model trigger. These scarce data cast further doubt on the applicability of the surface water exposure models for screening purposes.

Table 4-3 A summary of model calculations for surface water and peak drain flows in the UK field experiment with SCP (Boxall et al., 2002). All results are in [µg/L]. nc = not calculated.

Soil type	Nominal soil concentration [µg/kg]	VetPec Max. value Porewater	VetPec Surface water	EMEA groundwater	EMEA Surface water	RIVM Surface water	Peak flow from drainpipe (clay) or soil pore water (sand) (LOD 0.25 µg/L)	
							Year 1	Year 2
Sand	393	0.84	0.01	370	112	37	0	0
Clay	500	nc	0.01	nc	74	26	589	6

Veterinary pharmaceuticals are released into the soil as part of an organic matter rich matrix, and it is well known that organic amendments including manure can increase but also decrease the adsorption of chemicals to soil (Iglesias-Jimenez et al., 1997; Morillo et al., 2002). In addition, the direct transfers of the non-dissolved chemical fraction should be considered. The transfer of particle-bound fractions is particularly important for run-off, while colloidal associations should be considered for drainage since the fraction bound to small particles could also be relevant for preferential flow via macropores. For example the sorption of oestradiol-17β is associated with the surface area and/or the cation exchange capacity of the soil, with high correlation to particle size (clay) and organic matter. K_F values were in the range of 86 – 6670 L/kg, with K_{om} values of 1800-72500 L/kg (median 2600 L/kg) (Casey et al., 2003). In soil column leaching studies performed with oestradiol-17β K_{om} values ranging from 950 to 1700 L/kg were determined. The strong sorption did not appear to hinder degradation; degradation half-life values of 0.4-10 days were determined (Das et al., 2004). 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 second simulated storm event seven days later was 69% of the first loss, which is in proportion with the load remaining after the first event (Nichols et al., 1997; Busheé et al., 1998).

The screening level models tend to be conservative in predicting exposure levels, which satisfies the need to err on the safe side in the screening phase. However, the trigger value for surface water exposure clearly obscures the possibility of transport of strongly sorbing substances.

4.2.3 Functional validation for groundwater

With respect to groundwater, in Phase I of the EMEA Note for Guidance the concentration in the ground water is set equal to the concentration in the porewater (EMEA, 1997). The EMEA scheme suggests that the PECsoil as calculated for the upper layer is the input term for the calculation. Conceptually, in this model the groundwater table reaches to the mixing depth defined by the soil concentration module, or it supposes complete vertical transposition of the residue to the depth of the groundwater table. In this model partitioning depends on equilibrium sorption to solids, no saturation at binding places, and steady-state conditions. Movements, dilution, desorption, and transformation is not modelled. As explained in the previous section, in VetPec the distribution of chemicals between porewater and solids are calculated using fugacity functions.

In Table 4-3 a comparison is made between the predictions and the porewater measurements of SCP in the UK field experiment (Boxall et al., 2002). Again, these model approaches did not perform satisfactorily as a screening tool.

The accuracy of the partitioning model predictions depended strongly on the soil layer to which the measurements were standardised. If a 5 cm layer was chosen instead of the current 20 cm, calculated concentrations would have been four times higher. In any case, all

calculations have overestimated the actual porewater concentrations. The maximum *drainflow* concentration from the clay field in the first year (Table 4-3) corresponded reasonably with the EMEA *porewater* calculation. The field measurements are clearly the result of preferential flow of both solutes and manure-associated particles through soil 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 drainpipes, resulting in lower concentrations.

4.3 Scenario parameters

The exposure modelling can be split in three major subroutines: one for the animal husbandry phase, one for the slurry handling, and one for the environmental phase. The situation of interest that is to be modelled defines the model approach (mechanistic, deterministic) and the respective parameter values. A specific combination of parameter values is denoted a scenario. The following variables and scenario parameters need to be observed in the scario and have to be addressed, either as a parameter or as default:

1) Emission
   a) disease incidence and remediation: occurrence of infections 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: timing and amount
   e) slurry texture
3) Substance behaviour in slurry
   a) degradation in slurry
   b) distribution in slurry layers or fractions (solid-liquid)
4) Immission into soil
   a) dosage applied
   b) repetitions
   c) soil management
5) Substance behaviour in soil and water
   a) degradation in soil
   b) distributions in soil (spatial heterogeneity)
6) Environmental conditions
   a) climate
   b) soil
   c) hydrology
   d) topography.

Given the quantity of variables, it is virtually impossible to control all variables and examine one parameter at the time in an experimental setting. Below key parameters are discussed.

Emission. Disease incidence and dosage are static parameters, since in the risk assessment at registration the modelling is performed using a given prescribed dose to a target animal. Several target animals have more production cycles in one year, which may all need treatment. Depending on the relation between animal cycles and manure storage in the model, a certain number of cycles should be observed in the emission module. Excretion of residues is also an input-parameter in the model. Excretion patterns and cumulative excretion may differ depending on species, race, mode of application and dosage. Data reported in literature suggest that total cumulative excretion may range from 0.2 to 1, and the excretion time from 1 and 100 days, depending on species, substance, dosage, and route of administration (Short et al., 1987; Halley et al., 1989; Lumaret et al., 1993; Herd, 1995; Ramazza et al., 1996; Strong et al., 1996; Winckler and Grafe, 2001c). Although the parent compound may be transformed into transformation products, the conjugates may be reverted in the slurry to active compounds (Henschel et al., 1997; Panter et al., 1999) and others may have some activity themselves (Schowanek and Webb, 2002). Excretion of metabolites may hence contribute to the effects of the parent compound.

Storage. The input, storage and outflow of contaminated and uncontaminated slurry determine the loads that will reach the soil. Different animal types may contribute to the same slurry storage system and the treated animals do not exclusively determine final concentrations. Slurry production and quality is monitored more or less intensively in EU countries due to the restrictions in the use of fertilisers. 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). Body weights at treatment are proposed based on adult weights for parent animals and the mean of slaughter weight and starting weight for production animals. Depending on the physical state of the manure, the manure is kept in bedding, piled, or stored in tanks or lagoons. Proportions of manure types and storage systems differ considerably between countries (Menzi, 2002). Depending on structure and handling, the manure can be aerobic or anaerobic and there will be a great variation in temperature, redox potential, pH, and storage time of the slurry. Different manure types and storage systems will influence storage conditions and manure composition in different ways (Donham et al., 1988). Conditions like oxygen levels, manure age, microbial activity and temperature will determine the fate of organic contaminants to a large extent, but are highly diverse within and between storage systems (Hoeksma et al., 1987; Novem, 1991; Jenkins et al., 1997; Richard et al., 1998; Pitts et al., 1998; Qiang, 1999; Arogo et al., 1999; Schiffer et al., 2001; Moreira, 2001). Depending on climate, season, storage systems and manure structure, temperatures can range from ambient (freezing) to 65°C (composting) (Kelley et al., 1994; Eghball, 1998). For underground slurry storage systems this range can be narrowed down, because average soil temperatures for Europe range from 4 to 18°C (FOCUS, 2000). For storage of solid manure

(piles, containers) temperatures can be quite higher and varying due to composting processes (until anaerobicity is reached).

Manure models that describe manure loading, quality change, and fate of constituents (i.e. CO_2, NH_3) do exist, and could be adapted, but also have to be improved amongst others on insufficiently developed manure production submodels and the limitation to liquid manure facilities (Ni, 1999; Ni et al., 1999; Hilhorst and De Mol, 2002). The most realistic period in which storage can take place cannot be determined with a rule of thumb. Storage time is a function of manure output and substance and manure input. Since these functions largely depend on the (unknown and individual) substance prescriptions, disease patterns, and manure management, either a complete manure model with detailed inputs is needed, or a scenario for every livestock category of interest has to be defined. In the latter option, dosing, excretion and manure handling are made part of the scenario rather than of the model algorithms. Given the sheer endless variability described above, the slurry storage, production, and removal, should be defined in scenarios for screening level models.

Soil immision patterns. Agricultural practices will play a very important role in determining the concentrations of veterinary drugs in the environment. Livestock manure is the second most important source of nutrient inputs to agricultural land (Pau Vall and Vidal, 2001). The pattern of agriculture and manure use can vary widely from one region to another. The nutrient content of manure varies from country to country and from one region to another within a country (Provolo and Riva, 2002). The relative contribution of the animal types to the manure-N input to soil per EU country is depicted in Figure 4-1. These data indicate that bovine and pig manure are the main slurry types used to fertilise land (if organic fertilisers are used). However, sheep and poultry manure are regionally and locally important. We propose to use the lower nitrogen production standards presented to the European Resource Management of the European Commission DG XI (Ketelaars and Van der Meer, 2000) (Table 4). Storage systems differ between countries and animal types. The frequency at which slurry is taken out of the storage facilities can have a different timing. For example, for cattle and pig slurry, seven respectively three moments are considered by (Tijmensen et al., 2002). Another source reports average slurry storage times of 9 months (range 0 – 50 months; see table 5), but it is not specified to what animals the data apply (WRc-NSF, 2000). Spreading events are monitored at regional levels (ADAS, 1998; Berende, 1998; Van Staalduinen et al., 2001). In the Netherlands and in Belgium, over 95% of the agricultural (arable) land is manured one to three times per year. The manure produced by livestock will be applied to land, the amount applied being dependent on immission limits for nitrogen and phosphorus, fertiliser recommendations, soil type or crop tolerance for slurry. These limits which are designed to avoid the excessive input of nutrients in soils vary across member states. For example in Italy, at national level, the maximum amount of manure which can be applied to land that is not designated as a vulnerable area is the annual production from 4 tonnes of live weight per hectare without regard of animal species (Bonazzi, 2002). Based on

Table 4-4 Standardised nitrogen production standards (P_N), rounds (N_{animal}), and treatment body weights (m_{animal}), for different livestock categories (Ketelaars and Van der Meer, 2000).

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

[#] veal data based on (Van Staalduinen et al., 2001) and (Montforts, 1999)

the data in Table 4, the annual load may range up from 275 kg N/ha for turkeys to 550 kg N/ha for dairy cows. The exact amount that is allowed will depend on the way the production per hectare is quantified over the year, and the nitrogen content of the manure produced. The amount of slurry that is actually applied (in one time) will depend on many other practical factors. A different situation is found in the Netherlands, that designated its entire territory as a vulnerable area under the EU Nitrate Directive (91/676/EC). This directive applies to areas that are vulnerable to leaching of nitrate in all member states and the nitrogen immission standard of 170 kg N/ha can be considered as a realistic best case for these areas.

Incorporation depths have been recorded in an inventory by WRc-NSF (WRc-NSF, 2000) (see Table 4-5). Plough depths for slurry and solid manure were in the range of 0-28 cm, with averages of 16.5 cm.

Table 4-5 Amounts of slurry stored and applied, time of application and length of storage (WRc-NSF, 2000).

	% applied	quantity stored	storage time	application rate	plough depth	nitrogen content
Slurry		gallon	months	kg/ha/y	cm	kg N/m^3
Average	100	1042571	9.1	21213	16.5	2.69
Min	100	0	0	80	0	0.99
max	100	5000000	50	100000	28	7
Manure		tonne				tonne
Average	98.4	2359	8.06	12637	16.5	2.11
Min	25	0	0	3	0	2.11
Max	100	110000	48	55000	28	2.11

Environmental conditions. Climate and soils are important factors in the determination of chemical concentrations in the environment. In 1993, the European Commission and the European Crop Protection Association jointly established FOCUS (Forum for the Co-ordination of pesticide fate models and their USe) which, as one of its tasks, established standard leaching and surface water exposure scenarios for pesticide registration in Europe. The simple fact that this methodology encompasses the same agricultural fields that are relevant for manure application, predestined these scenarios to be applicable to residues spread by manure as well.

4.4 Scenario development for spreading of manure

Since at the screening stage of the exposure assessment already decisions are made on the acceptability of the environmental risk, regulatory authorities have to be careful in choosing exposure scenarios (Montforts et al., 1999). The emission route from slurry to soil and water is unmistakably an important route, and a complex one. A critical component for the registration procedure is the identification of relevant scenarios. The actual use pattern of the product should be explored, because repetitive use, season-related use, or concurrent uses over large areas, in relation to the timing and scale of emission to the environment (i.e. spreading of manure), have a significant impact on the actual exposure. A general lack of information on distributions of parameter values within and between systems forces one to propose the use of a deterministic model with empirical parameters defined in a simple scenario of realistic and standardised conditions.

The amount of slurry present in storage depends on the time of year; and depending on the number of cycles treated during this time, the concentration in the slurry is determined. A major difference between the two screening models that have been discussed is the time span in which clean manure is produced. The shorter this period, the higher the predicted manure concentrations will be. The amount of manure that is spread containing the residue of the treatment is delimited by the storage capacity of the system and the opportunities to take the slurry out. Most UK farms only have the capacity to store slurry for less than one month, and in several other European countries from 1 to 12 months (Menzi, 2002). The depth of

incorporation depends on the method of application. In the field, no incorporation has been registered as general agricultural practice. If manure is incorporated, the mean incorporation depth is 16.5 cm. Maximum slurry loads ranged up to 550 kg N/ha/year and more. Realistic worst case conditions are hence proposed in a simple scenario assuming:

- single treatment per animal place,
- standard European nitrogen production values,
- a manure production volume of 1 month (30 days) containing the full residue,
- a nitrogen application rate of 600 kg N/ha/year in one time onto agricultural land, which is distributed over 5 cm soil with a bulk density of 1500 $kg.m^{-3}$,
- no dissipation during storage, and no after-treatment of slurry.

If the exposure calculation in Phase I according to this scenario fails the trigger for further testing, safe use in all member states is possible.

If not, then realistic best case conditions, characterising a possible safe use in vulnerable areas under the Nitrate Directive in the European Union, are proposed in a similar scenario, now assuming

- active incorporation of slurry into 20 cm of soil,
- at a nitrogen application rate of 170 kg N/ha/year in one time.

If the trigger is exceeded, then a phase II assessment would be compulsory for all member states. Further assessments should be made at the member state level, since environmental concerns can be a reason to refuse (mutual recognition of) marketing authorisation[20].

The PECsoil can be used in conjunction with screening level models that predict mass transfer to groundwater and surface water. Both screening level and mechanistic models for distribution of residues from soil provided for pesticide registration by FOCUS are considered as suitable for veterinary drugs as for pesticides (FOCUS, 1995; Groen, 1997; FOCUS, 2000; FOCUS, 2001). The simple fact that this methodology encompasses the same agricultural fields that are relevant for manure application predestined these models and scenarios to be applicable to residues spread by manure as well. Application timers in the models should be set to relevant regional conditions for manure application. These may depend on spreading restrictions and where these do not apply, by worst case conditions (e.g. autumn vs. spring conditions).

Interestingly, the predicted initial soil concentration using the proposed scenario in conjunction with Dutch default conditions, for a high volume compound like (oxy)tetracycline used in pigs or broilers, amounts to 200 µg/kg soil, with a possible maximum of 1000 µg/kg soil (Montforts, 2003). The average concentration of antibiotics in soil based on the total annual consumption of 402 tonnes and the total capacity of agricultural fields to utilise manure in the Netherlands, amounts to approximately 100 µg/kg soil (Van Staalduinen et al., 2001; MARAN, 2002). The difference between these results is within an

[20] European Court of Justice, Cases 302/86 Danish Bottles [1988] ECR 4607 and 120/78 Cassis de Dijon [1979] ECR 649

order of magnitude, which suggests that regional scale modelling of soil concentrations may provide suitable approaches for protective risk assessments.

4.5 Fish cultivation

The scale of fish cultivation, dominated by land based fish nurseries, for commercial purposes, is limited in the Netherlands. The production amounts to about 0.2% of the production of animal husbandry (Luiten, 2002). In 1994 in total 26 and 10 companies were involved in cultivating eel and catfish, respectively. Most nurseries use water recirculation systems, in which the water is recycled after a water treatment by filtration. Catfish nurseries discharge on the municipal Sewage Treatment Plants (STP), but 40% of the eel nurseries discharge directly on surface water. Tropical fish nurseries may also contribute to the emission to surface waters (Schrap et al., 2003)

The surface water exposure scenarios discussed in Montforts et al. (1999) were based on a fish farm that breeds 50 tonnes of eel a year, the median production in The Netherlands. Two scenarios were distinguished, based on the incidence of administration of the product, and the type of wastewater treatment:

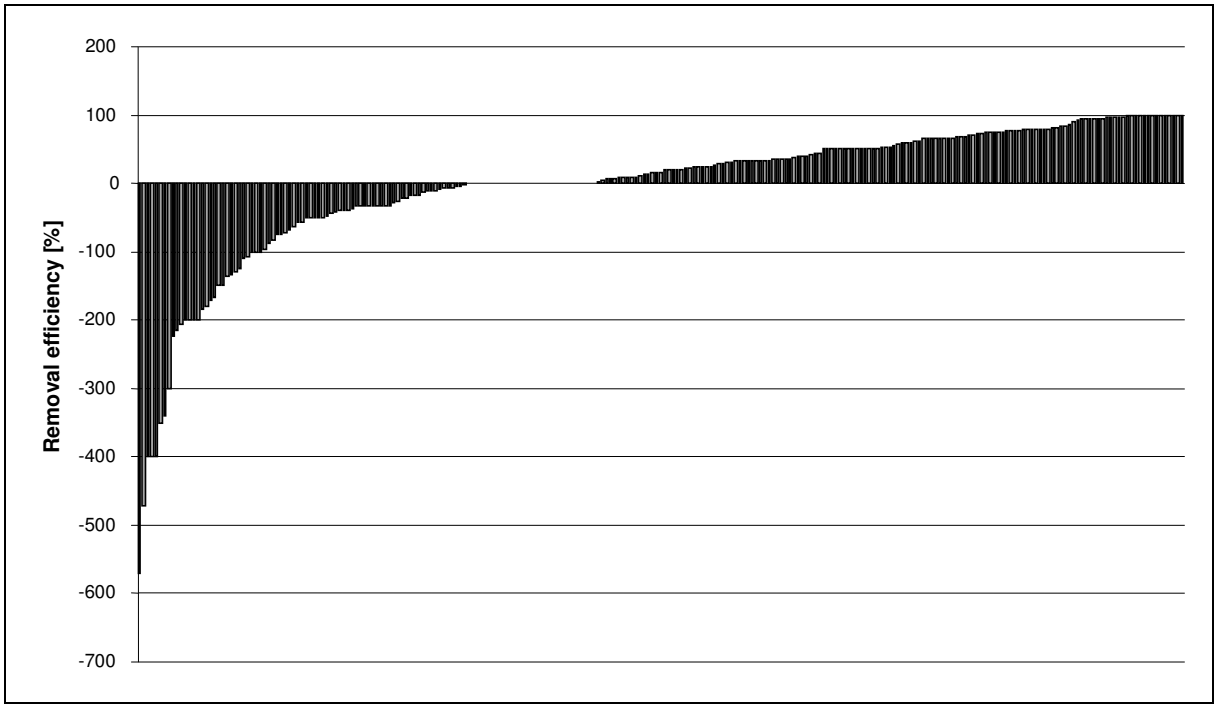
- continuous medication; without recirculation/filtration, followed by a settlement tank;
- occasional medication (≤4 times a year), without recirculation/filtration before discharge to the settlement tank.

The total water volume of the nursery was set at 70 m^3. After the settlement tank the water fraction is discharged to surface water. The settling tank is used in order to reduce the amount of precipitation in the waste water before discharge into surface water or waste water treatment systems, but it was also assumed to play a significant role in reducing concentrations of chemicals in the waste water. It was assumed that the settling tank had a removal efficiency of 50%. This 50% removal efficiency was taken from Wagemaker (1993) and is an estimated value based on the average difference between pesticide concentrations that were measured in the influent and effluent of settling tanks used at mushroom production plants, as reported in Van Beersum (1988). Due to the settlement tank the total amount of substance emitted was equally spread out over 25 days. In the exposure model the fish farm discharges on a small waterway that dilutes the effluent by a factor 5 or 3, depending on the water volume discharged. The surface water concentration was therefore constant for at least this emission period.

In addition to the Van Beersum (1988) data set, data on the concentration of pesticides in wash water from flower bulb processing facilities were analysed (Frijters, 2000). A critical evaluation of the available data was performed. The two sets of data analysed contained a total number of 251 observations:

- Individual concentrations of 41 pesticides in the influent and effluent of settling tanks at three different mushroom processing companies at five different time points (Van Beersum, 1988)
- Individual concentrations of 17 pesticides in the influent and effluent of settling tanks used in the washing of flower bulbs (Frijters, 2000).

Figure 4-2: Distribution of removal percentages based on selected influent/effluent concentration, expressed as a percentage of the influent. Average –2.35%, median 16.67%, 10th percentile –110%, n=251



The difference between influent and effluent concentration was expressed as a percentage of the influent concentration. The results are presented in Figure 4-2. The calculated 'removal efficiencies' based on 251 sample pairs varied from –571% to 100%. The available data show an averaged difference between the influent and effluent concentration of -2.35%. The median of the samples was at 17%. Thus, if an indication of the removal efficiency can be derived from these data, the settlement tanks removed a negligible amount of the total load.

Perhaps more important is the applicability of the model for the situation of interest. The reduction to safe levels of the excess organic substances in effluent and the increase of the fraction of the water reused are the major purposes of wastewater treatment in indoor fish production systems. In nursery water treatment systems, a very high concentration of organic matter in wastewater, but no soil, can be expected (Kamstra and Van der Heul, 1998). A sub-routine describing the effect of water treatment and sludge retention should be based on the systems of interest.

The emission pattern of treated water is of importance. Some treatments require stopping the water flow for some time, followed by transfer of fish to clean water. Therefore a 100% immediate release should be expected for a worst case estimate. In the original model, emission of the residue was modelled over 25 days, but this retention factor lacks empirical or mechanistic underpinning.

In the exposure model the fish farm discharges on a small waterway that dilutes the effluent by a factor 5 or 3, depending on the water volume discharged. In trench-like waters,

owing to the low flow, only poorly developed turbulence is likely to occur from time to time. Hence, in polder waters and the like, it is to be expected that noticeable lengths of these channels be gradually filled with poorly diluted effluent from waste water treatment plants or other point sources. In the Netherlands, about 60 domestic wastewater treatment plants (c. 20%) have a dilution factor of 2 within 100 metres (De Greef and De Nijs, 1990). Whether or not the effluent flows of the fish nurseries are similar to those of the domestic treatment plants is unknown. Monitoring of effluent from a tropical fish nursery in the Netherlands, with presumably batch-like treatment, revealed concentrations of 11 – 41 µg/L for six therapeutics. The highest concentration found was 120 µg/L for oxytetracycline. Downstream of the effluent oxytetracycline was measured at a concentration of 57 µg/L (Schrap et al., 2003). A dilution factor of two may be very well applicable to direct emission in low-flow ditches. Given the high sorption coefficient of oxytetracycline of logKoc 4 - 5 L/kg (Rabølle and Spliid, 2000), dissipation towards the sediment would be expected. For example at the emission point of a tank-based aquaculture system for trout in Northern Italy, a sediment concentration of 246 µg/kg dry weight was found (Lalumera et al., 2004).

4.6 Conclusions

The empirical validation of the soil concentration models with (oxy)tetracycline and sulphonamides conducted 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 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.

Surface water and groundwater models generated highly deviating predictions compared to the field results. What all models have in common is that soil porewater concentrations are exaggerated compared to the results of the sand soils, but not compared to the results of the clay soil. This provides reason to assume that surface water contamination is not controlled by sorption alone, and cut-off values on sorption properties are not warranted. Furthermore, the exposure model will always predict surface water exposure, which means that spreading manure containing residues of veterinary medicines always leads to pollution of surface water. This prediction always warrants a risk assessment, since all actions that may lead to surface water pollution are to be controlled under the surface water pollution Directive 76/464/EEC.

A scenario for a simplified soil exposure model has been made. The PECsoil can be used in conjunction with screening level models that predict mass transfer to groundwater and surface water. Both screening level and mechanistic models for distribution of residues from soil provided for pesticide registration by FOCUS are considered as suitable for veterinary drugs as for pesticides (FOCUS, 1995; Groen, 1997; FOCUS, 2000; FOCUS, 2001). The simple fact that this methodology encompasses the same agricultural fields that are relevant for manure application predestined these models and scenarios to be applicable to residues spread by manure as well. Application timers in the models should be set to relevant regional conditions for manure application. These may depend on spreading restrictions and where these do not apply, by worst case conditions (e.g. autumn vs. spring conditions).

Interestingly, the predicted initial soil concentration using the proposed scenario in conjunction with Dutch default conditions, for a high volume compound like (oxy)tetracycline used in pigs or broilers, amounts to 200 µg/kg soil, with a possible maximum of 1000 µg/kg soil (Montforts, 2003). The average concentration of antibiotics in soil based on the total annual consumption of 402 tonnes and the total capacity of agricultural fields to utilise manure in the Netherlands, amounts to approximately 100 µg/kg soil (Van Staalduinen et al., 2001; MARAN, 2002). The difference between these results is within an order of magnitude, which suggests that regional scale modelling of soil concentrations may provide suitable approaches for protective risk assessments.

The development of regional and catchment area models for assessing pesticides and other agrochemicals, but also manure-borne pathogens, is receiving a significant attention (Walker and Stedinger, 1999; Arhonditsis et al., 2002; Deelstra et al., 2002; Bicudo and Goyal, 2003). In particular run-off and erosion in vulnerable areas of the Mediterranean region would be suitable processes for regional modelling. Additional alternatives are the use of total losses, which may be implemented through probabilistic models (Pablos et al., 1998). It should be, however, recognised that addressing these transfer routes requires regional models, which cannot be implemented as generalised screening tools. Recent studies demonstrate the influence of particle-bound fractions in regional transport modelling and the relevance of erosion in the overall transfer of organic matter from soil to watersheds. These findings suggest that the contribution of these mechanisms can be higher than expected, particularly for chemicals with a high binding potential (McLachlan et al., 2002; Polyakov and Lal, 2004). Smith et al. (2001) observed relevant contributions of surface run-off including particles losses for manure applications over 2.5-3.0 t/ha slurry solids in UK arable lands. But due to the higher run-off/erosion risk of the Mediterranean region a lower threshold should be considered for this area (Arhonditsis et al., 2002). Tentatively, screening assumptions can be done on the basis of simplistic models, correlating directly the concentration in the top soil layer with the expected concentration in the run-off. These models were initially developed for herbicides. They describe non-linear relationships, with an extraction coefficient representing removal of the pesticide from the top 1cm soil layer, which ranged from c. 0.01 up to 0.2, and an exponent representing reductions in the

extraction potential with ageing (Leonard et al., 1979; Southwick et al., 2003). The suitability of these models for pharmaceuticals should be studied.

It is concluded that the original 50% removal efficiency of the settlement tank as used in Wagemaker (1993) and Montforts (1999) lacks empirical foundation since only part of the entire data set (the 'positive' efficiencies) had been used. There is no evidence that a settling tank contributes to removal of a substantial fraction of the total load of dissolved and particle-associated pesticides from wastewater. Furthermore the nature of the settling process of fish nursery sludge may be very different from that of soil particles. The data available are unsuitable for a mechanistic analysis of retention processes, due to a lack of detail in the description of system dimensions, water volumes and flow rates, sludge characteristics, and total pesticide load. A sub-routine describing the effect of water treatment and sludge retention should be based on the systems of interest. Monitoring data from a location with direct emission of fish nursery wastewater indicated a dissipation (or dilution) factor of 2 between effluent and downstream surface water concentrations. What dilution factor is most applicable to fish nurseries cannot be determined. Sediment contamination should be incorporated into the exposure model.

This validation indicates that the surface water exposure model was not well founded, and is based on generalised data derived from situations that were not applicable. With the aim to eliminate no-risk situations from further assessment, a worst-case assumption would be that the therapeutic concentration would be the environmental exposure concentration. Further risk assessment will require the development of an applicable exposure model.

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5. Effect assessment at the base of an exposure trigger in soil – a critical appraisal

5.1 Introduction

Environmental protection legislation both in the USA and in Europe requires an environmental impact assessment for projects and activities, including the use of chemical substances (Zeeman, 1987; Van Leeuwen, 1995; Kolluru, 1996).

The starting point of any risk assessment is the translation of the desired environmental quality in quantifiable goals. So far, protection goals have been associated with the structures and processes within the physical environment: water bodies, soil layers, systems, habitats, species and individuals (Van Leeuwen, 1995). It is the concept of risk that connects these protection goals with the possible presence and impact of substances.

In the risk assessment, effect and exposure are treated as variables that are determined by product use and substances properties. The exposure is generally quantified in a model under realistic worst case conditions. The effects of a given substance are assessed using toxicity models (e.g. single species tests) in order to determine the exposure concentration that will not result in unacceptable effects. This concentration is derived from the lowest experimental (no-)effect value and a safety factor that depends on the underlying dataset, or from a statistical analysis of these underlying data. This statistical analysis assumes a normal distribution of toxicity data representing the ecosystem sensitivity to the given substance (Posthuma et al., 2002). More complex studies involving multiple species under environmentally relevant conditions are also relevant in determining an exposure level of no concern (De Jong et al., in press). The ratio between calculated exposure and the predicted acceptable effect level is the risk quotient (PEC/PNEC or RQ): the risk information the regulator can use to make a decision for that product. Guidance on exposure and effect assessment is given in Technical Guidance Documents (TGD) used in the European Union (EU) frameworks of new and existing substances, biocides, and medicines for human consumption (Forbes and Calow, 2002c; EC, 2003).

In the EU, chemicals and products containing medicines, feed additives, pesticides, and biocides can only be notified or registered if risk-based standards laid down in the European legislation have been met, but at the same time non-risk standards are applied: triggers on production, emission, formation, occurrence, and hazard (Van Leeuwen, 1995; Bartell, 1996; Heyvaert, 1999b; SSC, 2002). Some examples are:

- The standard for groundwater and drinking water for pesticides and biocides is 0.1 µg/L in the 91/414/EC and 98/8/EC directives. The exposure standard itself is however not based on risk, but on hazard considerations in combination with best available detection techniques at the time the standard was set. The trigger might be underprotective for some substances (Crommentuijn et al., 2000). The Scientific Committee on Plants (SCP) and the Scientific Committee on Toxicology, Ecotoxicology and the Environment of the

European Commission DG Sanco stress the need for further risk evaluation at exposure concentrations below the 0.1 µg/L (SCP, 2000; CSTEE, 2002).

- The assessment of the risk to sediment dwelling organisms under the 91/414/EC directive is triggered by the exposure of the sediment phase: >10% after 14 days in a water/sediment degradation test, in combination with the hazard to *Daphnia magna* (ECCO, 2002).
- Persistence and bioaccumulation under the 91/414/EC and 98/8/EC directives have to meet hazard-based standards (DT50 <90 days, DT90 <1 year, BCF <100 L/kg), if not they trigger specific ecotoxicological testing.
- In the 2001/79/EC directive on feed additives, an exposure concentration of 10 µg/kg soil is used as a threshold for further risk assessment.
- In the guidance on risk assessment for veterinary medicines an exposure concentration of 100 µg/kg soil is used as a threshold for risk assessment (VICH, 2000).
- In the same guidance an effluent concentration of 1 µg/L for fish medicines is used as a threshold for risk assessment (assuming a dilution factor of 10 for surface water).

The Scientific Steering Committee of the Health & Consumer Protection Directorate General (DG Sanco) of the European Commission (EC) endorsed the research on triggers for prioritisation (SSC, 2000).

This paper discusses the scientific tools developed in ecotoxicology, environmental chemistry and risk assessment, employed to derive the trigger concentration in soil for the assessment of veterinary medicines.

5.2 Case study: the soil concentration trigger value

The EU directive 2001/82/EC on the registration of veterinary medicinal products calls for an environmental risk assessment (Anonymous, 2001b). An international harmonisation of the guidance and data requirements pertaining to the environmental risk assessment of veterinary medicinal products in the EU, USA and Japan was started in 1996 by the International Co-operation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH). The resulting guidance document on the environmental impact assessment (Phase I: exposure assessment) is now operational in the product registration for veterinary medicinal products in Europe (VICH, 2000; EC, 2001b; Koschorreck et al., 2002). The guidance document foresees no risk-based assessment for substances with a presumed negligible emission and exposure level. Science was called in to back up the numerical value of the soil exposure trigger, with an assessment of a dataset of several drug substances. Concepts and methods developed in ecotoxicology, environmental chemistry and risk assessment were introduced and applied to data which lead to the recommendation that the lowest toxicity value (100 µg/kg) in the available dataset should provide an acceptable measure of safety to protect the environment (AHI, 1997).

The soil concentration trigger decides if a risk assessment is needed. It is an exposure trigger and is based on effect data. All data that were at the base of the value of this trigger are presented in Table 1. Effect data for a given substance can be used to derive a safe

exposure level for this substance, if several assumptions are made and if necessary conditions have been fulfilled. Below the effect data and assessments that were the basis for the soil exposure trigger value are subjected to a critical appraisal. Three sections relate to data selection and interpretation, two sections relate to the context of the trigger value within the risk assessment scheme:

1. Are the data representative for the terrestrial ecosystem,
2. Are the data complete or have other data been neglected or overlooked,
3. Have the data been assessed correctly,
4. Is the trigger value standardised to the conditions specified in the risk model, and
5. Does the soil trigger have influence on other parts of the risk assessment scheme?

5.3 Reflection of the terrestrial ecosystem

The degree to which the data set represents the soil ecosystem is an important issue that may influence the outcome to a great extent. The relative importance of autotrophic and heterotrophic species, detrivores, producers, and predators should be reflected in the dataset. If population size is the protection goal, the endpoint should relate to population growth (2002b; Forbes and Calow, 2002c).

However, the studies reported focus on endpoints that are not the most sensitive regarding population growth: chronic effects on micro-organisms, mostly tested in agar; sub-acute effects on Oligochaeta (*Eisenia sp.*) in artificial soil; and long-term effects on plants, tested in quartz sand.

With respect to the microorganisms, the relation between the Minimum Inhibitory Concentration (MIC) in agar, and the desired level of protection in soil, is unclear. Firstly, the MIC is the lowest concentration that completely inhibits the growth and this value contains no information on the dose-response curve. Secondly, complete inhibition may occur at a very different concentration in soil than in agar. The bioavailability in agar plates during MIC studies can be much lower than in soils, since growth media contain a higher amount of organic compounds and complexing agents than most porewater (Van Dijck and van de Voorde, 1976; Lunestad and Goksøyr, 1990; Griebler, 2001). Thirdly, species were not identified in Table 1, so it is unclear if the lowest value relates to a bacterium isolated from soil-related bacterial communities, or to ascomycetes, moulds or algae, as explained in AHI (1997). Soil bacteria communities consist of a/o. gram-negative and gram-positive bacteria, with different sensitivities to contaminants (Rönnpagel et al., 1998). Fourthly, there are indications that the nitrifying organisms that can be cultured may not be representative of natural populations. Studies using 16S rDNA profiles have shown that *Nitrosomonas europaea*, which is readily isolated from most soils by classical methods, is not dominant before enrichment due to the high NH4+ concentration classically used to isolate nitrifying bacteria (Hiorns et al., 1995). This does not compromise the suitability of *N. europaea* as a model species, but does emphasise the gap between effect model results and impacts on ecosystem functioning in the field.

Table 1. Tabulation of the Lowest Environmental Assessment Endpoints from Environmental Assessment Reports Submitted to the US FDA/CVM from 1973 to 1997 (AHI, 1997). Units were originally presented as ppb.

Name of Drug	Molar mass	Class of Drug	Earthworm NOEC (ppb)	Microbe MIC (agar) or NOEC (soil) (ppb)	Plant NOEC (ppb)	PNEC ** (ppb)
Sarafloxacin	385	Antibacterial	1,000,000	30	**1,300**	130
Sarafloxacin	385	Antibacterial		300,000 with sediments		
Tilmicosin	869	Antibacterial	918,000		**100,000**	1000
Ceftiofur	546	Antibacterial		**250**		2.5
Florfenicol	358	Antibacterial		**400**		4
Pirlimycin	465	Antibacterial	1,000,000	**130**	400	13
Lincomycin	461	Antibacterial	1,000,000	**780**		7.8
Tiamulin	610	Antibacterial		**500,000**		5000
Apramycin	785	Antibacterial	100,000	**100**	160,000	10
Semduramicin	894	Anticoccidial		100,000	**310**	3.1
Maduramicin	934	Anticoccidial		250	**100**	1
Halofuginone	496	Anticoccidial		200,000	**24,000**	240
Salinomycin	773	Anticoccidial, Perf.enhancer		780	**400**	4
Narasin	765	Anticoccidial	500	**100**	150	10
Oxfendazole	315	Antiparasitic	971,000	9,000	**900**	90
Fenbendazole	299	Antiparasitic	56,000	1,000,000	**36,000**	3600
Ivermectin	861	Antiparasitic	12,000		**560**	5.6
Doramectin	899	Antiparasitic	2,000	40,000	**1,600**	160
Eprinomectin	913	Anthelmintic	**295,000**	1,000,000		2950
Clorsulon	381	Antiparasitic		**2,000**		20
Efrotomycin	1145	Perf.enhancer	1,000,000	20,000	**400**	40
Morantel	370	Perf.enhancer		**50,000**		500
Virginiamycin	535-823	Perf.enhancer		**10,000**		100
Lasalocid	613	Perf.enhancer		**200**	2,000	2
Monensin	693	Perf.enhancer	10,000		150	1.5
Laidlomycin	792	Perf.enhancer		400	**160**	1.6
Bacitracin	1421	Perf.enhancer		**10,000**		100
Melengestrol acetate	397	Perf.enhancer	**1,800**		2,000	18
DrugA	1000*	Anticoccidial	900,000	**10,000**	10,000	1000
DrugB	1000*	Antibacterial		1,000	**130**	1.3
DrugC	1000*	Perf.enhancer	8,110	64,000	**7,500**	750

* estimate

** based on assessment factors according to (ECB, 1996) and accepting the MIC as a relevant value. Values in bold were used for the derivation of the PNEC

The toxicity testing on earthworms did not take reproduction into account. The consequences can be illustrated with the data on Oramec R (0.08% ivermectin w/v) on

earthworms (Gunn and Sadd, 1994). An EC_{50} (acute) of 15.8 mg/kg was accompanied by a NOEC at 2 mg/kg because 27% reduction in fecundity (note that the hatching of the cocoons was not investigated), found in the next lower dose, was statistically not significant. This result is not satisfying and with log-logistic regression analysis, the EC_{10} would be 0.5 mg/kg, 30 times below the EC_{50} based on acute effects (Laskowski, 1995; Van der Hoeven et al., 1997). The assessment based on acute data would be underprotective for fecundity, because the assessment factors (AF) on acute and reproduction endpoints differ only a factor of 10 (EC, 2003).

Plants are tested on germination and growth, which can be considered as relevant provided they were determined in soil. Phytotoxicity of antibiotics differs between species (Jjemba, 2002). However, it is unclear to what species of monocotyles and/or dicotyles were tested and whether leguminose species were included.

Effects of antibiotics in soil on worms or on insects are not expected at the low-level toxic to bacteria. Effects on soil-dwelling *Collembola* and *Enchytraeids*, on the leaf-dwelling *Orius spp*. (a bug), and on larvae of the white-fringed beetle (*Graphognathus spp.*) were found in the range of 70-500 mg/kg substrate (soil or artificial food) probably due to interference with gut microflora (Bass and Barnes, 1969; Baguer et al., 2000; Arijs et al., 2002; Jensen et al., 2003). Toxicity of anthelmintics and antiparasitics on these groups might be quite the opposite.

In conclusion, the terrestrial ecosystem was not investigated in great detail, both with respect to the representation of test species and with respect to the selection of testing conditions and endpoints.

5.4 Data selection

The data set used for the trigger value consists of 64 test results on three taxonomic/trophic groups with 30 substances: three are anonymous (anticoccidial, antibacterial, and a performance enhancer), one hormone, 18 antibiotics/coccidiostats, three anthelmintics, three anthelmintics/antiparasitics, and one antiparasitic. For eight substances data are presented on three taxonomic/trophic groups; for seven substances one value is available. The general validity of such a small set of data may easily be refuted if new information is generated.

Alternative data on drug substances available in public literature have not been included. A critical evaluation of the sensitivity of the selected effect models has not been attempted (Berland et al., 1969; Berland and Maestrini, 1969; Necas, 1971; Kumar and Kaushik, 1971; Van Dijck and van de Voorde, 1976; Jacob and Talpaysayi, 1977; Harras et al., 1985; Thiele-Bruhn, 2003; Vaclavik et al., 2004).

Not all information that was available was presented, since only the lowest of the available endpoints were listed, and species names and test conditions were not identified. This may hamper further interpretation of the results.

5.5 Assessment factors

Three arguments were presented to justify the redundancy of assessment factors: the (low) bio-availability of substances in the presence of soil; the functional redundancy of microbes in soil; and the influence of degradation in soil. These arguments will be discussed to a greater extent below.

Bioavailability in soil

It was stated in the original assessment that due to sorption of the substances to soil matrix the availability will be reduced and thus the toxicity would be reduced compared to tests performed in quartz sand or agar. Binding of complex molecules to soil is depends on many factors, thus the partitioning of medicines cannot be generalised, although soil toxicity of several organic pollutants to earthworms has been correlated to porewater concentrations (Van Gestel and Ma, 1990; Tolls, 2001). An effect like mutagenesis is not necessarily diminished by sorption and, even though the contribution of this particular effect to reproduction and population growth rate is limited, it indicates that sorption and bioavailability are not mutual exclusive phenomena (Würgler and Kramers, 1992; Fretwurst and Ahlf, 1996). The argumentation on bioavailability provided is not used in a proper way to eliminate an assessment factor (AF). The AF intends to cover the uncertainty in the sensitivity of species, endpoints and exposure times, not the uncertainty in exposure concentrations. The latter uncertainty should be accounted for in the harmonisation between exposure calculations and effect assessment. The example of sarafloxacin in the original assessment is used here to demonstrate the effect of sorption. The EU-approach is followed as a model for calculation of concentrations in porewater (EC, 2003). The porewater concentration, representing the available fraction, depends on the concentration in the soil and the capacity of the substance to adsorb to the organic material in the soil.

$$PECporewater = \frac{PECsoil \cdot RHOsoil}{K_{soil-water} \cdot 1000}$$

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

Default settings and input parameters for this model are explained in table 2. The reported logKoc of sarafloxacin amounts to 6 L/kg. This sorption coefficient is in agreement with findings for related fluoroquinolones (Nowara et al., 1997). Assuming that no sarafloxacin partitions into air ($Fair_{soil}$*$K_{air\text{-}water}$ = 0), $K_{soil\text{-}water}$ equals 30000 and the relation between soil and porewater is described by:

PECporewater = 0.000057*PECsoil.

Table 2. Input and output parameters for the equilibrium partitioning model.

Symbol	Parameter	Unit	Default value
Input			
PECsoil	concentration of chemical [c] in the soil	$[mg_c.kg_{soil}^{-1}]$	
RHOsoil	wet bulk density of soil	$[kg.m^{-3}]$	1700
RHOsolid	density of soil solids	$[kg.m^{-3}]$	2500
$Fair_{soil}$	fraction air in soil	$[m^3.m^{-3}]$	0.2
$Fwater_{soil}$	fraction water in soil	$[m^3.m^{-3}]$	0.2
$Fsolid_{soil}$	fraction solids in soil	$[m^3.m^{-3}]$	0.6
Foc_{soil}	fraction organic carbon in soil (w/dw)	$[kg.kg^{-1}]$	0.02
Koc	partition coefficient organic carbon – water	$[dm^3.kg^{-1}]$	
VP	vapour pressure	[Pa]	
MOLW	molar mass	$[g.mol^{-1}]$	
SOL	water solubility	$[mg.l^{-1}]$	
TEMP	temperature at air-water interface	[K]	285
R	gas constant	$[Pa.\ M^3.mol^{-1}.K^{-1}]$	8.314
Intermediate results			
$K_{soil\text{-}water}$	partition coefficient solids and water in soil (v/v)	$[m^3.m^{-3}]$	
Kp_{soil}	partition coefficient solids and water in soil (v/w)	$[dm^3.kg^{-1}]$	
$K_{air\text{-}water}$	partition coefficient air and water in soil	$[m^3.m^{-3}]$	
Output			
PECporewater	predicted concentration in porewater	$[mg_c.l^{-1}]$	

The reported MIC_{agar} was 30 µg/L. If the concentration in porewater equals 30 µg/L, MIC_{soil} equals 530 mg/kg.

In the original assessment no attention was paid to the differences in volume fractions of solids, air, water, or binding places in soil compared to agar. The reported MIC_{agar} was 30 µg/L, the reported $NOEC_{soil}$ (with unknown organic carbon content) was 300 mg/kg, and the calculated MIC_{soil} equals 530 mg/kg. Taking into account that a MIC is expected at higher concentrations than the NOEC, and that most soil bacteria are not freely dispersed but associated with particulate matter, just like the compound, the difference between the agar result and soil result is not inconsistent with the partitioning theory (Griebler, 2001).

Also, mitigating effects due to functional redundancy may play an important role, since a calculated MIC based on an agar test for presumably one strain is compared to an empirical NOEC for a community process. This confuses the argument that the phenomenon of sorption actually reduced toxicity. Hence the result of MIC 30 µg/L in agar is not refuted by the test result with soil, and this MIC value remains the lowest representative endpoint in agar.

One can also assume that compounds that are hydrophilic (weak adsorption), are concentrated in the porewater and may thus be able to exert toxic effects at lower soil concentrations. A discussion on the implications of this argument for highly water-soluble compounds will be continued below.

Functional redundancy

The ecological concept of functional redundancy was brought forward: the loss of a few vulnerable species will not affect system functionality, as brought forward in Van Straalen and Van Gestel (1993). This concept was used as an argument for omitting an assessment factor: A different reasoning is given elsewhere: though redundant species may not have to be protected, safety factors to extrapolate from tested species to more sensitive – yet not redundant – species are still required (Forbes and Calow, 2002a).

For example in pesticide risk assessment effects on functional endpoints of bacterial communities are assessed on a soil community function level; a reduction of 25% after a time period of 100 days is considered acceptable in the EC Directive 91/414/EEC. However, this assessment is based on soil community tests, not on single strain growth tests.

There are also arguments against this application of the concept of functional redundancy:

- Not all microbes in soil are part of the same system: nitrifiers will not replace nitrogen fixers.
- Once redundant species are gone, the system remains more vulnerable to future impacts.
- Each species has its own function in the ecosystem and the replacement of microbial species by more resistant ones may have severe ecological consequences (Van Beelen and Doelman, 1997; Van Beelen and Fleuren-Kemilä, 1999).

The original assessment contained predominantly single strain tests. It was argued that some species may be lost, but the assessment could not indicate how many species exactly would not be protected due to the fact that a safety factor was not applied. This emphasises the insuperability of assessing soil community functionality with endpoints for single strains, a problem that was also addressed in Van Straalen and Van Gestel (1993).

Mitigation by degradation

Degradation in soil is presented as a factor that will reduce the effects in soil. This may be true for some substances, but offers no reason to abandon assessment factors. Firstly, if the test substance degraded in the test system, it has been reflected in the endpoint value. Secondly, an effect that is caused by the initial concentration in the test system may very well become apparent in the field as well. Thirdly, one uncertainty in the exposure-effect relationship in the field is the time-to-effect. Dissipation in the test system may be slower than in the field, which may give rise to an overprotective assessment. However, this should be addressed, rather than obscured, in the effect assessment.

Other uncertainties in the exposure-effect relationship in the field were not considered, such as the fact that degradation may also generate metabolites that need to be assessed separately for fate and effects. Also the other side of the medal, persistency, was not addressed. Substances may be very recalcitrant to degradation and give rise to long-term exposure and effects in other compartments, or trophic levels, than covered in the test systems.

Conclusions on the safety factors

It is common practice to use assessment factors on collections of endpoint values to derive predicted no effect concentrations (PNEC) (Crommentuijn et al., 2000; CSTEE, 2000; EC, 2003). The argumentation on the reduced availability due to sorption and degradation was substantiated only with examples that were representative for the argument. The exact exposure-effect relationships between agar and soil remain unsettled taking into account that most microbes in soil and sub-soil are associated with particles and are not dispersed in the porewater and that bio-availability in agar and nutrient broth may also be limited. The argumentation on the functional redundancy of microorganisms does not overcome the problems of assessing soil community functionality with endpoints for single strains.

If we accept the MIC in agar as a NOEC, the proper use of assessment factors would result in a trigger of 1 µg/kg (see Table 1). This PNEC would serve only for the terrestrial system and for the substances within the dataset.

5.6 The position in the risk assessment framework

Does the context of the risk assessment framework call for further considerations of the applicability of the soil trigger value? Standardisation to the conditions specified for the risk model, differentiation between emission routes, and harmonisation between environmental compartments are considered here.

Standardisation to risk model conditions

The reference dataset yielded a threshold value derived from a specific effect study. The study conditions may very well be different from the generic conditions within the risk model or the specific conditions in the field. For example, in agricultural soils with moderate organic matter contents (0.7-4.1% o.c.) the substances will be more available than e.g. in artificial soil for earthworms testing, containing typically 10% organic matter, equivalent to approx. 6% o.c.) (FOCUS, 2001). Within the frameworks of pesticide registration and quality standard setting, toxicity results are corrected based on the organic carbon and clay content, provided the substance is hydrophobic (logKow >2) (Crommentuijn et al., 2000). Such standardisation cannot be performed with the data in Table 3, because the matrix properties are not given.

Table 3. Properties and Environmental Quality Standards (EQS) of some substances used as pesticides and as veterinary medicines (Crommentuijn et al., 2000).

Substance	LogKoc	Lowest soil NOEC in µg/kg	Species	EQS soil (normalised to 10% o.m. and 25% clay) in µg/kg
Dichlorvos	1.83	75000	*Lampito mauritii*	0.0028*
Cypermethrin	4.87	-	-	0.39*
Diazinon	2.64	350	*Folsomia candida*	6.2

* harmonised with EQS for water

Differentiation between routes of emission

In the original assessment it was acknowledged that the dataset, containing plants, earthworms and microbes, does neither represent all mode of actions nor all species. It was therefore recommended that "ecto- and/or endoparasiticides used in pasture should advance directly to Phase II to address specific areas of concern, e.g. dung fauna" (VICH, 2000). Nevertheless, parasiticides, hormones, and other compounds that were not well represented in the dataset (Table 1), that are administered to stabled animals (not on pasture), will be subjected to the trigger.

Harmonisation between compartments

It is important to note that the soil trigger decides on the further assessment of the risk to soil, surface water, and also groundwater. The environmental compartments soil, water, air, and sediment are connected and substances may be transported between compartments. The trigger value in soil should protect water and sediment as well. The equilibrium-partitioning method has been applied to harmonise environmental quality standards (Crommentuijn et al., 2000; EC, 2003). The examples in table 2 illustrate that a safe level in soil does not necessarily protect surface water or groundwater. A trigger should –like quality standards– be harmonised between compartments.

Several veterinary substances are used as pesticides as well, for which in European legislation standards have been set to water, groundwater and drinking water contamination (Anonymous, 1976; Anonymous, 1979; Anonymous, 1998b; Anonymous, 2000). 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 effects (Health Council, 2001). This scientific opinion sides veterinary medicines with 'pesticides' and 'biocides' in the environmental legislation. A soil trigger that is not harmonised might result in violation of these water quality standards.

5.7 Conclusions on the scientific evaluation of the dataset

The conclusion of this section is that the potential of the scientific tools to assess the data has not been exhausted and the argumentation to discard assessment factors did not come up to the mark. The selection of species and test endpoints for establishing a trigger value was not optimal. A further correction of the reference data before the assessment, together with a harmonisation between terrestrial and aquatic compartments, based on agreements on boundary conditions within the risk model, was required.

5.8 Extending data selections and interpretations

Several tools from ecotoxicology, environmental chemistry and risk assessment can be used to improve the trigger value: partitioning calculations, statistical analysis of all data,

additional hazard identification, and harmonisation between compartments. These tools are examined below.

Data selection

New data provide information to lower the trigger without changing the original assessment approach. Twenty substances were tested on effect against *Vibrio fischeri*. EC90 values, indicative of the MIC ranged from 30 µg/l to 388 mg/L. EC10 values, indicative of the NOEC, ranged from 4 µg/l to 90 mg/l (Backhaus and Grimme, 1999). In another research, eleven substances were tested on growth inhibition of sludge bacteria, both in a batch system and in a pour-plate system, where individual cells are exposed. EC50 values ranged from 100 µg/l to >100 mg/L in the batch test, but from 28 µg/l to 449 mg/l in the pour-plate method. A test on growth inhibition of *Nitrosomonas europaea* yielded EC50 values ranging from 16 µg/l to >100 mg/l; using the pour plate method the EC50 values for four selected compounds ranged from 2 to 460 µg/l (Halling-Sørensen, 2001). These data provide the following 'critical values': for a single strain (*V. fischeri*) the EC90 was 30 µg/l, the corresponding EC10 was 4 µg/l (ofloxacin). The lowest EC50 was 2 µg/l (chlortetracycline on *N. europaea* pour plate method). For a community process the lowest EC50 value was 28 µg/l (chlortetracycline pour plate method). These last two results were obtained from a pour-plate method with low microbial density. Low seeding densities are regarded to yield sensitive indicators of in situ effects. For the protection of the gastro-intestinal microflora, the Joint Expert Committee on Food Additives (JECFA) selects not just the lowest MIC, but selects MIC values from all relevant sensitive species, and corrects test endpoint values for the higher microbial density in the gut (AHI, 1997). For the soil system, the desired level of protection in soil should guide the selection of species and assessment factors and it is not straightforward to downsize effects in order to correct for hypothetical different microbial densities in soil. Potentially, the trigger can be based on the results of 100, 30, 28, 16, 4, or 2 µg/l, depending on the preference for a given community process, a particular single species, a certain inoculum density, or specific endpoint.

Partitioning calculations

The concept of partitioning has been addressed above for effects on microbes mediated through agar or soil. For a substance with low partitioning to organic matter, the MIC_{soil} will actually be much lower than for a hydrophobic substance. Thus, what would be the result if the endpoint in agar for the hydrophobic compound was also to protect the soil for a potential hydrophilic substance? This is demonstrated here with the example of a hydrophilic substance (metronidazole) and the lowest MIC-value of 30 µg/L established for sarafloxacin (molar mass 385 g/mol) in table 1. Both the partitioning in the soil system, aiming at effective concentrations in the water phase, and the influence of differences in molar mass are accounted for in this example.

Based on molar equivalents, this lowest MIC in agar amounts to 30/385 = 0.078 µmol/L. The substance metronidazole has a molar mass (M) of 171 g/mol and a sorption coefficient Koc of 40 L/kg (Rabølle and Spliid, 2000). For this substance the threshold would

be 11 µg/kg soil dw, based on effective concentrations in the porewater calculated with the formulas in Appendix 1. This value is very different from the predicted and validated value (530 and 300 mg/kg soil) for sarafloxacin, and is below the trigger.

This partitioning approach can also be applied to a small hypothetical molecule with Koc equal to 0 L/kg. For this completely dissolved substance the trigger would be 0.01 mmol/kg. Would this worst-case hydrophilic behaviour apply to a small molecule with M = 100 g/mol, the trigger equals 1 µg/kg; in case of the highest molar mass in Table 1 of 1457 g/mol, the result is 15 µg/kg. For a hypothetical substance, with a mode of action covered in the reference data set, and a strong preference for the water phase, the predicted MIC depends on the molar mass and will typically range between 1 and 15 µg/kg.

If we accept the value of 2 µg/l as determined by Halling-Sørensen (2001) as the reference for the trigger, the result would be approximately an order of magnitude lower.

Statistical analysis

A collection of data can statistically be assessed to generate information on the distribution of no effect levels. This distribution reveals the potentially affected fraction of species at a given exposure concentration. One could assume that the data of interest are representative of a (log-)normal distribution. If all endpoints for all species had been listed, the distribution could be fitted on the data resulting in a threshold value at a chosen level (e.g. 5^{th} percentile) with a chosen confidence level (e.g. 95%) (Aldenberg and Jaworska, 2000). This technique is widely applied in standard setting and risk assessment (Van Beelen and Doelman, 1997; Posthuma et al., 2002). This Species Sensitivity Distribution (SSD) approach will be examined with the assumption that all substances that are present in the dataset have a similar distribution of effects. However, the dataset in Table 1 presumably does not show all available test results, which makes it impossible to assess the sensitivity distributions. For this purpose the data in Table 4 on soil microbes in agar are presented (Van Dijck and van de Voorde, 1976). In the table the Predicted MIC values (PMIC, using the lowest MIC and an AF = 10) and the Hazardous Concentrations (median level) at which 95% of the species are protected (HC5), are presented. The difference in numerical values between the PMIC and HC5 depends on the data set and is as high as a factor of 1-160. For this dataset, while the lowest value is 100 µg/L, a concentration of 10 µg/L would be the value that protects at least 95% of all species for the most potent compound, gentamycin.

The application of this statistical method for the sensitivity distribution of species to a single compound can be extended. It is applied here to the population of substances, under the assumption that the selection of substances is a representative sample of all possible substances. For matters of convenience the MIC values were not corrected for molar mass. The HC5 of each substance in Table 4 is taken as the representative effect endpoint per substance. The analysis of the collection of HC5 values results in a HC5 of 2 µg/L (Table 5). Continuing the line of reasoning in the original assessment from this point forward, the trigger would be 2 µg/kg.

Table 4. Sensitivity of environmental micro-organisms to antimicrobial agents (Van Dijck & van de Voorde, 1976). All results >1000 mg/L are transformed to logMIC = 4 and all results <1 mg/L are transferred to logMIC = –1. PMIC and HC5 are not log-transformed.

Antimicrobial agent	logMIC (mg/L) for strain[a]													PMIC	$HC5_{su}$
	mb	md	hy	citr.1	citr.2	flav	kl	th	cy	rh	hyph	r.sp	nitr	mg/L	mg/L
tetracycline	0	2	0.7	2	1	2	2	2	1	2	1	-1	3	0.01	0.40
polymyxin B	.	.	.	.	.	.	.	4	2	4	2	1	4	1	3.2
chloramphenicol	-0.3	0.3	0.7	0.7	1	1	2	2	1	3	1	.	4	0.05	0.22
streptomycin	1.7	2	1.7	2	3	3	.	4	2	2	3	-1	3	0.01	1.3
neomycin	0	0	0	0	.	-1	.	3	2	2	1	1	4	0.01	0.03
gentamycin	-1	-1	-1	-1	.	-1	.	3	2	2	1	1	3	0.01	0.01
kanamaycin	1	1	1	1	.	1	.	3	3	3	3	1	3	1	1.4
benzylpenicillin	4	4	4	4	4	3	4	3	2	3	2	2	1	1	21
ampicillin	3	3	1	4	.	1	.	-1	2	4	2	1	2	0.01	0.31
cloxacillin	4	4	4	4	.	4	.	.	.	.	.	.	.	1000	.
oxacillin	.	.	.	.	.	.	.	2	3	4	1	1	3	1	1.6
cephalothin	3	2	2	2	.	3	.	2	2	4	1	3	3	1	11
tylosin	2	2	3	4	.	2	.	3	1	2	2	1	4	1	4.2
oleandomycin	2	2	2	2	4	.	4	3	1	3	4	2	4	1	9.2
spiramycin	2	2	2	3	.	2	.	3	1	4	2	2	3	1	9.8
virginiamycin	1.3	2	2	2	4	4	4	1	1	3	2	2	3	1	3.7
flavomycin	1	1	3	3	.	1	.	-1	4	-1	2	.	3	0.01	0.05
novobiocin	1.3	2	2	2	4	.	3	-1	-1	3	2	2	4	0.01	0.17
bacitracin	2	2	3	3	.	1	.	4	2	3	2	1	4	1	5.0
nystatin	4	4	4	4	.	3	.	3	3	4	4	4	4	100	1356
sulfathiazol	4	4	4	4	.	3	.	-1	2	2	3	2	3	0.01	1.6
furoxone	4	4	2	2	1	1	1	2	1	3	2	2	3	1	2.3
$HC5_{species}$	0.21	0.54	0.51	0.93	1.56	0.24	5.7	0.17	1.0	7.3	3.24	0.29	90		

[a] Abbreviations: mb = *Mycoplana bullata* ATCC4278; md = *M. dimorpha* ATCC4279; hy = *Hydrogenomonas* sp.; Citr = *Citrobacter* sp 1 and 2; flav = *Flavobacterium* sp.; kl = *Klebsiella* sp.; th = *Thiobacillus thiooxydans* 504 DSM; cy = *Cythophaga johnsonae* 425 DSM; rh = *Rhodopseudomonas* sp.; hyph = *Hyphomicrobium* sp.; r.sp = *R. sphaeroides* 158 DSM; nitr. = *Nitrobacter* sp. HC5su = HC5 for substance; HC5species = HC5 for species.

Table 5. HC5 values calculated using individual HC5 values for all substances except nystatin, oxacillin, cloxacillin and polymyxin B, for all species except citr.1, kl and nitr. mentioned in table 4.

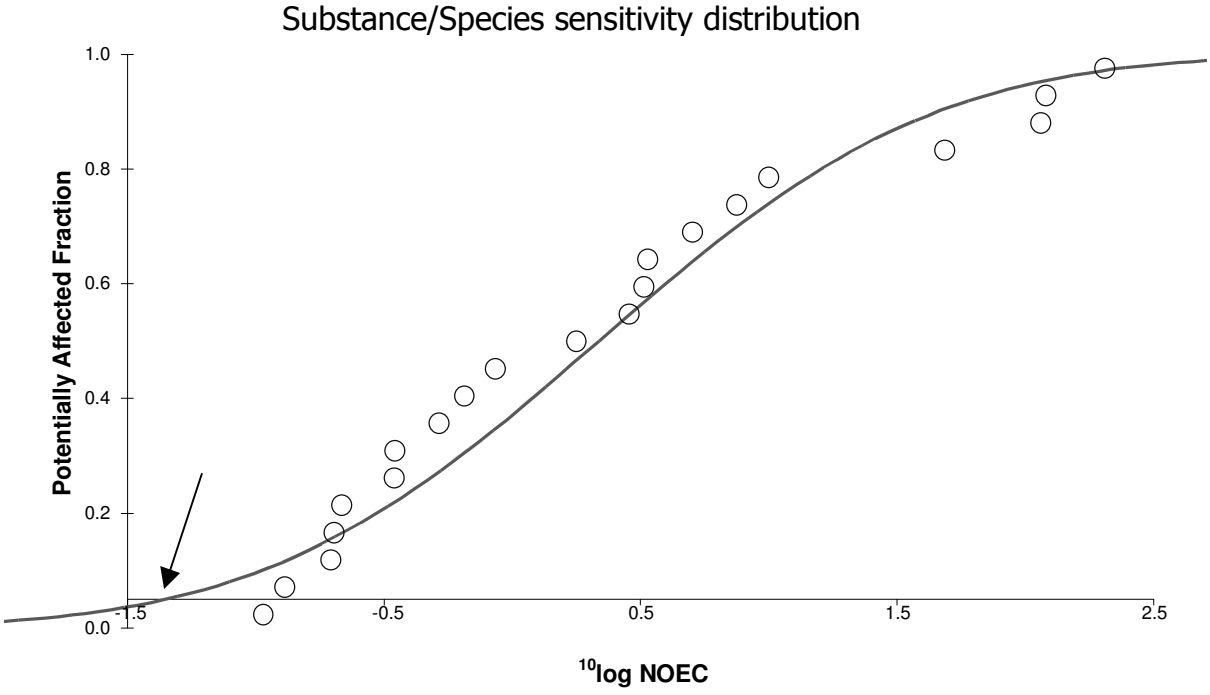
	HC5 [mg/L]	lower 95% confidence interval
lowest PMIC	0.01	
lowest HC5 for substances	0.01	0.0001
HC5 of all lowest values per substance	0.02	0.002
HC5 of all $HC5_{substances}$	0.02	0.004

PMIC = predicted minimum inhibitory concentration

HC5 = hazardous concentration at which 5% of all species or substances is not protected

Applying this approach to the endpoints for all substances in Table 1 using ETX1.0[21], the following distributions and thresholds after normalisation on molar mass are revealed. Figures 1 and 2 show the median HC5, but not the lower confidence intervals. Based on the data on plant species for all substances (Figure 1) the HC5 is 0.04 µmol/kg dw in soil with a lower 95-percentile confidence interval of 0.008 µmol/kg dw in soil. Based on the MIC data these HC5 values are 0.08 and 0.03 µmol/L in agar (Figure 2).

Figure 1. Substance/species sensitivity distribution for plants. The arrow indicates the HC5 concentration. The X-axis crosses the Y-axis at the 5th percentile.

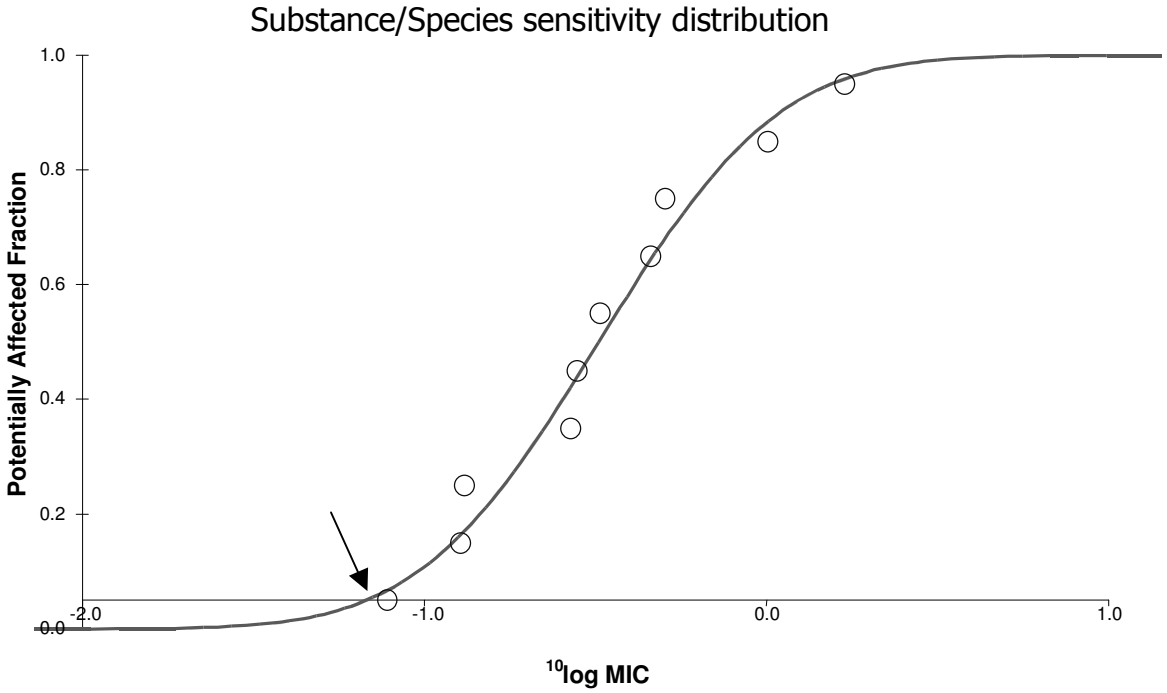


These HC5 values can be transformed to triggers based on the example of the hypothetical small hydrophilic substance (M = 100). This yields a trigger of 4 µg/kg for plants in soil, with a lower confidence interval of 0.8 µg/kg in soil, and a trigger of 1 (lower confidence 0.4) µg/kg for microbes in soil. The distributions can be used to assess the fraction of the different taxonomic groups that is not protected at a given exposure. At 100 µg/kg there is a 45% likelihood that the substance will affect plant species and an 80% likelihood that bacterial species are affected.

Incorporation of the new data presented above may lower the result of this analysis. Due to the different endpoints (MIC, EC90, EC50, EC10) this is not attempted here.

[21] See Van Vlaardingen et al. (2004).

Figure 2. Substance/species sensitivity distribution for micro-organisms (MIC). The arrow indicates the HC5 concentration. The X-axis crosses the Y-axis at the 5th percentile.



The hazard of resistance development

The effects of antibiotics can range from simple parameters like a decrease in biomass, respiration rate or denitrification rate, to more complex parameters like community shifts and the survival of new genetical information (Landi et al., 1993; Badalucco et al., 1994; Da Gloria Britto De Oliveira et al., 1995). Effects of some antibiotics on nitrification and decomposition in soil have been reviewed and the few studies available indicate effects at very high concentrations only (Jensen, 2001; Thiele-Bruhn et al., 2003). It was put forward that the substances will be an energy-source to other species rather than a pollutant, although this may be at a sub-therapeutic, thus a resistance-inducing concentration. Perhaps other assessment strategies could provide more relevant information.

Shifts in community tolerance caused by soil pollution have been shown to have impacts on e.g. extinction of sensitive species, competitive abilities, and metabolic diversities (Van Beelen and Doelman, 1997; Siciliano and Roy, 1999; Mcbain et al., 2002; Russel, 2002; Séveno et al., 2002). The survival of adapted bacteria in absence of the compound that the bacteria have adapted to, is usually said to be limited, but the acquired functionality (e.g. resistance genes) remains present at low levels (Cooke, 1983; Stappen et al., 1989; Zuidema and Klein, 1993; Séveno et al., 2002; Park et al., 2003). The costs for resistance can even be compensated for (Björkman et al., 2000). In some cases related compounds can uphold the resistance level against another compound (Aarestrup et al., 2001). Horizontal transmission of genetic information is very efficient in the gut of soil arthropods and resistance genes can

be transferred from manure to soil and groundwater, where low levels of antibiotics may be present (Hoffmann et al., 1998; Chee-Sanford et al., 2001; Halling-Sørensen et al., 2002; Sengeløv et al., 2003). Resistance development occurs already at the Minimum Effect Concentration (MEC) at which growth is reduced, that is tenfold below the Minimum Inhibitory Concentration (MIC), the endpoint used to derive the soil concentration trigger discussed in Chapter 5 (O'Reilly and Smith, 1999). Thus, even at concentrations below the Phase I trigger, resistance genes may be favoured, which can be transferred from manure to soil and groundwater (Halling-Sørensen et al., 2002; Sengeløv et al., 2003). The management of resistance development in water and sediment face comparable challenges (Grabow et al., 1976; Cooke, 1983; Linton et al., 1988; Rodgers, 2001; O'Reilly and Smith, 2001).

Should resistance development be identified as a hazard? According to the European Commission this hazard is addressed in the current guidance, even though it obviously is not (EC, 2001b). What kind of hazard are we dealing with? Is it a hazard for the ecosystem integrity or also a hazard for public health in general? How can we express the degree of damage? Currently both molecular and ecological methods are investigated. Pollution-induced community tolerance has been found suitable to detect community shifts at low concentration levels (Ares, 1999; Schmitt et al., 2004). If there is a genetic basis for these shifts is to be explored. It has been suggested to include not only the rate of appearance of the initial resistance mutations but also the possible counter-selection against the resistant variants as well as the rate of virulence-restoring compensatory mutations, which allows resistance to be maintained (Björkman et al., 2000). How can this information be used in decision making, knowing that it also applies to antimicrobial products used as pesticide and biocide (Björkman et al., 1998; Siciliano and Roy, 1999; Mcbain et al., 2002; Russel, 2002; Séveno et al., 2002; Midtvedt, 2004; Kümmerer, 2004a; Kümmerer, 2004b)? Resistance development occurs already at the Minimum Effect Concentration (MEC) at which growth is reduced, that is tenfold below the Minimum Inhibitory Concentration (MIC), the endpoint used to derive the trigger under discussion. This indicates that at the MIC level a selection pressure for resistance development is present. Since these effects may indeed occur at sub-therapeutic levels, a safety factor of 10 for this aspect would have been warranted.

Harmonisation between environmental compartments

The HC5 for the most sensitive trophic level of bacteria in soil based on the original data was calculated at 1 µg/kg. Harmonisation between compartments is however required. As the soil threshold is based on a substance that is completely in the porewater, the aquatic compartment would be protected as well by this trigger. However, the porewater concentration at 0.1 µg/kg is 8 µg/L, which is above the trigger value set by VICH for effluent water (1 µg/L). Harmonisation of the soil trigger to the water trigger would lead to a porewater concentration of 1 µg/L, and hence to soil concentrations of 0.0125 µg/kg for the completely dissolved substance. The relevance of the aquatic data set for this purpose should however be assessed.

Representativiness for other substances

Although the information status of the trigger suggested above is higher than that of the operative trigger, they both are derived from an empirical exercise with a given set of substances and endpoints. The question is whether this set of compounds and endpoints is representative for the new compounds to be evaluated. There is no easy answer to this. One has to take into consideration what part of the population of substances was tested, what endpoints were tested, what endpoints will be tested if a full effect assessment is to be performed, and how the information was aggregated to derive the threshold.

The compounds in the reference set were identified as a group because of two properties: they were applied as veterinary medicines and data were available. If the endpoints in the reference set are the same as those to be established in a full effect assessment, the threshold is certainly valid for the compounds that were in the reference set, but not naturally for other compounds. The fact that a substance was applied as a veterinary medicine is a property that contains no information on the likelihood that another compound will have comparable ecotoxicological properties. More information and more discriminating properties are needed to allow for a case based reasoning that extends from substances with identified common characteristics to substances that yet have to be developed. For instance, threshold levels for flavouring substances were based on numerous substances and endpoints, and correlated to structural classes (Munroe et al., 1996; Munroe et al., 1999). Statistical methods developed to derive substitute confidence intervals around tiny data sets can also be applied easily, but only have meaning, if the substitute data are representative (Aldenberg and Luttik, 2002). Case-based prediction of ecotoxicological effects of pesticides relies heavily on structural class of the compound under investigation (Van den Brink et al., 2002). This is fundamental to the derivation and application of (Quantitative) Structure-Activity Relationships ((Q)SARs) that might be very useful for the environmental risk assessment for medicinal products. Acceptability criteria relating to the accuracy of the predictions do have to be set, as accuracy of predictions of e.g. ECOSAR is only 67% (Breton and Boxall, 2003; Hulzebos and Posthumus, 2003).

5.9 Discussion

Risk management of veterinary pharmaceutical products is one of the tasks of EU governance. The authorisation procedure for (veterinary) medicinal products is recognised to be the outstanding example of risk based decision making, because efficacy and side effects of the product, also in comparison to other products, are to be considered (Di Fabio, 1994). It has been considered that the registration process, and the risk models used, should reduce the costs to society in terms of environmental and economic damage, and the assessment process itself should neither hamper product development nor timely action (Cranor, 1997). The introduction of an exposure trigger can be a powerful tool in realising these objectives. A scientific analysis of data can strengthen the choice of the numerical value. A number of conclusions on the data and arguments to support a soil concentration trigger were drawn.

The dataset was not very comprehensive in number of substances and types of endpoints. Existing information available in literature was not considered. New information on effects of veterinary medicines on microbial species have become available, and provide reason to lower the trigger value even without changing the assessment approach.

More information and more discriminating properties are needed to allow for a case based reasoning that extends from substances with identified common characteristics to substances that yet have to be developed. Next to the selection of the data, the selection of ecotoxicological tools determines the outcome of the scientific assessment of the same dataset. Most importantly, the argumentation on the use of assessment factors did not comply with the EU-guidance given in the frameworks of new and existing substances and pesticides and biocides (EC, 2003). It was demonstrated that further considerations of the given arguments for a soil threshold concentration provide for strong arguments to set a threshold at 1 µg/kg. Complementary reasoning gives rise to set the trigger at 0.0125 µg/kg. This degree of variability in outcomes is troublesome, yet justifiable with a transparent consideration of starting points and assumptions, selection criteria, arguments and related uncertainties.

In comparison to the information contained in the sensitivity distributions, the soil exposure trigger value of 100 µg/kg may be underprotective for 80% of soil microbial species. Another implication of the use of this trigger value is that, if Environmental Quality Standards will be derived for substances using the TGD, these may be below the operative trigger value. For some parasiticides that may enter the environment through the manure of stabled animals this may be the case, as shown in table 2.

Also, the exposure trigger based on the soil compartment determines if a further assessment of the surface and groundwater compartments is performed, but it is not harmonised with the possible no-effect levels in these compartments.

6. European medicines and feed additives regulation are not in compliance with environmental legislation and policy

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Mark H.M.M. Montforts and Joop A. de Knecht

6.1 Introduction

For the product categories of medicines, veterinary medicines and feed additives, the environmental risk assessment (ERA) procedure at registration is currently under development. The purpose of this paper is twofold: firstly, it investigates what limitations environmental legislation sets to the use of medicinal products and how an environmental assessment within the registration process can be of help in achieving environmental quality standards; secondly, it investigates whether the registration process and the assessment meet these expectations. As a case study, special attention is given to veterinary medicines. For a general overview of the knowledge, problems, and research concerning sources, fate and effects of medicines in the environment we refer to (Kümmerer, 2001).

6.2 EU Environmental legislation and the relation with product registration

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 binding law, according to the (Anonymous, 1976), (Anonymous, 1979), (Anonymous, 2000) and (Anonymous, 1998a) directives on water, groundwater and drinking water, respectively (Wösten et al., 2001; Van Rijswick, 2001). Specific substances of concern have been identified and listed (List I and II). 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.

Within the Framework Directive on Water (2000/60/EEC) all acts of discharging or spreading of waste-material, polluting and deleterious substances that might lead to contamination of surface water and groundwater are forbidden, unless the competent authority has granted authorisation (a permit). The permit specifies the receiving water body, the discharged substance(s), and the measures to be taken to prevent further pollution, for the legal person (e.g. a farmer) on a case by case basis. The use of a product (e.g. pesticide, fertiliser) or the use of a treated product (impregnated piling, treated cattle) are such acts that

have to be authorised by the competent authority (Ministry of Public Works, water-bodies, river-bodies, provinces, municipalities). These authorities are facing a huge administrative burden that would be relieved if all individual permits could be replaced by one single authorisation. Although registration of a product cannot be regarded as a permit nor as such a legal authorisation, registration can be a useful 'start-of-pipe' measure, because it reduces the need for regulating emission: general conditions and restrictions have already been identified and certain substances or uses will not be allowed. The competent authority can now focus on site-specific circumstances.

It is possible that environmental directives on the quality of water already contain qualitative standards for substances used in medicines and feed additives, even though the products groups 'medicines' and 'feed additives' are not named in the environmental directives. 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.

Are medicines to be categorised as 'pesticidal'? The Dutch government published in 1989 a document on quality criteria for substances in soil and groundwater (TK, 1989). It was considered necessary to specialise 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 discern 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. Also 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 effects (Health Council, 2001).

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 (TK, 1989). This point of view is reflected in the directives on pesticides and biocides (Anonymous, 1991a) and (Anonymous, 1998b) 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 (NW4, 1998). Furthermore, to all substances that qualify as pesticidal, a qualitative standard is already available for drinking water, and at least in The Netherlands also for

groundwater (0.1 µg/L). And last but not least, the ERA should assess the risk to both surface water and groundwater for every use in order to fulfil its role as a tool in the environmental protection.

The registration process should thus meet the following demands in order to be an effective tool for environmental policy:

- An ERA is performed at every registration or renewal in order to take new data or methodologies into account;
- Decision criteria are in compliance with the environmental directives;
- Principles and practical procedures for the assessment at registration are operational;
- The methodology for the setting of environmental quality criteria should be harmonised with the ERA methodology for products.

6.3 Product registration and the relation with EU environmental policy and laws

The EC unfolded its vision on chemicals in Europe in the White Paper (EC, 2001c). The European Union chemicals policy must ensure a high level of protection of human health and the environment as enshrined in the Treaty both for the present generation and future generations while also ensuring the efficient functioning of the internal market and the competitiveness of the chemical industry. Fundamental to achieving these objectives is the Precautionary Principle. Whenever reliable scientific evidence is available that a substance may have an adverse impact on human health and the environment but there is still scientific uncertainty about the precise nature or the magnitude of the potential damage, decision-making must be based on precaution in order to prevent damage to human health and the environment. Another important objective is to encourage the substitution of dangerous by less dangerous substances where suitable alternatives are available. The White paper puts particular focus on substances which are carcinogenic, mutagenic or toxic to reproduction, and on substances which are PBT (persistent, bio-accumulative and toxic) or which otherwise give rise to high concern.

The EC White paper on existing substances is to be regarded as the underlying principle for the regulation of substances. To underline the importance, we point out that the European Council already incorporated the principles of the White paper in their reaction to the evaluation of the pesticide directive 91/414: “The Council calls on the Committee to develop a new pesticides policy in line with the relevant aspects of the forthcoming EU Chemicals Policy based on the principles endorsed by the Council Conclusion in June 2001 … In principle, these (PBT) substances should be avoided in plant protection products” (EC, 2001a).

Although the regulation of existing substances does not apply to products that are regulated in other frameworks, the same principles should (eventually) be applied in these frameworks. An ERA should be performed in order to determine the likelihood of effects in the environment. This implies that at product registration environmental data should be available, at least a risk classification should be made, and depending on the mode and scale

of use some substances are not wanted. In order to accomplish this in an effective manner, it should be clear:

- What the protection goals (criteria) of the assessment should be;
- What is acceptable (standards or levels) and what is not;
- How the assessment should be performed (methodology).

A regulatory problem arises when a product registration procedure is harmonised at a European level by the Communautarian authority, while the authorities at the national level are responsible for maintaining the desired environmental quality. This may lead to a less effective implementation of the ERA as a tool for environmental policy. The product registration process also determines the availability of a product on the common market; therefore the registration process should meet the following additional demands:

In order to perform an ERA at registration for the common market, common (harmonised) environmental protection goals are required. European environmental legislation provides a common basis for environmental goals for all products.

The ERA should be developed under the supervision of competent authorities (with respect to environmental quality), for example through national interdepartmental steering groups that prepare the national points of view.

Implementation of the ERA procedure is an act that will have legal consequences for stakeholders (producers, users, and third parties). Formalisation of the contents and the procedure should be transparent and open to input by regulators, scientists, industry and other interested parties; a view shared by the EC (EC, 2001b).

6.4 Product directives on medicines and the environmental assessment

Medicines and feed additives can reach the environment at production, at use, after use (excretion) and as waste material. Only emission at production is outside the scope of the registration and is not dealt with here. Given the elaborate risk assessment schemes and methodologies formalised in a regulation on existing substances (Anonymous, 1993b), and directives for new substances (Anonymous, 1993a), plant protection products (91/414/EEC) and biocides (98/8/EC), where both the organisation (competent authorities, technical meetings and working groups) and the deliverables (uniform principles, dossier requirements, guidance for decision making and listing, guidance on risk assessment, guidance on models, guidance on preparing a monograph) are comparable and have been tested in practice, one would expect a similar system for medicines.

The Directive on human medicines (Anonymous, 1965) recognises that an application for the marketing authorisation for a medicinal product for human use must be accompanied, if applicable, by reasons for any precautionary and safety measure to be taken for the storage of the medicinal product, its administration to patients and for the disposal of waste products, together with an indication of any potential risks represented by the medicinal product for the environment.

The directive on veterinary medicines (Anonymous, 2001b), that replaces the 81/851 and 81/852 directives, states that with a request for registration of a veterinary medicinal

product information is to be provided to enable an assessment of the safety for the environment. Both administration and excretion of the products, and the disposal of unused material or waste, should be assessed. The assessor is free to determine what information should be delivered.

The directive on feed additives (Anonymous, 2001a) considers that the existing regulations on feedstuffs should be supplemented by the establishment of criteria for the assessment of the risk of the additive having an adverse effect on the environment. In contrast to the medicines, the decision-making criteria for feedstuff are fastened down in law, but hardly any methodology is provided.

The directives of the three product groups all require an environmental assessment at registration. The quality of the assessment depends not only on the information in the directives. The directives may have been elaborated upon in national law and guidance documents, and the availability of operational procedures, assessment tools, and expertise at the evaluating and decision-making authorities are of importance. A further investigation into the process, the actors, and the deliverables is made. The veterinary medicinal products are explored in most detail as a case study.

As discussed above, in relation to environmental policy making the registration process with respect to the environmental assessment should have the following five features:

1. Formalisation of the contents and the procedure should be transparent and open to input by regulators, scientists, industry and other interested parties;
2. European environmental legislation provides a common basis for environmental goals;
3. Standards and harmonised methodology are (made) available;
4. An ERA is performed at every (re-)registration;
5. Principles and practical procedures are operational.

Therefore not only the details of the scheme and the methodology are of importance, but also the organisation (who), implementation (what) and operationalisation (how) of the risk assessment procedure are to be considered.

6.5 The development of the ERA for Veterinary Medicinal Products (VMPs) in Europe

Figure 1 depicts the organisation of the registration process of VMPs, in which administrative, scientific and regulatory responsibilities are separated. The Directorate-General (DG) Enterprise is responsible for the European legislation on the registration of VMPs. The registration process is mandated to the European Agency for the Evaluation of Medicinal Products (EMEA)[22]. Within EMEA, the scientific committees advise on the requests for Marketing Authorisation with respect to quality, efficacy and safety of the products. These committees are the Committee for Veterinary Medicinal Products (CVMP) for veterinary medicines and the Committee for Proprietary Medicinal Products (CPMP) for

[22] Commonly referred to as the European Medicines Evaluation Agency

human medicines. The Standing Committee on VMPs, as part of the DG Enterprise, decides on the proposal of the CVMP and turns it into binding law. DG Enterprise has also the Veterinary Pharmaceutical Committee (VPhC) at its disposal, which was installed by Directive 75/320, for advice on interpretation of the directives, compulsory consultation when changing directives, and other issues. Member states are involved in the registration process through their representations in the SC VMP and VPhC. Member states also appoint two independent experts to the CVMP.

The EMEA was mandated by DG Enterprise to elaborate on the old 81/851 and 81/852 directives (Blasius and Cranz, 1998). This has resulted in guidance documents for performing the environmental risk assessment of veterinary medicines (EMEA, 1996; EMEA, 1997), but this was not the end of the process. 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)[23] to which both DG Enterprise and the EMEA are committed (DG Enterprise, 2000). 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 (VICH, 2000) and replaces the EMEA 1997 guidance on Phase I. This guidance document is at this moment leading for the registration procedure. To understand the contents of this guidance, one has to understand the organisation that created it. The VICH Steering Committee (VICH SC) authorised formation of a working group to develop harmonised guidelines for conducting environmental impact assessments (EIA) for veterinary medicinal products. The mandate of this VICH Ecotoxicity/Environmental Impact Assessment Working Group (VICH Ecotox WG), as set forth by the VICH SC, is as follows: "To elaborate tripartite guidelines on the design of studies and the evaluation of the

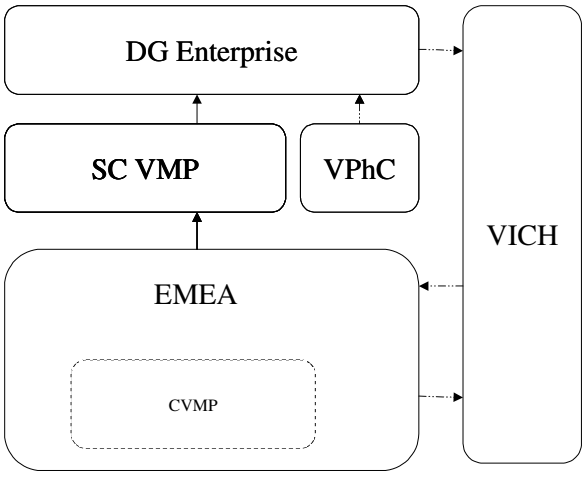


Figure 1 The relations between the EC, EMEA, CVMP, VICH, and SCVMP. Dotted lines: information and advice. Straight lines: proposals and decisions on Marketing Authorisation for products.

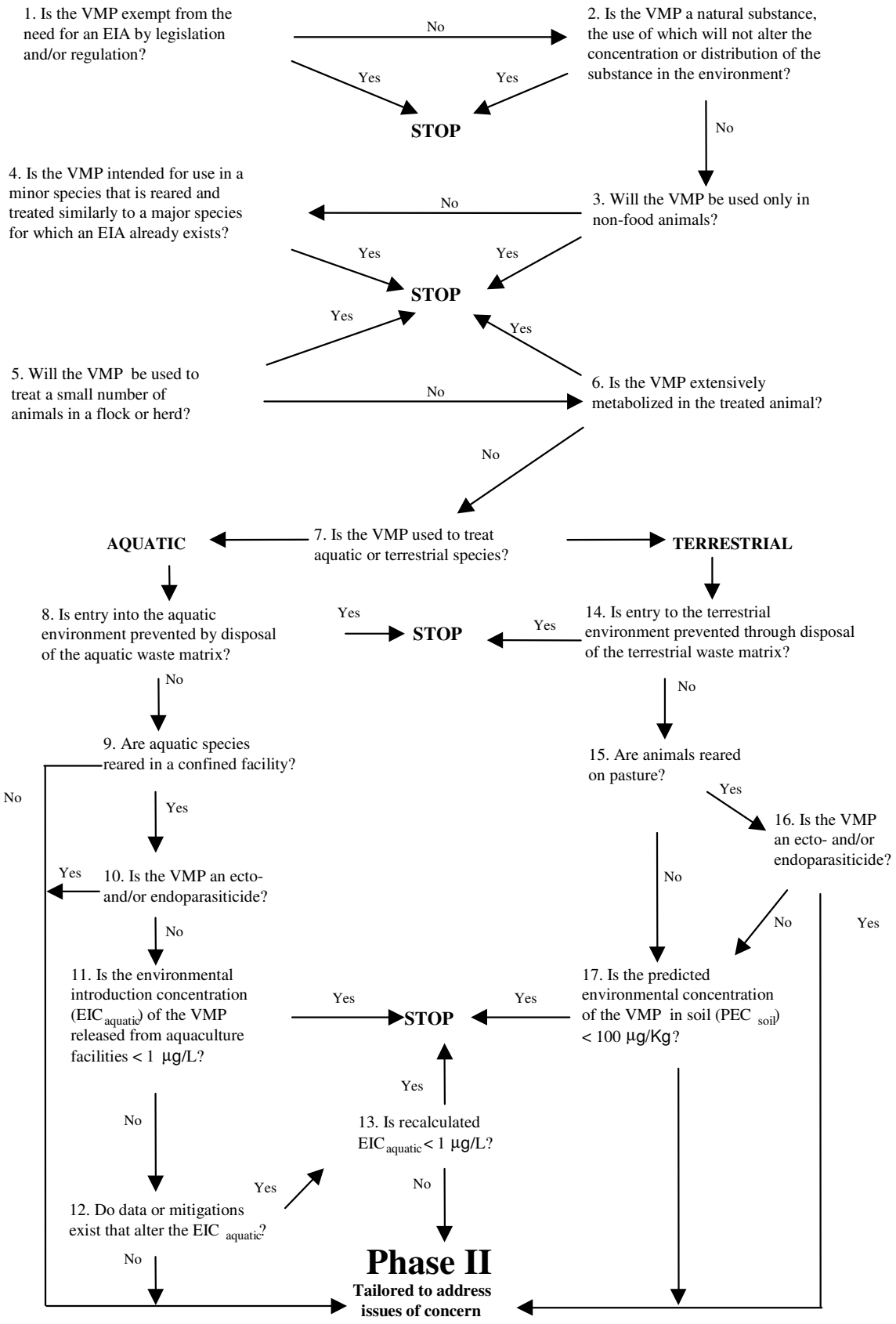
[23] Commonly referred to as the Veterinary International Conference on Harmonisation.

environmental impact assessment of veterinary medicinal products. It is suggested to follow a tiered approach based on the principle of risk analysis. Categories of products to be covered by the different tiers of the guideline should be specified. Existing or draft guidelines in the US, the EU and Japan should be taken into account." The VICH working group consists of three representatives of industry and three of the regulatory authorities, one of each continent. The VICH Ecotox WG elected to develop harmonised guidance in two phases (Phase I and Phase II). Phase I identifies VMPs that require a more extensive investigation of their potential to have environmental effects on non-target organisms. The VICH SC recommended to the WG that Phase II should include a list of studies needed for VMPs that enter Phase II and that decision-making or interpretative criteria should be included in Phase II. The working group was advised not to incorporate risk management options into Phase II.

In other words, the working group had to deliver harmonised guidance using limited resources (six experts) defending primarily industrial and governmental interests, secondarily environmental and regulatory interests. The interests of the three industrial representations are very close: maximum result (registration) and at minimum costs (in terms of both dossier requirements and consumer image). The interests of the three governmental representations were however more different: apart from opening the markets and removing trade barriers, the existing registration procedures should not be compromised too much, the availability of products should not be hampered, and the environment in the three 'continents' (with different legislative criteria) should be protected. The product of such a setting is likely to be clear on intentions, but not on details and procedures.

The draft guidance documents published by the VICH are circulated for consultation to members of industry, the CVMP, and the DG Enterprise. These interested parties have the opportunity to provide their comments to their respective representatives. The EMEA working party member has to deal with (conflicting) comments from experts from all member states (represented in the CVMP), and from various experts from (governmental) science laboratories. Not until October 2001 (i.e. after the Phase was approved) a working group on ecotoxicology was established under the CVMP to advice on matters related to preparation of guidelines on environmental risk assessment, in particular providing comments on the VICH phase II guidelines, which are in preparation; to provide advice on issues not covered by existing guidelines such as developing agreed exposure calculation models; and to provide further advice at the request of the CVMP, on other issues related to environmental risk assessment of VMPs, in particular for facilitating a harmonised implementation of guidelines. After adoption by the Steering Group of the VICH the guidance is published by the EMEA and distributed to the member states, i.e. the national agencies for the authorisation of VMPs. Although the EC is informed on the progress of the work and contents of the guidance, it is not involved in regulatory approval of the guidance. Therefore the guidance has no legal status, even though it is an elaboration of the directive and it influences the registration process to a large extent.

Figure 2. Phase I Decision Tree



The policy making process on the ERA for VMPs has the following characteristics:

- Consensual approach between industry and registration agencies[24];
- No participation of other interested parties;
- Little or no involvement of policy makers from DG Enterprise or DG Sanco, a concern expressed by the European Parliament (EC, 2001b);
- The guidance and interpretative criteria have no legal status;
- Until recently no scientific opinion was formed that represented the CVMP point of view;
- There was no formal exchange between the VICH and EMEA working groups and steering groups or technical meetings for biocides or pesticides, even though these product groups share active substances and emission routes to the environment.

Thus the first two of five features for a proper assessment (see page 99) are not present. The formalisation of the contents and the procedure is neither transparent nor open to input by scientists and other interested parties such as competent authorities (with respect to environmental quality); the formalisation has no legal status, and European legislation cannot provide common protection goals.

6.6 Contents of the ERA for veterinary medicines

Let us now look at the contents of the guidances. As discussed above, a proper assessment at this point should involve environmental goals based on European environmental legislation, and should contain standards and harmonised risk assessment methodology. The ERA for medicines and feed additives consists of two phases. In Phase I, products are assessed on their intrinsic hazard and their level of exposure (see Figure 2 on veterinary medicines).

If the product fails to meet the triggers or exemptions, Phase II testing is needed. This step is not rigid, as some VMPs that might otherwise stop in Phase I may require additional environmental information to address particular concerns associated with their activity and use (VICH, 2000).

Question number 8 highlights a crucial component of the decision scheme underlying the decisions also made in questions number 3, 5, 7, and 12: what are the emission routes at and after administration, and can one be certain that the emission is absent or insignificant? The burden of proof is at the applicant, and the decision is to the assessor. In the guidance emission is limited to four routes: no emission, emission to surface water, emission to soil and direct emission into the environment (pasture animals). Emission to water and groundwater via soil and direct emission at application are not considered. Emission in the waste-stage of the product is not included in the guidance, although it should be part of the safety assessment as required by Directive 2001/82, and notably, there is no EU policy on the quality of soil (which is assessed), but there is legislation on the quality of groundwater (which is not assessed).

[24] For a further characterization of this policy-making style see the commentary of O. Renn in Löfstedt et al. (2001).

A total residue approach on the active substance is adopted, relieving the applicant from performing degradation route studies (animal, manure, soil, and water). Although this is a worst-case approach concerning the effect exerted by the parent compound, the fate of different metabolic fractions is not considered.

The exposure level that is considered irrelevant is quantified both for water and soil for antibiotics. For certain compounds (non-parasiticides) trigger values for exposure are introduced: 1 µg/L in water and 100 µg/kg in soil. These triggers are substantiated with an assessment of a dataset of toxicity values of several antibiotics, assuming:

- The data set (substances) is representative for all substances with the same pharmacological mode of action;
- The data set (endpoints) is representative for the aquatic respectively the terrestrial environment;
- Safety factors on the lowest experimental effect value are redundant due to:
  - the availability of substances in the presence of soil (due to sorption);
  - the functional redundancy of microbes in soil;
  - the mitigating influence of degradation.

The approach as such, i.e. determining the level of toxicity that will have a very small likelihood of being present in a product, and thus the level of exposure that can be considered an acceptable risk considering all products, is in fact equivalent to the practice in the exposure assessment, where the 'insignificant' emission routes are not considered in view of all other emission routes. However, the assessment to determine a safe level is found to be of poor quality from an ecotoxicological point of view (De Knecht and Montforts, 2001). The soil trigger for feed additives is, on the contrary, 10 µg/kg in the 2001/79 Directive.

The VICH has not yet agreed upon a Phase II guidance. The European registration process has to rely on the available guidance on Phase II as published by (EMEA, 1997), that consists of the hazard quotient approach, where predicted exposure and effect are combined. Breaching of risk ratio triggers (e.g. PEC/EC50) leads to refinement of the assessment and inclusion of field studies. The guidance is however ambiguous on the decision schemes: what compartments have to be assessed: surface water and groundwater are not assessed directly in Phase I: do they have to be assessed in Phase II? What data are compulsory, what exposure models and effect models must be used, and how should field studies be designed or interpreted? And how should persistency and accumulation be expressed in exposure modelling and effect assessment? Methodological problems resulting from the lack of guidance have been discussed in (Montforts et al., 1999; Halling-Sørensen et al., 2001; Montforts, 2001).

The available guidance is ambiguous on the ERA:

- no clear data requirements;
- testing protocols are lacking;
- decision making criteria are not clearly defined;
- there are no clear standards expressing acceptability;
- the methodology has not been elaborated to a satisfactory extent;
- justification of the trigger values is scientifically unsound;

- the ERA does not cover all Communautarian environmental legislations (i.e. groundwater).

The content of the VICH Phase I and the EMEA Phase II guidance do not bear the required characteristics of a proper assessment: it does not contain all Communautarian environmental quality criteria, clear acceptability standards, or harmonised methodology.

6.7 Implementation of the ERA

Let us now look at the implementation of the guidances. As discussed above, a proper assessment at this point should be performed at every registration to be effective. Because science is developing as well, also at every renewal the ERA should be reconsidered. The authority should apply environmental expertise at assessment and at decision-making, have methodology and criteria to its disposal, know how to weigh different interests, have expertise in the realism of risk mitigation measures, and should be able to deal with gaps in knowledge. Let us see if these requirements are met.

In Article 13 of directive 2001/82 exemptions are made to the dossier requirements and the extent of the safety assessment, as a result of which all member states, except the Netherlands and the UK, do not assess existing substances on environmental safety (De Knecht et al., 2001). If this interpretation is juridical correct, the registration process cannot function as a tool in environmental policy and is not in accordance with the White Paper intentions.

In order to prepare and make sound decisions on the safety of a product, ecotoxicological expertise is required at preparation and at decision making. Neither the members of the Netherlands Committee and Working Group on the Authorisation of VMPs, nor of the CVMP reflect the fact that ecotoxicology is a safety aspect on its own right. In several member states the ERA is not performed by qualified environmental chemists or ecotoxicologists, but by staff with a veterinary background (De Knecht et al., 2001), although dossier evaluation is a sensitive step in the registration process (Mensink et al., 1995), (Boesten, 2000), (Tiktak, 2000), (Pontolillo and Eganhouse, 2001).

The authorities do not have, as discussed above, clear methodology and criteria to their disposal. The authorities have little experience with risk mitigation measures and the available guidance does not, as advised by the VICH, deal with this matter. Risk mitigation measures at registration usually target the emission of the product to the environment. Measures that target the necessity or redundancy of the product are not expressed in the risk assessment and are also outside the scope of the registration (which considers the use (and should also consider the disposal) of the product.). Product labelling intended to reduce risk can only influence the use and disposal of the (prepared) product and the treated animal, but not the use and disposal of the contaminated manure and slurry. Two examples are found in the literature (Greiner and Rönnefahrt, 2001). The first example is on the restriction on spreading of manure from animals treated with the product. A label to keep treated animals stabled is enforceable, but a label to spread the contaminated slurry not within some distance from the ditch is not; at the moment the veterinary practitioner or farmer uses the product, he

cannot foresee the eventual spreading of the manure. The inspector can check the quality of the slurry, but not whether the medicinal product had been applied according to the label or not. The second example is the registration of a product containing alkylphenols. It is EU policy to abandon the use of these compounds (Footitt et al., 1999), but this cannot be considered a risk mitigation measure at registration. Not only does the product *in casu* contain this substance, but also the use in medicines is exempted from the general risk reduction strategy and the safety of the products with these compounds should be assessed at registration.

The authorities and have not made explicit how to weigh different interests nor how to deal with gaps in knowledge. The guidance on human medicines states "since for medicinal products the benefit for humans has relative precedence over any environmental risks, the environmental risk management procedures adopted for industrial chemicals and pesticides (i.e. prohibiting or restricting their use if an unacceptable risk to the environment is evident) is neither possible nor desirable in this case. Precautionary measures through product labelling are therefore the recommended risk management procedures for medicinal products, when concerns for the environment are present." This guidance indicates that environmental risk is at most a reason to suggest risk mitigation measures and undermines the legitimacy of the ERA: why impose a burden on the producers that will not discriminate the products? The guidances for veterinary medicines and feed additives do contain decision-making or interpretative criteria, which only lead to requests for further assessment. No information is provided on weighing of risks versus benefits, on provisional approval given an expected (low) level of risk. As there are not strict dossier requirements, it is not clear how lack of information should be included in the decision making.

The ERA at registration does not bear the last features for a proper assessment. Assessments are not made for all products, and the decision-making principles and practical procedures are not operational. It is therefore unlikely that any result of an ERA can be taken into consideration.

6.8 Discussion and conclusions

It has been argued that environmental protection in itself is not an issue to be dealt with at EU level. There are considerable problems that cross borders (e.g. groundwater depletion) or arise on a specific location due to actions of several states, but the level of quality desired on each location and the specific member states involved in each case are not uniform. Environmental legislation should thus be a case for member states only, or multi-lateral negotiation (Golub, 1996). To illustrate this line of reasoning: the EC has issued directives on water and groundwater (trans-national relations), but not on soil. The framework directive on water (2000/60/EC) is a fine example of the awareness of the EC of both the subsidiarity principle (regulate at the lowest appropriate level) and the complexity of the existing regulations on water quality. The framework directive formulates common objectives, leaving a great deal of decision making to the member states, and provides for a coherent approach of water management, recognising the relationships with other policy

areas, such as environment, nature, spatial planning, agriculture and product policy. It is argued here that the subsidiarity issue on environmental regulations is not only defined by scale of the revelation of the effects, but also by the mechanics that lead to the effects, including the working of the common market for products. This is why environmental legislation serves a purpose in European registration procedures for products.

A regulatory problem arises when a product registration procedure is harmonised at a European level by the central registration authority, while the authorities at the national level are responsible for maintaining the desired environmental quality. This may lead to a less effective implementation of the ERA as a tool for environmental policy. This problem can be tackled in two ways:

- the ERA should be based on common principles based on EU regulations and policy that steer the national authorities;
- the ERA should be developed under the supervision of competent authorities, for example through national interdepartmental steering groups that prepare the national points of view.

Both options are not reflected in the forging of the ERA for medicines and feed additives. The formalisation of the contents and the procedure is neither transparent nor open to input by scientists and other interested parties; the formalisation has no legal status, and European legislation cannot provide common protection goals in a global setting. The VICH Phase I and the EMEA Phase II guidance do not contain all Communautarian environmental quality criteria, nor clear acceptability standards, nor harmonised methodology. The scientific validity of the registration procedure is compromised (cf. (Heyvaert, 1999a)). Assessments are not made for all products, and the decision-making principles and practical procedures are not operational. It is therefore unlikely that any result of an ERA can be taken into consideration at registration, which undermines the legitimacy of the process.

What is the ultimate effect of these developments? Assessors at the registration agencies do not know if and how to perform or conclude an ERA. Applicants do not know what effort the authorisation process will place upon them, which makes it difficult to take management decisions on the development of new products, or the renewal of old products. It is not the ERA as such, but the lack of clarity in procedure and requirements that may ultimately compromise product availability. Products that pose a threat to environmental quality at or after use or disposal may now be registered, forcing the authorities responsible for water and land quality to regulate and enforce product use and slurry use on a case by case basis.

The major efforts recently made by the regulators and scientists within EMEA and VICH need to be founded on clear policy decisions and embedded in a uniform and transparent decision-making procedure. It should take little effort to postulate Communautarian decision-making criteria together with their levels of acceptability. These will provide a solid basis for the implementation of the existing risk assessment methodologies, and subsequently help to clarify the (compulsory) data requirements and (realistic) risk mitigation measures. These five elements (criteria, standards, methodology,

data requirements, and mitigation measures) will then provide a reference for deciding on the environmental acceptability, both for the producers and for the decision-makers.

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7. Legal constraints in EU product labelling to mitigate the environmental risk of veterinary medicines at use

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MHMM Montforts, National Institute for Public Health and the Environment, Bilthoven
HFMW van Rijswick, Faculty of Law, Utrecht University
HA Udo de Haes, Institute of Environmental Sciences, Leiden University

7.1. Introduction

European and national regulators are involved in managing environmental risks of veterinary pharmaceuticals from two perspectives. One is the registration of pharmaceutical products (Blasius and Cranz, 1998), and the other is the management of good environmental quality (Montforts and De Knecht, 2002). The framework of the registration procedure for veterinary medicines consists amongst others of the European Community legislation, Member State legislation, case law, as well as global (trade) agreements. In this paper we investigate what possibilities and obligations are created for applicants and authorities within this framework to assess the environmental impact of the use of a veterinary medicinal product, to take the results of the risk assessment into account in decision-making, and to bind users and third parties to precautions in the labelling and packaging. The objective of this article is then to investigate methodological and legal restraints that render the precautions in the labelling and packaging ineffective as risk mitigation measures within the European Union. For further reading on the legal context in the US the reader is kindly referred to Daughton and Jones-Lepp (2001), Haskell et al. (2003a; 2003b), and Nidel, (2003).

7.2. The obligation to take environmental risk into account at registration of veterinary medicinal products

The codified EU Directive 2001/82/EC lays down rules for, amongst others, placing products on the market, labelling and package leaflet. Placing on the market evolves around the permit to market the product, the so-called marketing authorisation (MA), the procedures for granting the marketing authorisation, and procedures for mutual recognition of marketing authorisations within the EU. The Directive addresses both regulatory authorities and applicants, but not the consumers of the marketed products. The recently adopted Directive 2004/28/EC amends the 2001/82/EC Directive. In this new Directive, any risk of undesirable effects on the environment is included in the definition of risks relating to the use of the product (worded in Article 1(19)). Article 12(3)j. requires the applicant ‘to provide tests assessing the potential risks posed by the medicinal product for the environment. This impact

shall be studied and consideration shall be given on a case-by-case basis to specific provisions seeking to limit it.'

The risk assessment is to be examined by the registration authority. This examination is performed by a scientific committee, since the European Court of Justice (ECJ) ruled in case C212-91 (Angelopharm) that "the Scientific Committee is the only party involved in the policy-making process that is competent to make those scientific and technical assessments on which the legal validity of the measures depends" (Heyvaert, 1999a). At the European level Regulation (EC) 726/2004 (re)installed the Committee for Veterinary Medicinal Products (CVMP) to provide these risk-based opinions in the centralised procedures (Blasius and Cranz, 1998). The CVMP is also involved in decentralised procedures, where a marketing authorisation obtained in one Member State is taken for mutual recognition to other member states. When disputes between member states about public health or environment remain unsolved, the case is also referred to the CVMP, which will provide for a binding opinion on the matter.

The framework of the registration procedure for veterinary medicines thus generates a scientific opinion on the environmental risk. There are two possible options for the authority in response to an identified environmental risk. The first option is to eliminate the risk by denying marketing authorisation. This option is based in the articles 30 and 33 to the Directive. In the Directive 2004/28/EC, amending the 2001/82/EC, Article 30 states that marketing authorisation is denied if the risk-benefit balance of the product is, under the authorised conditions of use, unfavourable. A risk/benefit balance is defined as 'an evaluation of the positive therapeutic effects of the veterinary medicinal product in relation to the risks'. In Article 33, it is stipulated that a mutual recognition of a marketing authorisation can be denied if there are concerns for a potential serious risk to human or animal health or for the environment.

The second option is to mitigate the predicted risk to an acceptable level by addressing the user of the veterinary medicine through the information that accompanies the product (Koschorreck et al., 2002). This option has the intention of establishing a code of conduct that is reaching further than the Good Agricultural Practice taken as a starting point in the risk assessment. This option is held in high esteem, since it is explicitly worded in Article 12.3.j of the 2004/28/EC Directive and the recital. This option is further investigated in this article, by examining the requirements set in the Directive towards the risk assessment methodology and the obligations towards the user of the medicinal product.

7.3. The structure of the environmental risk assessment

The EU Directive and the Notes for Guidance provide for a methodology for assessing environmental risk following the use of the product under representative conditions. It is stated in Annex I, Part 3, chapter 1.5, to the EU Directive 2001/82/EC that:

'the assessment shall normally be conducted in two phases. In phase I, 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 treated animal 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 compartments,
- 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 phase II, taking into account the extent of exposure of the product to the environment, the investigator shall then consider whether further specific investigation of the effects of the product on particular ecosystems is necessary. 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 have to be taken into account. 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.'

Thus the Directive has specified the scope and boundary conditions for the environmental risk assessment to be performed. Article 33(2) of the recent Directive 2004/28/EC coerces the Commission to adopt guidelines defining a potential serious risk for human or animal health or for the environment. This is essential to make the risk/benefit-based decisions (Di Fabio, 1994). The European Medicines Agency (EMEA)[25] and the CVMP have published guidance on the environmental risk assessment, that was implemented in 1997 in the European registration process (EMEA, 1997). A revised guidance document on Phase I has been implemented by July 1st 2001 (VICH, 2000). For Phase II the 1997 guidance is still leading, but a new Phase II guidance is under preparation that is expected to come into force in 2005 (VICH, 2003). The guidance documents consider the use stage of the products. The waste stage of the products is however not guided by these guidance documents, and neither will it be considered here.

The Notes for Guidance identify acceptable risks of applications: generally when the level of exposure is below a predicted no-effect concentration. If the predicted exposure level were to be greater than the predicted no-effect level, the assessment proceeds to a next tier where the Note for Guidance requires more data and more advanced methods to refine the risk assessment. Regarding the exposure assessment, the identified and consolidated emission routes are direct emission to the environment, emission through dung of grazing animals, emission of contaminated water, and emission through spreading of slurry from treated animals. The assessment is performed taking codes of conduct according to Good Agricultural Practice into account. Good Agricultural Practices to the use of manure on land

[25] Formerly named the European Medicines Evaluation Agency

may differ between members states and are amongst others set by the Nitrate Directive for vulnerable areas, advisory standards for crop fertilisation, and tolerance of crop for excessive manuring (Montforts and Tarazona, 2003). This allows for the use of generalised data on animals, manure production, storage, handling and spreading, under worst case conditions. A second important assumption is that spreading of manure is a given fact, and that the contamination by the veterinary medicinal products does not restrict the spreading of the slurry.

The EU Directive and the Notes for Guidance provide thus for a methodology for assessing environmental risk following the use of the product under representative conditions. The most important conclusion is that risks arising from direct exposure, at treatment, or from exposure to treated animals, and indirect exposure, by the spreading of contaminated materials such as dung and manure, are within the scope of the registration assessment. Further details on the risk model and the available methodology will be addressed below, where relevant. We will now investigate what the possibilities are for risk reduction by provision of instructions to the user of the veterinary medicine.

7.4. Risk mitigation by labelling and packaging

Together with the marketing authorisation, several documents and particulars with relevant information are issued at registration. These entail a summary of product characteristics (SPC) and an assessment report, as stipulated in Article 14 and 25, the containers and outer packages (Article 58), and a package leaflet (PL, Article 61). All of these particulars should contain 'precautions (as a special class of prescriptions) for disposal of unused medicinal products or waste material from medicinal products, if any'. The SPC should also contain explanations of these precautions together with an indication of any potential risk to the environment. All these precautions shall conform to the particulars and documents pursuant to Article 12 of Directive 2001/82/EC. Precautions should therefore *demonstrably* reduce the environmental risk. We will now consider these documents and particulars in greater detail. It will be investigated what the subject of the measures can be, who the addressee is (the object of the precaution), and what the disposition of the precautions is (precept, prohibition, or recommendation).

A precaution is not a mandatory enactment under the Directive 2004/28/EC. The Directive does neither elaborate on obligations to consumers to obey the documents and particulars nor on supervision and sanctions. Precautions are hence not legally binding through the Directive. Although it can be expected that the precautions will have their intended effects in a certain number of instances, the reasonable worst case situation remains the one where the precautions are not followed. In that sense, the precautions are merely recommendations. Paradoxically, all precautions should therefore be considered as ineffective risk reduction measures, unfit for inclusion in the labelling. However, national

legislation concerning the veterinary practice should turn these prescriptions into legal injunctions. The situation in the Netherlands is presented here as an example.

In the Netherlands, rules on precautions have been laid down in the Veterinary Medicines Act (Diergeneesmiddelenwet) (Anonymous, 1985). It is established in Articles 7 and 40 that it is forbidden to act against the prescriptions in the documents and particulars issued at registration. This prohibition applies to the users of the veterinary medicine, provided that the prescriptions are stated in the Package Leaflet, the container, or the outer packaging. Information in the SPC alone is however not legally binding, but may assist the veterinarian in selecting the appropriate treatment. Ignoring the prescriptions issued at registration is a penal offence, supervised and sanctioned, under the Economic Offences Act (Wet op de economische delicten) (Anonymous, 1950). The Veterinary Medicines Act also controls the availability of veterinary medicines. There are three classes of veterinary medicines: freely available products, products under prescription that can be administered by the keeper of the animals, and products that can only be administered by the veterinarian.

The subject of instructions (the 'what' question) in the labelling may be the product (e.g. dosage and posology), the treated animals, or animal products such as eggs and milk (e.g. withdrawal times). Likewise, the excreta of treated animals can be addressed by special instructions, since these are under control of the keeper of the treated animals. The addressee of these precautions (the 'who' question) may be the veterinarian or the keeper of the treated animals. Other persons or subjects are not the users of the products and cannot be addressed.

It is also very important that the precaution addresses the right addressee with reasonable demands. Unreasonable demands will not only be ineffective, but may also delay the registration procedure. An illustration of unreasonable demands can be found in the precautions concerning the application of biocidal products for the impregnation of wood. The precautions of concern addressed the person that impregnates the wood with instructions on the selection of the product for certain types of wood. The precaution distinguished between the different final destinations of the wood: use in contact with soil and water, or not. A Netherlands Court, the Board for the Appeal of Private Enterprise (CBB) ruled that restrictions on the use of wood preservation products should only have bearings on destinations (of the treated wood) that were to be determined reasonably clear and objective at the time of use of the product (CBB, 2000). Restrictions bearing on the anticipated use of the wood in contact with soil or water were considered not to meet this requirement. It was taken into account, that the person applying the product for impregnation was not the person who determined the destination of the treated wood. When deciding on using the product on a given batch of wood, the destination of the wood would not be reasonably clear for him to make the right decision. The precaution that distinguished between contact with water and soil or not, was unreasonable and the authorisation was nullified.

If precautions refer to the handling of treated animals or manure that has been contaminated with residues of the medicinal product, such precautions only should have legal force if the user of the product also controls these animals or this manure. Such precautions would have no legal force if another person than the user of the product actually determines

the destination of the animal or the manure. Without legal force, the precaution cannot be considered to mitigate the risk. There are two situations where this applies.

Firstly, regarding the products that are to be administered by the veterinarian only, the precautions cannot instruct him or her on the destination of the treated animals or the manure, since the farmer controls these. Second, for products that are administered by the keeper of the animals, the precautions do have binding force. However, once the animals or manure have been sold to a third party, the precautions are no longer binding. For these open ends a solution must be developed.

All precautions should be based on factual information provided in the dossier and generated in the risk assessment. To what extent the effect of the precaution is demonstrable by the risk assessment methodology will be explored in the next section.

7.5. The demonstration of the effect of risk mitigating precautions

In European Member States, several medicinal products have been registered after decentralised procedures, with special precautions contained in the SPC, Package Leaflet, container and outer packaging. All these precautions shall conform to the particulars and documents pursuant to Article 12 of Directive 2001/82/EC. Precautions should therefore demonstrably, i.e. quantifiably, reduce the environmental risk. This means that the impact of the precaution should be expressed in the risk assessment, in conformity with the dossier and the risk assessment methodology. The methodology available typically targets realistic worst case conditions of use that cover all possible situations in the field. Special precautions should apply without exemption to the worst case conditions.

Below some examples of special precautions for the environment are discussed with respect to the methodological demonstration of the efficacy of the precautions[26].

Many products containing parasiticides for pasture animals carry a precaution that dictates that treated animals should not enter surface water at or after treatment. Apparently, the aquatic environment is at risk when treated animals have access to surface water, since residues of parasiticides are excreted with dung for days after treatment (Lumaret and Errouissi, 2002). According to the Notes for Guidance, the risk for surface water is based on an exposure model where 1% of the dosage (per hectare) is excreted in a ditch (100 m^3) adjacent to the field. The resulting exposure concentration is compared to the toxicity of the crustacean *Daphnia magna*, taking an assessment factor of 100 into consideration. A risk quotient >1 indicates risk and calls for refinement of the assessment or risk mitigation measures. A few examples of products with this precaution are presented here.

The package leaflet of Eprinex Pour On (containing eprinomectin) carries the precaution ‘treated animals should not have direct access to surface water and ditches’.

Without access to surface water, the treated animals will not expose the aquatic environment to excreted residues. The precaution on Eprinex Pour On eliminates demonstrably the risk to the environment since treated animals are not allowed to have access to surface water anymore. The precaution is technically sound. However, there is apparently no time period after which the risk of Eprinex Pour On would have become acceptable. It could be discussed whether this precaution is proportional since treated animals will have no longer access to fields with adjacent surface water.

The package leaflets of both Equimax oral gel for Horses (containing ivermectin and praziquantel) and of Noromectin 1.87% oral paste for Horses (containing ivermectin) carry the precaution: '*treated* animals should not have direct access to surface water and ditches *during treatment*'. Apparently, the treatment poses a risk to the aquatic environment, not the excretion of residues after treatment, which would be expected. Based on the Notes for Guidance the predicted concentration ivermectin in surface water would be 25 ng/l after the treatment of ponies (0.2 mg/kg bodyweight, 250 kg bodyweight, 5 animals per hectare). Halley et al. (1989) reported an EC50 of 25 ng/l for ivermectin in *Daphnia magna*. Applying the assessment factor of 100 results in a toxicological threshold of 0.25 ng/L. The risk quotient of 100 is above the threshold of 1. This precaution does not eliminate the risk of surface water contamination due to entry of residues excreted by the horses *after* treatment, which most likely was the intention.

The package leaflet of Triclaben 10% (containing triclabendazole) carries the precaution 'Cattle should not have access to surface waters within 7 days after treatment.' The package leaflet of Clik 5% Pour-on (containing dicyclanil) carries the precaution 'The treated sheep should be kept away from water courses for at least one hour after treatment.' Apparently, the risk to the aquatic environment is acceptable after 7 days, respectively 1 hour after treatment. These precautions provide clear instructions and the potential effect of these precautions can be demonstrated with the risk assessment methodology, since information on the excretion pattern of the active substance should be available (Montforts et al., 1999; Taylor, 1999).

Apart from the technical aspect, other legal aspects will determine the conformity with the EU Directive, as discussed above. Third parties will not be bound by the precautions stated above; inferring that treated animals will pose a risk to the environment after they have been sold to third parties within the stipulated time periods. The proportionality of the precautions should also be observed.

The product Sebacil Pour On (containing phoxim) is applied to pigs. The package leaflet contains the precaution: 'When spreading manure from treated animals on agricultural lands a safety distance of 10 m to adjacent surface waters must be kept to avoid exposure of the aquatic environment.' Apparently, the risk to surface water after manuring of land was

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[26] The German federal registration number for Eprinex Pour On is 400629.00.00, for Triclaben 10% 400661.00.00, for Sebacil Pour-on 12201.00.00. The other SPCs were retrieved from http://www.hevra.org/vmri_spc/.

not acceptable. In the methodology provided by the Notes for Guidance in Phase I, the concentration in surface water depends on the concentration in soil as a result of spreading of slurry. The model assumes a dilution factor of 3.3 on the porewater concentration and describes the partitioning function as follows:

$$PECporewater = \frac{PECsoil}{Foc_{soil} \cdot Koc}$$

where PECsoil is concentration in the soil in [$mg.kg_{soil}^{-1}$], Foc_{soil} is the fraction organic carbon in soil in [$kg.kg^{-1}$], Koc is the partition coefficient organic carbon – water in [$l.kg^{-1}$], and PECporewater is the predicted concentration in porewater in [$mg.l^{-1}$]. The degree of surface water contamination in this exposure model is neither related to the actual transport processes (erosion, run-off, and drainage), i.e., the ratio between treated soil and receiving surface water, nor to the distance to the surface water. The water contamination depends on the equilibrium concentration between soil solids and soil porewater, and a dilution factor between soil porewater and surface water. The distance to the surface water is not modelled. The precaution must therefore have been based on an exposure assessment that handled this parameter of distance-to-edge, taken from a different source of exposure modelling. The German package leaflet contains the precaution: 'Whenever slurry of animals treated with Sebacil Pour-on is applied on agricultural fields, *because of the hazard of run-off*, a minimum distance of 10 m to surface waters should be observed.' The hazard of run-off is indicated here. The German EXPOSIT model is known to contain a function that calculates a reduction in run-off when a vegetative buffer strip is observed between the treated soil and the surface water. A 10 meter vegetative buffer strip would reduce surface water contamination with 67% due to run-off (Winkler, 2001). Evidently, next to the process of run-off, drainage is a process to be considered (Kay et al., 2004), and the recommended no-spreading zone does not influence the contribution by drainage to the same extent. Moreover, in all operative drainage models, used in pesticide registration, the drainage model contains only a single soil column. The effect of a no-spreading zone, which would be a second soil column in the exposure model, is not demonstrable in drainage calculations with the currently available models in the frameworks of registration of veterinary medicines or plant protection products (VICH, 2000; FOCUS, 2001; WRc-NSF, 2001; Winkler, 2001).

Since the Notes for Guidance do not define the relative contribution of the process of run-off to the final water concentration, the influence on the final exposure concentration could not be quantified. The methodology should be improved on these aspects in order to make these precautions demonstrably effective. Apart from the technical aspect, other legal aspects will determine the conformity with the EU Directive, as discussed above. Third parties will not be bound by the precautions stated above; inferring that the manure from treated animals will pose a risk to the environment after it has been sold to third parties.

Another example concerns the effect of the precaution on Nuflor Drinking Water Concentrate for Swine (containing florfenicol): 'Manure from treated pigs should be stored for 3 months prior to spreading and incorporating into land'. Apparently, the concentration of the residue in the manure was too high. The precaution addresses the manure storage in the exposure model, which is in potential important in limiting exposure of the environment (Pierini et al., 2004). The precaution may generate a necessary certain amount of dilution of the residue with clean manure during these 3 months. However, the Notes for Guidance refer for an example of the calculation of the soil exposure concentration to the paper by Spaepen et al. (1997). In this paper, the shortest dilution period is about 5 months for slaughtering pigs, making it less conceivable that the intended risk mitigating effect is dilution. It is more likely that the effect of degradation on the concentration of the residue was assessed in the dossier. The assessment of fate and distribution of veterinary medicines in manure during storage is complicated, due to the lack of technical guidance both for conducting degradation studies and for interpretation of the results and subsequent exposure modelling. The performance and evaluation of laboratory studies on the degradation in manure have been investigated (Bouwman and Reus, 1994), but have not yet resulted in internationally accepted test guidelines (Van Vlaardingen et al., 2001). Also there is currently no scenario that lays down representative worst case conditions for the modelling of degradation during manure storage (Montforts and Tarazona, 2003). Proportions of manure types and storage systems differ considerably between countries and will influence storage conditions and manure composition in different ways (Donham et al., 1988; Menzi, 2002). Conditions like oxygen levels, manure age, microbial activity and temperature will determine the fate of organic contaminants to a large extent, but are highly diverse within and between storage systems (Hoeksma et al., 1987; Novem, 1991; Arogo et al., 1999). Manure models that model manure loading, quality change, and fate of constituents do exist for nutrients, but are not operational for organic contaminants (Ni, 1999; Ni et al., 1999; Hilhorst and De Mol, 2002). Therefore, the waiting period would probably contribute to risk mitigation, assuming at least some degradation of the relevant residue, but the exact effect under relevant worst-case conditions cannot be quantified using available methodology. The methodology should be improved on these aspects in order to make these precautions demonstrably effective.

Again, apart from the technical aspect, other legal aspects will determine the conformity with the EU Directive, as discussed above. Third parties will not be bound by the precautions stated above; inferring that the manure from treated animals will pose a risk to the environment after it has been sold to third parties.

7.6. Discussion, conclusions and recommendations

In this paper we investigated what possibilities and obligations are created by the EU Directive 2001/82/EC, to bind authorities, applicants, and users, to instructions and prohibitions in the labelling to the product. The regulatory framework obligated applicants and authorities to assess the environmental risk of the use of the product. The assessment is to

be performed by a scientific committee. The CVMP Notes for Guidance provide for a methodology for establishing environmental risk following the use of the product under representative conditions. Risks arising from indirect exposure, by the spreading of contaminated materials such as dung and manure, are within the scope of the registration assessment.

Doubts on the acceptability of environmental risks may constitute a reason for the applicant to change product characteristics or target species, and for the authority to deny marketing authorisation. The present article focuses on the alternative option to mitigate the risk to an acceptable level by special precautions in the information that accompanies the product.

The retrieved precautions address the fate of treated animals or the contaminated excreta, seeking to rule out or diminish the exposure of the environment. The grazing of treated animals in fields adjacent to surface water, the storage of manure, and the distribution of manure on land adjacent to surface water, are the components of the exposure methodology that are altered by the precautions, which subsequently ought to demonstrate the necessary reduction in risk. The intended addressee is therefore the keeper of the animals and the manure. Should the intended addressee not be addressed and bound by the precaution, or the risk reduction not be demonstrable, it has to be accepted that the risk will not be mitigated.

Several constraints have been identified that make risk mitigation measures technically or legally ineffective, hence unsuitable for labelling and packaging (see Table 1 for an overview).

First, through the Directive precautions are not legally binding to veterinarians and farmers (the consumers). In that sense, no precaution can be considered an effective risk reduction measure. National legislation concerning the veterinary practice must turn these recommendations into legal injunctions, in order to make the precautions work. The way precautions are worded, in relation to the national legislation determines the national legal status, and thus their efficacy as risk reduction measure. It is imperative that the legality of the precautions and the possible subjects and addressees of the precautions are defined in national regulation, and that this is harmonised between Member States. One way would be to incorporate in the Directive that consumers are bound to the precautions. Member States will have to transpose this into national legislation. By means of a Regulation this prescription would have direct effect on the consumers in all Member States.

Second, precautions can be used to control the fate of the treated animal and the manure containing excreted residues, provided the legal person addressed is the keeper of the treated animals. If the product is to be administered by the veterinarian, environmental precautions regarding the treated animals or manure are thus not binding. The legislation at hand also does not transfer precautions regarding the treated animals and the manure to third parties. The solution to these shortcomings is to include this transfer of responsibilities to second and third parties, either in the precautions themselves or in the legislation, and to prohibit both trade and use of the animals and manure in the precautions during the time that the precaution is operative.

Table 7-1 Overview of selected precautions included in the package leaflet of veterinary medicines with a view to mitigate environmental risk.

Product	Precaution	Problem	Solution
Eprinex Pour On	treated animals should not have direct access to surface water and ditches	addressee, proportion	Include transfer of liability to other parties, define waiting period
Equimax oral gel for Horses ; Noromectin 1.87% oral paste for Horses	treated animals should not have direct access to surface water and ditches during treatment	addressee, efficacy	Include transfer of liability to other parties, define waiting period
Triclaben 10%	Cattle should not have access to surface waters within 7 days after treatment	addressee	Include transfer of liability to other parties
Clik 5% Pour-on	The treated sheep should be kept away from water courses for at least one hour after treatment	addressee	Include transfer of liability to other parties
Sebacil Pour-on	At application of slurry of treated animals on agricultural fields a minimum distance of 10 m to bordering surface waters is to be observed	addressee, efficacy	Include transfer of liability to other parties; improve exposure assessment methodology
Nuflor Drinking Water Concentrate for Swine	Manure from treated pigs should be stored for 3 months prior to spreading and incorporating into land	addressee, efficacy	Include transfer of liability to other parties; improve exposure assessment methodology

Third, precautions are only acceptable under the Directive if their potential effect can be demonstrated using the risk assessment methodology. Thus, the precautions forbidding release of treated animals or manure containing residues into the environment are technically effective, since the effect can be demonstrated in the methodology. The impact of temporary storage of manure containing residues cannot be quantified because of a lack of standardised model conditions. Likewise, the precise effect of the precautions prohibiting the spreading of manure within a certain distance to the surface water can as yet not be quantified with available exposure assessment methodology. The flexibility of the risk assessment methodology to deal with temporal and spatial differentiation in the exposure and effect assessment should be improved accordingly.

Fourth, whether the precautions on confinement of the animals or the manure (for a time period or infinitely) leaves the farmer with reasonable alternatives is an issue of proportionality. Precautions that are impossible to incorporate in Good Agricultural Practice should be avoided.

Discharges of slurry and chemical substances are in the EU also regulated by community legislation such as the Nitrate Directive and the Directives on water pollution 76/464/EEC, on groundwater protection 80/86/EEC, and in the near future the Water Framework Directive (2000/60/EC). This type of legislation operates from the starting point that all actions that may lead to pollution are forbidden unless a permit is granted by the national competent authority. This legislation addresses different authorities than the Directive 2001/82/EC does. The permit ought to regulate the emission (e.g. by prescribing

application or purification techniques) as well as the maximum permissible concentration of the substance in the environment. The Marketing Authorisation is not a permit in this sense, but could provide for a firm scientific basis for the decision making by competent authorities. Ineffective precautions coerce the competent authorities to regulate the emission of residues. Also for products where the risk/benefit balance was favourable despite an environmental risk, the use or subsequent emission of residues necessitates regulatory consent. For example, for the use of Slice (containing emamectin) in the UK it will be necessary to obtain consents from the local environmental authorities (Anonymous, 2003). No-spreading zones are already Good Agricultural Practice in some Member States, for example in the UK (DEFRA, 2002). Alternative solutions to the use of precautions in the product information may thus be found in establishing precautions in permits, or in codes of Good Agricultural Practices, issued in these frameworks (Van Rijswick, 2001; Van Rijswick, 2003). Inevitably, the scientific and juridical underpinning of the precautions in these frameworks should be as meticulous as in the framework of registration, and will also require a flexible risk assessment methodology to quantify the impact of temporal and spatial differentiation of residue emissions.

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8. Discussion

The objective of this thesis has been the validation of the environmental risk assessment methodology for the marketing authorisation of veterinary medicines[27].

The fate and behaviour of pharmaceuticals in the environment has been studied incidentally since the second half of the 20th century. In some instances, this was not with the intention of identifying ecological damage, but rather to find substances that combated (pathogenic or harmful) bacteria and had no side-effects on the particular organisms of their interest, such as fish for commercial breeding, algae for taxonomic studies, or soil fungi for research on soil nutrient cycling (Wellborn, 1969; Berland and Maestrini, 1969; Ingham and Coleman, 1984). The possible effect of residues on the quality of the environment was a concern that had been recognised since shortly after medicines were actually found in the environment towards the end of the 1960s. About a decade later, these concerns about pollution and effects had reached the regulatory agenda and were addressed in the 81/852/EEC Directive on marketing authorisation for veterinary medicines.

Effect studies with a variety of veterinary medicines on non-target organisms have been published in both environmental and agricultural literature. Exposure assessments of veterinary medicines by the spreading of manure were developed in the 1980's comparable to those applied to heavy metals, nutrients, pathogens, and biocides. Notably from 1994 onwards, when the EU Committee for Veterinary Medicinal Products (CVMP) initiated the drafting of a guidance document for the risk assessment of veterinary medicines, an increasing number of reviews on use, emission, fate, occurrence, effect, and risk of pharmaceuticals has been published in public literature. Several authors have provided suggestions on a risk assessment methodology for the Marketing Authorisation of veterinary medicines, thus increasing the body of information useful for this research into risk assessment.

The validation exercise performed here addresses the quality of the science applied, which should target the high level of protection of the environment that the EU Treaty intends to reach (EC, 2002c). Performing a validation contributes to a better understanding of the information generated in the risk assessment. The representation of the environment in the risk model and the consequences of the choices regarding variables and relationships in the natural system were addressed in light of existing legislation and policy on environmental quality. Where possible, empirical validation of individual exposure models was performed, demonstrating the accuracy of the model for a specified use. The following research topics on model validation and on the interaction between science and regulation have been addressed in this thesis.

[27] This research is restricted to the methodology developed and implemented between 1994 and 2001.

1. **Harmonisation of protection goals and risk assessment methodology**
- What relevant environmental protection goals can be considered?
- Does the integral risk model address the protection goals?
2. **The conceptual and empirical validation of models and precautionary labelling**
- Are screening level exposure models for surface water in aquaculture, for dung, and for soil and water in intensive animal husbandry well founded and applicable?
- Is the soil trigger value based on effect data functional and validated?
- Can the efficacy of mitigation measures be demonstrated by the methodology used to predict the risk?
3. **The use of science in the registration framework**
- Is science applied transparently and impartially in the development of risk assessment methodology and in the decision making for product registration?

The findings are discussed below.

8.1. Harmonisation of protection goals and risk assessment methodology

In order to protect the environment, next to animal health, consumers, and professional users, the marketing of veterinary medicinal products is actively regulated in the European Union. In order to market veterinary medicines, one needs a Marketing Authorisation. This Marketing Authorisation is issued together with all information that can and should be made available when selling the product, after a scientific assessment of the products properties concerning quality, efficacy, and safety. An environmental risk assessment is to be performed as part of this assessment, and there is a clear policy and regulatory infrastructure to deal with this issue. This infrastructure consists of regulatory, administrative, and scientific bodies, at supranational, international, and national levels, and includes legislation, jurisprudence, trade agreements, and a number of regulatory guidance documents on the environmental risk assessment (EMEA, 1997; VICH, 2000; DG Enterprise, 2000; Anonymous, 2001b).

The guidance documents are of particular interest, since scientists forge these documents in order to guide other scientists in risk assessment. The first guidance document, or Note for Guidance, was prepared and released by the CVMP in 1997 and provided a comprehensive risk assessment methodology that followed the Directive in its prescription that the assessment had to be performed in two phases. A new guidance document on Phase I was prepared by the International Co-operation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH)[28] and was implemented by July 1st 2001. This guidance replaced the CVMP 1997 guidance on Phase I, the phase in which predominantly only the exposure of the environment to the veterinary medicines is assessed, but not the risks. The major changes concerned the soil concentration that should trigger a complete risk assessment. Not only was the trigger value increased tenfold, also groundwater

[28] Commonly referred to as the Veterinary International Conference on Harmonisation.

was no longer assessed in Phase I as an independent protection goal. This change drew the attention of both the scientific press and of the European Parliament[29].

As indicated in the introduction, environmental risk assessment is a scientific discipline that investigates the possible damage that certain activities, such as the use of veterinary medicines, may have on the environment. Often the risk is assessed in hierarchic levels, from screening level to advanced levels, where the estimations in higher levels overrule those in lower levels. At all levels, the same protection goals are observed, but the approach in the lower levels tends to be more conservative, less complicated, and with a low information need. When a risk in a lower level is deemed acceptable, no further assessment is required.

The tiered approach in the risk assessment scheme of the registration procedure of veterinary medicinal products is depicted in Table 8-1. The registration procedure is divided in two phases, Phase I and II. Phase I has the objective to identify the level of exposure that determines if there is enough concern to proceed to Phase II. The objective of Phase II is to identify the risk and possible risk mitigation measures. From the table, it follows that the practice of applying exposure concentrations as triggers for risk assessment is viewed, by the Scientific Steering Committee of DG Sanco, as unscientific. In my opinion, the use of threshold values, or concentration triggers based on effect data, is still part of the scientific domain of risk assessment. This research is predominantly concerned with Phase I.

My contradictory view on the scientific status of the use of thresholds is based on my perception of the scientific realm of risk assessment. The collection of protection goals, exposure models, and effect models, together with the conventions to apply the models and to harmonise their results, is by itself a model. It is a risk model to assess the risk of the use of veterinary medicines. The risk model includes all risk assessment methodology and all

Table 8-1. The tiered approach in the risk assessment of veterinary medicinal products (SSC, 2002).

Stage in regulatory evaluation scheme	Stage in scientific risk assessment scheme	Objective of stage	Methods employed	Requirements or applicability
Phase I		Identify exposure	Action limits	No test requirements
Phase II tier A	Screening	Rapid prediction of (absence of) risk	Risk assessment	Base data set on fate and toxicology
Phase II tier B	Primary	Standard approach to ensure consistent decision making	Risk assessment	Extended data set on emission, fate and effect
	Secondary	Substance and site-specific refinement		Case-by-case; depending on approach

[29] Currently (April 2004), the draft guidance on Phase II, the phase in which the risk assessment is carried out, is still under the consultative process. For this reason this guidance document is not considered here (VICH, 2003).

activities employed in the risk assessment procedure at registration. The objective of the risk model is to provide comprehensive information on all risks related to the use of the veterinary medicines in order to optimise the risk-based decision (Di Fabio, 1994; Cranor, 1997). The aim of the tiered approach within the risk model of the registration process is to eliminate the no-risk situations from further regulatory actions.

In risk assessment, both exposure and effects are modelled; the models are abstractions of reality and the outcomes are projections of what may happen. It depends on the level of protection what modelling scale is most suitable. Different scales of assessment aggregate information differently. Regional scale modelling may overlook local hot spots, and local modelling, in turn, may overlook spatial and temporal differences. The level of uncertainty and variability in the data associated with the assessment differs between the alternative scales of exposure and effect assessment. The choice of the scale hence influences both the modelling and the effect assessment approach.

The objective of every individual exposure model is to predict sufficiently accurate exposure concentrations. Exposure models describe transport, partitioning, and degradation processes, and enable us to model the fate of a veterinary medicine and to predict concentrations in soil or water because of its use. Effect models attempt to reproduce effects in model organisms or model systems as a result of exposure to a veterinary medicine, and enable us to make an estimation of adverse effects in the environment. All model results need to be translated to the situation of interest, taking variability and uncertainty in the model predictions into account. The combination of harmonised exposure and effect data results in estimates of risks, for example expressed as risk quotients, or margins of safety, for the situation under examination. The collection of all risk parameters (i.e. all results for all compartments, all routes of exposure, all species of concern) is the basis for the classification of the risk associated with the specific use of the veterinary medicine. In general, the classification in deterministic risk assessment is binary: there is a risk or not.

In the risk model, all relevant environmental protection goals are to be addressed. This statement sounds straightforward. The risk model should address all environmental compartments that are possibly exposed by the use of the veterinary medicine, at the high level of protection of the environment pursued by the European Union (Art. 174 of the EU Treaty (EC, 2002c)). The stage of hazard assessment is an important founding level for the protection goals and the geared risk model (Chapter 2).

The risk model is to be developed to address qualitative hazards (i.e. damage to environmental assets) and, where applicable, quantified standards. The fact that neither the Treaty nor the veterinary medicines Directive define the concept of environment suggests that there is no ambiguity expected. It appears that this stage has been insufficiently explored in the drafting of the VICH Note for Guidance, since not all possibly relevant protection goals are targeted in Phase I. In Phase I, only soil is assessed for veterinary medicines applied to stabled animals. Surface water and groundwater are only assessed in Phase II. The picture emerges that policy makers and scientists have not engaged themselves in a reconnaissance

of regulatory goals, assessment scales, model approaches, and the uncertainty and variability of data associated with the assessment.

The organisational setting of the process in which the guidance was forged has contributed to this situation, as addressed in Chapter 6. Both the CVMP and the VICH guidance documents were drafted in an international working group of acknowledged environmental scientists, with experience in the environmental risk assessment for other chemicals, such as pesticides. In the drafting of the VICH Guidance, the experts involved, six in total, represented industry (three) and 'regional' governments (one for each region: EU, USA, and Japan). The experts had to operate with limited resources, instructions and responses, to design a methodology that suited different environmental interests. The EU expert had indeed considered the EU Directives on environmental quality in the drafting process. The expert had noted that the reference made in this legislation to 'pesticides' (in Directives 76/464/EEC and 80/778/EEC) and to 'biocides' (in Directive 80/68/EEC) was to affect 'some veterinary medicines, but not all'[30], further considering 'that in specific cases there would be restrictions/provisions applying to products containing these substances'[30], without a clarification of who would be responsible for these provisions. In light of this interpretation, the protection goals of surface water, groundwater, and drinking water quality should have been integrated in the risk assessment methodology. Only when this would have been accomplished, then for given applications it would be possible to assess whether the use of the veterinary medicine was to be within the reach of any environmental legislation. The purport of the European 'regional' environmental legislation was however not considered as a *conditio sine qua non*. Consequently, surface water, groundwater, and drinking water quality were not taken into account in the risk model as a protection goal in Phase I.

A regulatory problem arises when a product registration procedure, through harmonisation at a trans-national level, neglects environmental quality objectives for which preservation authorities at the national level are responsible. It is clear and without dispute, that the legislation on registration of veterinary medicines is not an environmental legislation (based on Article 174 of the Treaty); and it is certainly not subsidiary to environmental legislation. However, there should be harmonisation of protection goals, standards and methodology between the relevant laws; else this may lead to a less effective implementation of the environmental risk assessment as a tool for environmental policy[31]. Not observing groundwater as an environmental criterion is not merely a technical flaw like having difficulty in establishing an acceptable effect[32], as suggested by Long and Crane (2003), but a grave conceptual flaw in the risk model.

For the implementation of the methodology in the European Union, a further elaboration of the technical guidance is therefore needed, since European environmental legislation forces national authorities to issue permits or letters of consent for the use of veterinary medicines and the emission of its residues. For example, the UK government has

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[30] Personal communication with the CVMP representative in VICH on Phase I, C. Long, June 2001.

[31] The implementation of the Framework Directive Water 2000/60/EC, with respect to the standard setting for pesticides, provides a recent example of this struggle.

[32] See also Crane and Newman (2000) and De Jong et al. (in press).

been subject to infringement proceedings from the European Commission over a complaint regarding groundwater pollution from organophosphate sheep dip, a veterinary medicine use (Maynard, 1997). This decision reflects the Commission's concern that groundwater resources should be fully protected in accordance with Council Directive 80/68/EEC. Even, so to speak, if the soil concentration is below 100 µg/kg.

Several (groups of) substances have been listed in this European environmental legislation as 'priority substances' or 'substances of concern'. These groups of substances are for example 'pesticides', 'biocides', 'chlorinated hydrocarbons' or 'heavy metals'. The decision of including a veterinary medicine in any of the special categories of, for example, 'pesticides' or 'biocides' should be based on the state of scientific knowledge[33]. The Netherlands Health Council advised the Ministers to treat (veterinary) medicines in a way comparable to 'pesticides and biocides' because they are pharmacologically active, are spread continuously, and little is known on their effects (Health Council, 2001). This scientific opinion puts medicines on a par with 'pesticides' and 'biocides' in the environmental legislation[34]. Therefore, not only should the quality of various water compartments be one of the endpoints in the Phase I assessment, but also the quantitative standard for pesticides in drinking water (and in the Netherlands also for groundwater) of 0.1 microgram/L should apply.

8.2. The conceptual and empirical validation of models

I have performed the conceptual and empirical validation of exposure models in a number of publications. In the first instance, I presented and discussed in Chapter 3 four models for three routes of emission and distribution of veterinary medicinal products (aquaculture, grazing animals, and stabled animals) that reflect realistic agricultural practice in the Netherlands. Thereafter, an empirical validation of the slurry-soil-water models was attempted. For the different routes of emission, the findings are summarised below.

Exposure assessment begins with the use and the emission routes. All relevant emission routes are covered in the methodology: by slurry, by dung, direct emission at application, and by wastewater, directly or after treatment, and by sludge. Distribution models follow on all these emission models[35]. The models that describe emission, distribution

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[33] The European Court of Justice decided in the Van Bennekom case, which addressed the question whether vitamins should be classified as medicinal products, that this classification must be decided on a case-by-case basis, "having regard for the pharmacological properties of each vitamin to the extent to which they have been established in the present state of scientific knowledge" C 227/82 [1983] ECR 3883. The core of this ruling is –in my opinion–, that the required classification of substances should be based on their properties that were established by scientific knowledge.

[34] The European Court of Justice regards pesticides and biocides as - per se - dangerous substances (Heyvaert, 1999a). The scientific opinion that medicines should be treated like pesticides transfers this qualification to medicines as well.

[35] Aerial emissions are not considered in the current guidance documents. Based on data from Zahn et al. (2001), air-borne emission of tylosin from medicated feed out of the barn was calculated to be 35% of the dosage (Powers, 2003). For veterinary medicines administered by water rather than feed, or directly in or on the animal, this percentage is likely to be much less. Differentiating this pathway will not change the worst-case exposure models for other pathways and is not further considered here. However, distribution of airborne resistant bacteria may cause a potential hazard (Gibbs et al. 2004).

and exposure are made up of model parameters and algorithms. The model parameters have dimensions and values, determined by the model structure and the environmental conditions that the model should cover. The different parameter values are hence not selected at random, but are selected from confined ranges. The combination of agronomic and environmental conditions that realistically represents an area in which a substance is to be applied, is named a scenario. Models can be run for different scenarios of interest, and provide exposure data for different situations of interest.

Aquaculture. An exposure model for surface water through emission of waste water from in-house fish nurseries had been proposed for the registration procedure in the Netherlands (Montforts, 1999), and has been taken as a case study for this research (Chapter 3). The results of the exposure modelling for fish medicines indicated that concentrations of fish medicine up to 1 mg/L might be present in surface water bodies near discharge points of fish nurseries (without passage through a sewage treatment) for a substantial period of time after treatment (25 days). However, depending on the degradation rate in a sewage treatment plant to which the nursery conceivably discharges, the calculated concentration in surface water could be 30 times lower.

Apart from the possible connection to a sewage treatment plant, the functionality of the conceived settling tank in the water circulation system was validated (Chapter 4). The removal efficiency of settling tanks was verified using pesticide data in wash water from predominantly mushroom industries. Since the available data gave no proof for a settling tank contributing to the removal of pesticides from waste water, it was recommended for risk assessment purposes to consider an efficiency of 0%. The pesticide data available were unsuitable for a mechanistic analysis of the retention process, due to a lack of detail in the description of system dimensions, water volumes and flow rates, sludge characteristics and total pesticide load. Furthermore, the nature of the settling process of fish nursery sludge may be very different from that of soil or compost particles. A model sub-routine describing the effect of water treatment and sludge retention should be based on the system of interest.

In order to empirically validate this model I had suggested monitoring of surface water for antibiotics and anthelmintics used in fish nurseries. Monitoring data from the effluent from a tropical fish nursery in the Netherlands supported the possibility that under low flow conditions of the receiving surface water the dilution of effluent water by surface water may be very small. Following these findings, I have revised the model. As a screening approach the environmental exposure levels are to be expected at the water concentration in the fish tank.

Grazing animals. The concentration in dung was a critical component in the Phase I assessment proposed by the CVMP Note for Guidance, since all concentrations greater than 10 microgram per kilogram would trigger a Phase II assessment. Alternative methods to predict the concentration to be compared with this trigger were assessed in Chapter 3 of this thesis.

The validation of the model for the concentration in dung (produced by grazing animals) shows that the worst-case calculations are overestimating actual concentrations, which suits the objective to err on the safe side, but fails the objective of realism. The proposed exposure assessment based on concentrations derived with actual excretion profiles and empirical dung production data appeared to lead to quite accurate results compared to field measurements. The trigger value was deemed, however, inappropriate: too low to discriminate between different dosages of substances (all would lead to higher concentrations), and too high to protect for the potent ivermectins. In the VICH Note for Guidance, the trigger value has been abandoned, and a Phase II assessment is warranted for all applications of anti-parasitic substances in grazing animals.

The Phase II assessment will have to answer to the question whether field populations of insects and possibly higher trophic levels will be reduced due to the use of the medicine. The effect of anthelminthic treatments in grazing livestock on dung insects, worms, and organic matter breakdown in Europe, has been debated several times (Madsen et al., 1988; Madsen et al., 1990; Wratten and Forbes, 1996; McKellar, 1997; Montforts, 1997b; Suarez, 2002; Svendsen et al., 2002; Sommer and Bibby, 2002; Lumaret and Errouissi, 2002; Svendsen et al., 2003; Floate et al., 2005). Contaminated dung may support some species, but may hypothetically function as an ecological trap for dung-dependent species that produce offspring only once, or feed on dung fauna (Donovan and Thompson III, 2001; Ries and Fagan, 2003). All papers address the population issues qualitatively, in the sense that short excretion periods, or availability of dung from untreated herds, are reasoned to sustain populations of dung dependent species. Exclusive use of these products, long excretion periods or use during reproductive seasons, are expected to seriously impact populations. However, no solutions for a quantitative approach of exposure and effect dynamics, with a concurrent level of acceptability were presented. Sherrat et al. (1998) demonstrated the impact of periodically contaminated dung on population levels of selected dung insects over longer periods using population modelling. To my opinion, this type of modelling is very useful in the registration procedure. A further development of the model approach, incorporating food-web modelling and the identification of indicator species and their life-cycle strategies is needed (Petney, 1997).

Slurry from stabled animals. The emission from slurry to soil and water is unmistakably an important and complex route. Given the many animal categories and slurry qualities, storage conditions and fertilising regimes, a realistic worst-case approach in a deterministic model is a choice that is well to understand. The actual use pattern of the product should be explored since repetitive use, season-related use, or concurrent uses over large areas, in relation to the timing and scale of emission to the environment (i.e. spreading of manure), have a significant impact on the actual exposure. The importance of storage of slurry has been noted in the guidance, but no clear guidance on the most relevant conditions for this parameter could be given. Therefore, scenarios for the risk assessment under different European conditions, incorporating information on realistic agricultural and veterinarian practice, land use, geomorphology and climate, were developed in this thesis (Chapter 4).

The CVMP Note for Guidance issued in 1997 contained relatively straightforward deterministic exposure models for soil. These models, as proposed by Spaepen et al. (1997), are capacity models based on fixed volumes and masses. Time windows are fixed in the parameter dimensions. The volumes of administered substances and produced slurry were based on a full year and the emission to soil was modelled as a single event. I hypothesised that the practice of slurry management, with repetitive spreading on grassland, would change soil exposure significantly. The influence of changes in values of parameters such as phosphate content of the slurry, mixing depth, and degradation rate, was also incorporated in the validation. The comparison of the CVMP models with the RIVM models demonstrated that differences in predicted exposure concentrations could be as high as a factor of 40. It was obvious that arbitrarily chosen values concerning for example the percentage of the herd treated, and variation in national nutrient immission standards or nutrient contents of the slurry, would lead to further deviating results.

There are indications that the soil model by Spaepen et al. (1997) is under-protective when compared to the results of German and UK field experiments, since several field measurements were higher (Figure 1, Chapter 4). The model I have conceived for a single spreading event was more successful, although some field measurements were still higher. Since the slurry volume that dilutes the residue strongly determines the concentrations, slurry concentrations should be related to a realistic short time frame for the production and the dilution of the contaminated slurry in order to optimise the realistic worst case predictions. I also concluded that the available field data did not allow for validation of separate parameters in the soil exposure models. The screening models calculate homogeneous distributions of the residue, which represent the median concentration in soil. The field data provided some estimates on variability in the slurry-soil model results: not only may field concentrations vary a factor 30 between samples within one field (Boxall et al., 2002); also patches of slurry may contain concentrations 30 times above those found in more homogeneous soil (Hamscher et al., 2002). This variation is unavoidable, but results in a situation where the modelled concentration in half of the area is 1 to 10 times (or more) lower than the actual concentration. The risk due to a higher concentration in one spot is however not compensated by the absence of risk in another.

The empirical validations of the CVMP and RIVM models on field data of (oxy)tetracycline and sulphonamides indicated that it is impossible to analyse the contribution of every single model parameter to the variability in the model predictions. The validation of the models was complicated by not only variation in doses (a function of dosage and animal body weight at the moment of treatment), excretion factors, dilution and degradation, slurry application rates and soil variability, but also factors such as representative sampling in slurry and soil, and field residue history.

Surface water and groundwater models generated high deviation in results compared to the controlled field results. The common element of all models is that soil porewater concentrations are over-estimated compared to the measurements for sandy soils. Again, since these model outcomes provoke further assessment of the risk in Phase II, protection of

the environment will be secured. However, there is also evidence that surface water and groundwater contamination is not controlled by equilibrium sorption between soil and porewater, but also by non-equilibrium sorption, preferential flow, and movement of particles, run-off and soil erosion. Hence, cut-off values based on sorption properties that determine whether a risk assessment is performed do not guarantee sufficient protection of surface- and groundwater. This is further elaborated below.

The degree of surface water contamination calculated in the screening level exposure models is not related to the physical transport processes taking place (erosion, run-off, drainage), the possible incorporation of the slurry into the soil, or the distance to the surface water. The soil-surface water models are merely simple transport coefficients that express the assumed mass transfer from soil to water. Models that describe these transport processes in physical terms are however available (FOCUS, 2001). Both screening and advanced level models share the presupposition that the area of land that connects to the ditch or stream is proportional to the size of the water body. In all models used in pesticide registration, the drainage model contains a single soil column from which drains, or run-off, discharge into the surface water. This single soil column makes it impossible to assess the impact of spatially restricted manure spreading on the surface water concentration. More advanced distribution models are needed to yield better judgements for differentiated land use and spatial risk mitigation measures.

Based on the validation performed, I propose realistic worst case conditions in a simple scenario assuming:

- single treatment,
- standard European nitrogen production values,
- an accumulated manure production volume of 1 month (30 days) containing the full residue,
- no dissipation during storage, and no after-treatment of slurry.
- a nitrogen application rate of 600 kg N/ha/year in one time onto agricultural land, which is distributed over 5 cm soil with a bulk density of 1500 $kg.m^{-3}$,

If the exposure calculation in Phase I according to this scenario does not exceed the trigger for further testing, safe use in all member states is possible. If the exposure meets the trigger, then realistic best case conditions, characterising a possible safe use in vulnerable areas under the Nitrate Directive in the European Union, are needed. I propose a similar scenario, now assuming active incorporation of slurry into 20 cm of soil, at a nitrogen application rate of 170 kg N/ha/year in one time. If the trigger is exceeded, then a phase II assessment should be compulsory for all member states. If the trigger is met, further assessments should be made at the member state level, since national environmental concerns may be a reason to refuse (mutual recognition of) marketing authorisation.

The resulting concentrations in soil can be used in conjunction with the discussed screening level models that assume a certain mass transfer to groundwater and surface water, preferably without triggers on substance properties or exposure concentrations (Chapter 4 of this thesis). The main reason is that the predictions are in general worst case, thus protective.

However, I consider the mechanistic models provided by FOCUS equally suitable for both veterinary drugs and pesticides. The simple fact that this methodology applies to the same agricultural fields that are relevant for manure application and for pesticide use, predestined the accompanying scenarios to be applicable to residues spread by manure as well (FOCUS, 2000; FOCUS, 2001).

Interestingly, the difference between the results of this local soil modelling approach and of a mass balance at national scale appears rather small. The predicted initial soil concentration using the proposed scenario in conjunction with Dutch default conditions, for a veterinary medicine that is used in large quantities, like (oxy)tetracycline used in pigs or broilers, amounts to 200 µg/kg soil, with a possible maximum of 1000 µg/kg soil (Montforts, 2003). The average concentration in soil, based on the total annual consumption of antibiotics of 402 tonnes, and the total capacity of the agricultural area to utilise manure in the Netherlands, amounts to approximately 100 µg/kg soil (Van Staalduinen et al., 2001; MARAN, 2002). The difference between the model and the mass balance is just one order of magnitude, which suggests that regional scale modelling of soil concentrations may provide alternative approaches for protective risk assessments in areas with intensive animal husbandry and manure surplus.

8.3. The validation of precautionary labelling

Two possible management options in response to an identified environmental risk were discussed in Chapter 7. The first is to eliminate the risk by denying marketing authorisation of the veterinary medicine in question. The second is to mitigate the predicted risk to an acceptable level by special precautions included in the information that accompanies the product. The intention then is to establish a code of conduct that is reaching further than the Good Agricultural Practice observed in the risk assessment.

If risk mitigation measures are ineffective, they are unsuitable for labelling and packaging. The risk mitigation measures are considered effective, when the risk model can demonstrate their efficacy, and when the precautions have legal force. However, precautions are not legally binding through the Directive on its own. In that sense, no precaution can be considered an effective risk reduction measure. It is imperative that the legality of the precautions and the subjects and objects of the precautions are defined in national regulation, and that this is harmonised between Member States. Otherwise differences in national legislation may obstruct mutual recognition of registrations where this is not needful.

Further, the wording of the precautions in relation to the national legislation determines the legal status. Under Dutch law, precautions can be used to control the fate of the treated animal and the manure containing excreted residues, provided that the legal person addressed is in fact the keeper of the treated animals. If the product is to be administered by the veterinarian, environmental precautions regarding the treated animals or manure are not legally binding for the keeper of the animals or of the manure. Similarly, for veterinary

medicines that are administered by the keeper of the animals, the current legislation does not transfer precautions regarding the treated animals and the manure to third parties (when the animals or the manure is sold). The solution to these shortcomings is to prohibit both trade and use of the animals and manure in the precaution. Another option is to transfer all responsibilities to the keeper and to other parties, either in the precautions, in EU legislation, or in the national regulation.

Precautions forbidding release of treated animals or manure containing residues into the environment, when addressing the keeper of the animals, are not only legally, but also technically effective: the impact can be demonstrated with the methodology. Whether or not the prohibition of spreading the manure (for a time period or infinitely) leaves the farmer with a real alternative for the disposal of this contaminated manure, is an issue of proportionality. The alternative for the farmer would be to resort to alternative products.

The impact of temporary storage of manure cannot be quantified however, because of a lack of standardised conditions. Likewise, the precise effect of the precautions prohibiting the spreading of manure within a certain distance to the surface water could not be expressed in terms of the exposure assessment methodology. In order to avoid marketing authorisations that would not comply in a legal sense with the Directive, because precautions are not demonstrably effective, the capacity of the risk assessment methodology to deal with temporal and spatial differentiation should be improved.

8.4. The use of science in the registration framework

In the introduction, I stated that there are potential controversies that require a carefully designated playing field, where science can be impartial and authoritative. One is at the demarcation line between science and regulation when deciding what should be investigated or protected, and when this protection goal is achieved. The second is the choice of scientific disciplines: what science is allowed and who selects the scientists? The third is the actual weight that science is given in the decision-making. A critical appraisal (Chapters 2, 5, and 6 in this thesis) of the settings of the development of the guidance documents and of the risk assessment at registration has dealt with these aspects.

The interconnections between science and regulation require some elucidation here. In the organisation of the European registration process of veterinary medicines, regulatory, administrative, and scientific responsibilities are separated. The Directorate-General (DG) Enterprise is responsible for the European policy and legislation on veterinary medicines. The European Medicines Agency (EMEA)[36] handles the administration, and the CVMP provides scientific opinions. According to the European Court of Justice (case C212-91 Angelopharm): "the Scientific Committee is the only party involved in the policy-making

[36] In a 1987 Council decision (87/373/EEC) the phenomenon of comitology was formalised, through which the EU sought to ensure that Commission decisions would be taken with due regard for the political preferences of the Member States, and for scientific expertise (Heyvaert, 1999a). EMEA (European Medical Products Evaluation Agency) was installed by

process that is competent to make those scientific and technical assessments on which the legal validity of the measures depends" (Heyvaert, 1999a). To my opinion, both the technical guidance and the risk assessment at registration resort under the responsibility of science. CVMP and VICH have issued Notes for Guidance. These scientific guidance documents are crucial in the registration process.

In the underpinning of the soil concentration value that triggers a Phase II assessment, science had been applied. This strategic effect assessment I have critically validated in this research. Validation is used here in the meaning of establishing whether the trigger value is 'well founded and applicable' (Addiscot et al., 1995). I drew a number of conclusions on the data and arguments to support a soil concentration trigger in Chapter 5.

Before the effect and risk assessment for substances spread with slurry from stabled animals is performed in Phase II, the concentration in soil because of this spreading is compared to a trigger value. The exposure trigger in soil, that determines whether a full risk assessment for all environmental compartments is performed, has been raised by a factor of 10 in the VICH Note for Guidance. This new trigger value was based on an effect assessment performed on a data set of existing veterinary medicines. The original data set was based on dossiers that have been submitted to the US authorities for the registration of veterinary medicines (Zeeman, 1987; AHI, 1997; Nidel, 2003).

If the data would have been assessed in the same way as the risk model for registration does, then the threshold will certainly be valid for the substances in the data set. For other compounds and for other risk models, this is not straightforward. The data set was very limited in the number of substances and types of endpoints. More substances and more discriminating properties were considered required for a case based reasoning that extends from substances with identified common characteristics to substances that yet have to be developed.

Next to the selection of the data, the selection of ecotoxicological tools determined the outcome of the scientific assessment. In order to derive 'no effect' concentrations from a limited number of effect endpoints from a small group of species, assessment factors are applied. The argumentation presented by the VICH on the redundancy of assessment factors did not comply with the EU-guidance given on these matters (EC, 2003). Moreover, the argumentation deviates from the risk characterisation performed in the CVMP Note for Guidance, which constitutes the risk assessment component of the risk model.

Because of these diverging methodologies, the predicted 'no effect' concentrations derived in Phase II may be found below the trigger value of Phase I. Consequently, the trigger in Phase I is not protective for its own risk model in Phase II. The trigger is not even protective for the compounds that were in the reference set. Applications that result in predicted exposure concentrations below the trigger, are not further assessed, but may cause damage to the terrestrial and aquatic ecosystem. Based on the distributions built from the

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the European Commission (EC) through Decree 2309/93 and began its activities in 1995. The name was changed by the Regulation (EC) 726/2004 to European Medicines Agency.

experimental evidence, the soil exposure trigger value of 100 µg/kg may already affect soil microbial species by 80% of the substances covered by its data set.

I took the validation of the soil trigger value a step further by introducing other readily available effect data. In contrast to the reference data set discussed above, the risk model under consideration has not generated these data. It was demonstrated that further consideration of available effect data would lower the trigger. In combination with alternative interpretations of the given arguments for the abandonment of assessment factors, a case was made to set a threshold at 1 µg/kg. It should be kept in mind that the exposure trigger based on the soil compartment determines whether a further assessment of the surface and groundwater compartments is performed in Phase II. The trigger was, however, solely based on soil data and was not harmonised with the possible quality criteria in these other compartments. Even if the soil compartment would be protected at the trigger value, protection of groundwater and surface water quality may not have been secured. In fact, this may not have been the case after all. Harmonisation of effect endpoints between compartments, using the equilibrium partitioning method, gives reasons for soil triggers as low as 0.0125 µg/kg.

The technical guidance documents not only elaborate on the requirement set in the legislation but also effectively define the protection goals, as argued in Chapter 2 and 6. From a regulatory point of view, a transparent input of scientists, Member States and interested parties (other regulatory authorities, manufacturers, users and consumers) is expected, in order to agree on protection goals and assessment methodology for the guidance documents (ESC, 2001). These protection goals were however not laid down in the legislation on product registration, quite to the opposite for the feed additives, pesticides and biocides, where protection goals and standards were incorporated in the EU Directives themselves, subjected to regulatory and political supervision. The VICH had appointed a working group for the drafting of the guidance documents. This working group was in my perception a scientific committee, but not a representation of all interested parties. The selection of protection goals is not the prerogative of science, and groundwater as a protection goal has unjustly been omitted from the risk model.

Addiscot et al. (1995) stated that some form of critical evaluation procedure is essential both to maintain the integrity of modelling and to ensure that the use of models by regulators does not result in the propagation of misleading information. Although models for exposure were meant, it is clear that this statement also applies to the use of effect models and to the use of risk models. Both the definition of protection goals and the underpinning of the soil exposure trigger were not well founded.

Should the development of resistance in bacterial communities due to the environmental exposure to antibiotics have been identified as a hazard? And what kind of hazard are we dealing with? Is it a hazard for the ecosystem integrity or also a hazard for public health in general? How can we express the degree of anticipated damage? Currently both molecular and ecological methods are investigated. Pollution-induced community tolerance has been found suitable to detect community shifts at low concentration levels. If

there is a genetic basis for these shifts is to be explored. Shifts in community tolerance caused by soil pollution have been shown to have impacts on e.g. extinction of sensitive species, competitive abilities, and metabolic diversities. Resistance development occurs already at the Minimum Effect Concentration (MEC) at which growth is reduced, that is tenfold below the Minimum Inhibitory Concentration (MIC), the endpoint used to derive the soil concentration trigger discussed in Chapter 5. Thus, even at concentrations below the Phase I trigger, resistance genes may be favoured. The survival of adapted bacteria in absence of the compound that the bacteria have adapted to is limited, but the acquired functionality (e.g. resistance genes) remains present at low levels. The costs for resistance can even be compensated for. Horizontal transmission of genetic information occurs for example in the gut of soil arthropods. Resistance genes can be transferred from manure to soil and groundwater, where low levels of antibiotics may be present (Rysz and Alvarez, 2004). Since this hazard, that is not yet addressed in the risk model, also applies to antimicrobial products used as pesticide and biocide, a harmonised approach for all antibiotic use should be developed.

8.5. Concluding remarks

In conclusion, I have outlined and discussed several technical and conceptual flaws in the use of risk assessment in the registration process. Foremost, and perhaps for the better, the formalisation of the protection goals by the Note for Guidance has no legal status. The VICH Phase I guidance does not contain all European environmental quality criteria and includes a trigger for the full risk assessment that was not well founded. Screening level exposure models are not always protective and lack standardised scenarios for uniform assessment. Risk mitigation measures are not validated, and give a false impression that the risk to the environment is reduced to acceptable levels. Some products have been registered with precautions that are not in agreement with the Directive. Products that pose a threat to environmental quality at or after use may now be registered and be used. Authorities responsible for water and land quality cannot infer from the registration of a veterinary medicine that the use complies with the quality standards set by environmental legislation. This legislation now obliges these authorities to regulate and enforce product use and slurry use on a case by case basis through permits or letters of consent.

All of the shortcomings identified above undermine, to a greater or to a lesser extent, the legitimacy of the registration process and the use of scientific opinions. Particularly the variability in outcomes for the soil concentration trigger is troublesome, since it gives the impression that science may produce information that will not lead to better judgement. The respectable efforts made by the regulators and scientists within CVMP and VICH need to be founded on clear policy decisions and embedded in a uniform and transparent decision-making procedure. A further elaboration on European agricultural and environmental conditions, regulatory and legislative constraints, and risk model design, is therefore

warranted. It should take relatively little effort to postulate European decision-making criteria together with their levels of acceptability, some of which this research has exemplified.

The recently adopted Directive 2004/28/EC amends the 2001/82/EC Directive and will have to be implemented at member state level by October 30th, 2005. In this new Directive, any risk of undesirable effects on the environment has been added to the definition of risks relating to the use of the product. Marketing authorisation is to be denied if the risk-benefit balance of the product is, under the authorised conditions of use, unfavourable. Compared to the text in the previous Directive, it is noteworthy that the qualification 'harmful' has been replaced by 'unfavourable risk-benefit balance', meaning 'an evaluation of the positive therapeutic effects of the veterinary medicinal product in relation to the risks'.

Policy makers, regulators, and scientists should engage in a reconnaissance of regulatory goals, assessment scales, model approaches, and uncertainty and variability of data associated with the assessment. Only then, a risk model (with or without triggers) can be applicable to the situation of interest and provide a solid framework for making risk-benefit decisions to the satisfaction of producers, decision-makers and consumers.

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Synopsis

Chapter 1

1. Veterinary medicines are pharmacologically active substances. This property categorises them under substances that are denoted as pesticides or biocides. Likewise, the use of veterinary medicines caused environmental damage comparable to the use of pesticides and biocides.

Chapter 2

2. The collection of protection goals, exposure and effect models, and the conventions to apply and harmonise their results constitutes a risk model. The risk model is a tool to make scientific assessments of the environmental risks of the use of veterinary medicines. The selection of hazard based criteria (thus not based on the risk of the use of the veterinary medicine) that trigger formal risk assessment is also part of the risk model and hence part of the scientific domain.
3. Present European legislation regulating the registration of veterinary medicines does not specify environmental protection goals. The risk model employed at the registration of veterinary medicines does not systematically address all environmental concerns identified in EU environmental legislation. The neglect of the risk to surface water and in particular to groundwater is a structural error in the risk model.

Chapter 3

4. Actual excretion profiles of residues in dung from grazing animals provide reliable input for modelling of concentrations of veterinary medicines excreted in dung. Contaminated dung may act as an ecological trap for dung dependent species, it attracts invertebrates without providing them the opportunity for reproduction. Population models need to be developed for indicator species in dynamic agro-ecosystems, taking different reproduction strategies and dependency relationships into account.
5. Variation in common agricultural practices with respect to slurry storage time and the use of slurry on land throughout the year gives rise to a variation in soil exposure concentrations within two orders of magnitude.

Chapter 4

6. The original exposure assessment model suggested for concentrations in soil suggested is not worst case. Dilution of residues by clean slurry and degradation during storage are the main parameters that control the concentrations of residues in slurry. Environmental parameters that control degradation of residues in slurry, like temperature and oxygen

levels, are highly variable in the field. No degradation of residues represents a realistic worst case. Concentrations on slurry 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.

7. Distribution of slurry in the field generates a considerable spatial variability. Actual concentrations may be an order of magnitude above or below the averaged concentration.
8. The complete slurry-soil distribution model cannot be validated by merely field soil measurements, due to the complexity and variability of all environmental and agricultural parameters involved.
9. Realistic worst case conditions for protective risk assessment are hence proposed in a simple scenario assuming a single treatment per animal place, standard European nitrogen production values, a manure production volume of 1 month (30 days) containing the full residue, and a nitrogen application rate of 600 kg N/ha/year in one time onto agricultural land, which is distributed over 5 cm soil with a bulk density of 1500 $kg.m^{-3}$, no dissipation during storage, and no after-treatment of slurry. If the exposure calculation in Phase I according to this scenario fails the trigger for further testing, safe use in all member states is possible.
10. Realistic best case conditions, characterising a possible safe use in vulnerable areas under the Nitrate Directive in the European Union, are created in a scenario assuming active incorporation of slurry into 20 cm of soil, at a nitrogen application rate of 170 kg N/ha/year in one time. If the trigger were exceeded, then a phase II assessment would be compulsory for all member states. Further assessments should be made at the member state level, since environmental concerns can be a reason to refuse (mutual recognition of) marketing authorisation.
11. The original surface water and groundwater distribution models are not founded on empirical data or on physical relationships. Consequently, trigger values for aquatic risk assessment following spreading of slurry to soil, based on substance properties, are not justified. The simple fact that the groundwater and surface water exposure methodology applied at the registration of plant protection products encompasses the same agricultural fields that are relevant for manure application predestines these models and scenarios to be applicable to residues spread by manure as well. In areas with intensive animal husbandry and manure surplus, simplified regional scale modelling of surface water contamination over larger time windows may well prove to be sufficiently protective.
12. Settlement tanks for coarse soil particles in wash water do not contribute to the removal of pesticide from the total load in water phase.
13. For the aquatic risk assessment of the use of veterinary medicines in aquaculture the worst-case environmental exposure levels are to be expected at the therapeutic concentrations in the fish tank.

14. For the underpinning of the value of the soil concentration trigger a reference dataset was used. The terrestrial ecosystem was not investigated in great detail, both with respect to the representation of test species and with respect to the selection of testing conditions and endpoints. Not all information that was available was presented.
15. Resistance development occurs already at the Minimum Effect Concentration (MEC) at which growth is reduced, which is tenfold below the Minimum Inhibitory Concentration (MIC), the endpoint used to derive the trigger under discussion. This indicates that at the MIC level used in the reference dataset, a selection pressure for resistance development is present.
16. For the underpinning of the value of the soil concentration trigger, the case that sorption reduces bioavailability, hence removing the need for assessment factors, has not been argued correctly. In the derivation of the value of the soil concentration trigger, the effect values have not been corrected for the argued influence of test conditions compared to standard conditions, or for the relative molar weight of the tested substance.
17. The concept of functional redundancy does not provide an argument to refrain from using assessment factors to extrapolate laboratory results with model species to vulnerable species.
18. For the underpinning of the value of the soil concentration trigger, the case that degradation in the field may mitigate effects, hence removing the need for assessment factors, has not been argued correctly. Not only may degradation have been reflected in the test result, also the risk may be increased due to persistency of the substance.
19. For the underpinning of the value of the soil concentration trigger, harmonisation with protection levels in water and groundwater was not observed. A soil trigger that is not harmonised with protection levels for water might result in violation of water quality standards.
20. Based on a statistical analysis of the reference data set, there is 45% likelihood that a substance represented by the reference data set will affect plant species and 80% likelihood that bacterial species are affected. The soil trigger is not even protective for the substances in the dataset under discussion.
21. For a hypothetical substance, with a mode of action covered in the reference data set under discussion, and a strong preference for the water phase, the predicted MIC depends on the molar mass and will typically range between 1 and 15 µg/kg. The trigger for antibiotics can be based on other experimental bacterial test results, yielding no-effect-levels at 100, 30, 28, 16, 4, or 2 µg/l, depending on the preference for a given community process, a specific single species, an certain inoculum density, or a specific endpoint.
22. For the underpinning of the value of the soil concentration trigger, the representation of different mode of actions of veterinary medicines was acknowledged to be insufficient to set a trigger for parasiticides administered to grazing animals. The trigger value is however applied to these and other ill-represented compounds, spread with slurry. More information and more discriminating properties are needed to allow for a case based

reasoning for a soil concentration trigger that extends from substances with identified common characteristics to substances that yet have to be developed.

Chapter 6

23. The technical guidance documents on the environmental risk assessment contain environmental protection goals and acceptability standards. Unlike the protection goals and standards codified in the directives on feed additives, biocides and pesticides, the goals and standards for veterinary medicines have not been subject to regulatory and political scrutiny. Since scientists developed the technical guidance documents, regulatory powers have been unobtrusively transferred to the scientific community. Implementation of the ERA procedure is an act that will have legal consequences for stakeholders (producers, users, and third parties). Formalisation of the contents and the procedure should be transparent and open to input by regulators, scientists, industry and other interested parties.
24. Registration authorities and scientific panels should reflect in their composition the fact that environmental concerns are to be taken into account in the risk/benefit profile.
25. Not all veterinary medicines are subjected to an environmental risk assessment at registration.
26. A positive opinion on the environmental risk at registration is by no means a charter for the use of the product with respect to liabilities set by environmental legislation to the emission of residues.
27. Communautarian decision-making criteria together with their levels of acceptability are needed. These will provide a solid basis for the implementation of the existing risk assessment methodologies, and subsequently help to clarify the (compulsory) data requirements and (realistic) risk mitigation measures. These five elements (criteria, standards, methodology, data requirements, and mitigation measures) will then provide a reference for deciding on the environmental acceptability, both for the producers and for the decision-makers.

Chapter 7

28. The registration framework has created an obligation for applicant and authority to assess the environmental risk of both the use and the disposal of the product. Potential risks arising from indirect exposure, i.e. by the spreading of contaminated matrices such as dung and manure, are within the scope of the EU Directive 2001/82/EC. At the European level, the CVMP is competent to make those scientific and technical assessments on which the marketing authorisation depends. At national level national scientific bodies are. The scientific body may decide what information is to be generated and when the assessment is conclusive on the risk/benefit profile. The dossier, to be delivered by the applicant, should contain all the information needed to perform the assessment, else the authorisation shall be withheld. Doubts on the acceptability of environmental risks

constitute a reason to deny (mutual) recognition of a marketing authorisation. The alternative option is to mitigate the risk to an acceptable level by special precautions in the information that accompanies the product.

29. Precautions are not legally binding through the Directive 2001/82/EC on its own. In that sense, no precaution can be considered an effective risk reduction measure. National legislation concerning the veterinary practice must turn these recommendations into legal injunctions, in order to make the precautions work. This is realised under the Dutch legislation. Precautions can then be used to control the fate of the treated animal and the manure containing excreted residues, provided the legal person addressed is the keeper of the treated animals. The Dutch legislation at hand does not transfer precautions regarding the treated animals and the manure to third parties. The solution to these shortcomings is to include a transfer of responsibilities, either in the precautions themselves or in the national legislation, to second and third parties, and to prohibit both trade and use of the animals and manure in the precautions during the time that the precaution is operative.
30. Precautions are only acceptable under the Directive if their potential effect can be demonstrated using the risk assessment methodology. The flexibility of the risk assessment methodology to deal with temporal and spatial differentiation in exposure should be improved accordingly.
31. With respect to liabilities set by environmental legislation to the emission of residues to soil, water and groundwater, alternative solutions to the use of precautions in the product information may be found in establishing precautions in permits, or in codes of Good Agricultural Practices, issued in these legislative frameworks. Inevitably, the scientific and juridical underpinning of the precautions in these frameworks should be as meticulous as in the framework of registration, and will also require a flexible risk assessment methodology to quantify the impact of temporal and spatial differentiation of residue emissions.

Samenvatting

Diergeneesmiddelen en risico's voor het milieu

In de loop van de jaren zestig werd een verband gelegd tussen het gebruik van landbouwbestrijdingsmiddelen en de achteruitgang van de roofvogelstand. Ook waren er meldingen van massale sterfte van vogels ten gevolge van het gebruik van met bestrijdingsmiddelen behandeld zaad en lokmiddelen voor knaagdieren. De overheid reageerde hierop met een verbod op het gebruik van verschillende middelen, en met de invoering van een beoordeling van het risico voor het milieu bij de registratie van bestrijdingsmiddelen. Enkele incidenten met vogels en vissen waren echter te wijten aan het gebruik van diergeneesmiddelen.

Diergeneesmiddelen zijn middelen die in potentie ziekte bij dieren kunnen genezen of afwenden, of middelen die gebruikt kunnen worden om ziekten vast te stellen, of om lichaamsfuncties te beïnvloeden. Wanneer we ons beperken tot de chemische stoffen, dan kunnen we vaststellen dat we te maken hebben met een groep van stoffen die biologisch actief is. Duidelijk is dat een deel van de stoffen die gebruikt worden om parasieten te bestrijden, ook in gebruik is als bestrijdingsmiddel. Daarnaast blijken diverse families van chemische verbindingen zowel diergeneesmiddelen als bestrijdingsmiddelen te omvatten. Een recent voorbeeld betreft de dramatische achteruitgang van populaties van verschillende soorten gieren in Pakistan aan het einde van jaren negentig, waardoor deze met uitsterven bedreigd worden. Deze achteruitgang kan verklaard worden door het gebruik van een ontstekingsremmer in runderen. Dit diergeneesmiddel is bij de concentratie die bij zoogdieren therapeutisch is, al dodelijk voor deze aaseters.

De Europese Unie streeft een hoge kwaliteit van het milieu na, zoals verwoord in het Verdrag van Europa. Er is daarom voldoende reden voor het uitvoeren van een risicobeoordeling voor het milieu bij de registratie van diergeneesmiddelen. Risicobeoordeling voor het milieu is een wetenschappelijke activiteit, waarbij onderzocht wordt welke mogelijke schade een activiteit of een handeling aan het milieu kan toebrengen. Daarbij wordt informatie over eigenschappen en de wijze van gebruik van het geneesmiddel bijeengebracht, zodat een nieuw soort informatie ontstaat: een schatting van de kans op, en de ernst van, effecten. In de praktijk wordt gezocht naar het niveau van blootstelling, of concentratie, waarbij geen effecten meer optreden. In feite wordt dus een 'geen-effect' concentratie gezocht en een 'geen-risico' beoordeling uitgevoerd. Daarbij wordt de risicobeoordeling doorgaans zo uitgevoerd, dat in eerste instantie zoveel mogelijk onzekerheid en variatie wordt afgedekt. Op deze wijze kan men er zeker van zijn, dat een toepassing die leidt tot een blootsteling die lager is dan de concentratie van 'geen-effect', niet verder onderzocht hoeft te worden. Is hieraan niet voldaan, dan zal de risicobeoordeling met inachtneming van meer informatie over blootstelling en effecten moeten worden herhaald, totdat het risico aanvaardbaar wordt geacht.

Voordat diergeneesmiddelen verhandeld en gebruikt mogen worden in de Europese Unie, moeten ze geregistreerd worden. Bij de registratie worden kwaliteit, veiligheid en werkzaamheid van het middel nauwkeurig beoordeeld. Het risico voor het milieu is een van de onderdelen van deze beoordeling. Het milieu wordt beschouwd als een verzameling van compartimenten, zoals water, bodem, grondwater en lucht, en organismen, zoals planten, dieren, en bacteriën. Het milieu wordt op verschillende manieren blootgesteld aan diergeneesmiddelen en het kan op verschillende manieren op deze blootstelling reageren. Ten behoeve van deze risicobeoordeling is er een wetenschappelijke beoordelingsmethodiek voorhanden, die is vastgelegd in een aantal leidraden.

De vraag is nu voor welke milieucompartimenten, op welke wijze en met welke nauwkeurigheid, deze risicobeoordeling uitgevoerd moet worden. Dit onderzoek wordt validatie genoemd. Valideren van een methode of een model levert inzicht op in de betekenis van de risicovoorspellingen.

Ten eerste zijn daar de rekenkundige modellen waarmee blootstellingconcentraties worden berekend. Deze modellen kunnen beoordeeld worden op de wijze waarop zij geconstrueerd zijn, wat hun vermogen bepaalt om de werkelijkheid na te bootsen, of op de wijze waarop de relevante processen wiskundig benaderd worden, maar ook door proefondervindelijke resultaten te vergelijken met de voorspellingen. Indien modelvoorspellingen weinig nauwkeurig of beperkt relevant zijn, moeten de resultaten met grote voorzichtigheid gebruikt worden. Testen met organismen in het laboratorium zijn in feite ook modellen. Er kan slechts een beperkt aantal soorten getest worden; daarom dienen deze soorten alle andere organismen in het milieu te vertegenwoordigen. Daarnaast is men ingeval van het milieu niet zozeer bezorgd om de effecten op individuen, maar om de invloed op populaties en het functioneren van het grotere geheel van het ecosysteem. Deze samenhang is niet aanwezig in eenvoudige laboratoriumproeven, waarvan de uitkomsten gepaard gaan met grote onzekerheden.

Ten tweede is daar het proces van de uitvoering van de beoordeling en de daarbijbehorende afspraken over het gebruik van gegevens en interpretatiewijzen van de grote verzameling van modeluitkomsten. Aangezien er vele soorten van gebruik van diergeneesmiddelen zijn, bij vele soorten doeldieren, zijn er ook diverse routes van blootstelling en verspreiding. In samenhang met een verscheidenheid aan beschermdoelen (denk bijvoorbeeld aan de kwaliteit van oppervlaktewater, sediment, grondwater, drinkwater, en bodem, de bescherming van soorten en van gebieden) zal de methodologie van de risicobeoordeling bij registratie bestaan uit een verzameling beschermdoelen, normen, aannames, rekenregels en conventies. Dit noem ik het risicomodel; het model dat het risico van het gebruik van diergeneesmiddelen voorspelt. Dit model moet worden toegepast bij de registratie en de uitkomsten moeten worden begrepen. Deze interpretatie van het risico wordt vervolgens gebruikt bij de besluitvorming.

Bij de interpretatie van het risico komen de wereld van de wetenschap en die van het beleid bijeen. Op vragen als: ‘Wat moet beschermd worden?’ ‘Hoeveel risico is aanvaardbaar?’ en ‘Welke rol speelt de wetenschap bij de besluitvorming?’ kan de

risicobeoordeling geen antwoord geven. Het moge duidelijk zijn dat het antwoord op deze vragen aan de basis van de risicovoorspelling moet liggen, en niet omgekeerd. Het zou ideaal zijn als het antwoord in de samenspraak van wetenschap en beleid ontwikkeld wordt.

Zoals hierboven aangegeven, zijn er wetenschappelijke leidraden beschikbaar die de toepassing van het risicomodel ondersteunen en die in het registratieproces gehanteerd worden. De ontwikkeling van deze leidraden en het gebruik van de risicoinformatie, de toepasselijkheid van het risicomodel voor het milieu, de betekenis van de resultaten van de blootstellingmodellen en de rol van gebruiksvoorschriften op de bijsluiter van het diergeneesmiddel in de risicobeheersing, vormen het onderwerp van dit proefschrift. Meer concreet waren de vraagstellingen de volgende:

1) Over de overeenstemming tussen beschermdoelen en risicomodel.
   a) Welke beschermdoelen zouden beoordeeld moeten worden?
   b) Richt het risicomodel zich op deze beschermdoelen?
2) Over de validatie van de rekenmodellen en de gebruiksvoorschriften.
   a) Zijn de blootstellingmodellen voor water bij gebruik in de viskweek, voor mest bij gebruik in grazers, en voor bodem en water bij gebruik in de intensieve veehouderij, deugdelijk onderbouwd en toegepast?
   b) Is de drempelwaarde voor de bodemconcentratie, die gebaseerd is op effectgegevens, functioneel en deugdelijk onderbouwd?
   c) Kan de effectiviteit van gebruiksvoorschriften met het oog op risicobeheersing worden aangetoond met de beschikbare modellen?
3) Over het van gebruik wetenschap in het kader van de productregistratie.
   a) Wordt de wetenschap inzichtelijk en onpartijdig toegepast in de ontwikkeling van de risicobeoordelingsmethodologie, en in het besluitvormingsproces bij productregistratie?

Deze vragen zullen nu achtereenvolgens behandeld worden.

De overeenstemming tussen beschermdoelen en risicomodel

Het risicomodel dat gebruikt wordt bij de registratiebeoordeling bestaat uit een aantal fasen en stappen. De eerste fase is beperkt tot een verkenning van enkele belangrijke eigenschappen van het diergeneesmiddel en een berekening van de blootstelling. Hoewel in principe elke blootstelling een zekere schade kan opleveren, is een met wetenschappelijke redenen omklede drempelwaarde voor de concentratie in de bodem vastgesteld, waaronder een risicobeoordeling niet noodzakelijk wordt geacht. In het risicomodel is daardoor het milieu in eerste instantie beperkt tot het compartiment bodem.

Uit mijn analyse blijkt dat het risicomodel voor de risicobeoordeling niet gefundeerd is op gemeenschappelijke Europese beschermdoelen, maar dat de beschermdoelen geformuleerd zijn vanuit de overeenkomsten tussen de beschermdoelen in de Verenigde Staten, Japan en de Europese Unie. De bescherming van grondwater en oppervlaktewater weegt niet even zwaar in deze drie regio's. Het belang dat de EU hecht aan de bescherming

van grondwater en oppervlaktewater, zoals vastgelegd in Europese wetgeving, is onvoldoende onderkend in de voorbereidingen van de leidraden, en vervolgens ook niet in de uitvoeringspraktijk in Europa. Er ontstaat een probleem wanneer de beoordelingsprocedure voor de registratie wordt geharmoniseerd op een internationaal niveau, terwijl lokale of regionale autoriteiten verantwoordelijk zijn voor het bereiken en handhaven van de gewenste milieukwaliteit. De beoordeling bij registratie zal onvoldoende functioneren als een filter ten dienste van het milieukwaliteitsbeleid, en het bevoegde gezag zal ter plekke de emissie van diergeneesmiddelen moeten beperken middels vergunningen omdat de milieuwetgeving op het gebied van de waterkwaliteit dit vereist. Niettegenstaande het gegeven dat de registratiewetgeving geen milieuwetgeving is, moeten beschermdoelen, normen en methodologie tussen deze toelatings- en milieubeschermings-kaders in overeenstemming met elkaar zijn. Ik ben van mening dat het niet beoordelen van de blootstelling van grondwater in de eerste fase van het risicomodel een conceptuele fout in het risicomodel is. Immers, wanneer in de eerste fase de concentratie in de bodem beneden de drempelwaarde wordt berekend, stopt de risicobeoordeling. De blootstelling van grondwater en oppervlaktewater hebben geen rol gespeeld bij het vaststellen van de drempelwaarde in de bodem. De drempelwaarde is niet geharmoniseerd met de beschermingsniveaus voor water en grondwater. Een bodemconcentratie beneden de drempelwaarde kan dus risico opleveren voor het grond- en oppervlaktewater, die immers in contact staan met de bodem.

Op basis van de bescherming die Europese wetgeving biedt aan grondwater en oppervlaktewater, is het hanteren van dit risicomodel naar mijn mening onjuist, temeer omdat in deze wetgeving voor enkele stofgroepen, waartoe diergeneesmiddelen ook kunnen behoren, al normen opgenomen zijn. De Gezondheidsraad heeft de overheid geadviseerd geneesmiddelen op een vergelijkbare wijze als bestrijdingsmiddelen te behandelen, en volgens het Nederlandse beleid betekent dat, dat de concentratie in het grondwater niet hoger dan 0,1 microgram per liter mag zijn. Deze concentratie kan al bereikt worden indien enkele grammen per hectare op het land gebracht worden. Het gebruik van een geregistreerd diergeneesmiddel zou daarmee een aanvaardbaar risico voor de bodem kunnen hebben, maar desondanks in strijd zijn met de waterkwaliteitswetgeving.

Validatie van blootstellingmodellen en gebruiksvoorschriften

De beoordeling van de blootstelling start met het gebruik van het middel: hoe wordt het middel gebruikt, met welke frequentie, wat komt er vrij bij gebruik of na uitscheiding door de dieren? De emissie is het startpunt van de modellering van de verspreiding, de verdeling, de ophoping en de afbraak door en in de milieucompartimenten die met elkaar in verbinding staan.

De vraag die bij validatie gesteld wordt is in hoeverre het gehanteerde model deugdelijk en toepasselijk is. We hebben er immers mee te maken dat het onmogelijk is om alle processen die in de werkelijkheid plaatsvinden, in het model te vangen. Een model is per definitie een vereenvoudiging, en deze vereenvoudiging levert onzekerheid op over de nauwkeurigheid en de toepasselijkheid van de voorspellingen.

De modellen bestaan in feite uit rekenregels en variabele of constante grootheden. Een grootheid heeft een waarde met een eenheid (of dimensie) die gekozen moet worden uit de werkelijkheid die het model beschrijft. De waarden worden gekozen uit specifieke bereiken die typisch zijn voor de situatie die het model dient te beschrijven. Bij de keuze van de waarde hebben we dus te maken met een zekere variabiliteit. In plaats van een waarde te selecteren, kan een model met behulp van kansberekeningen gebruik maken van het volledige bereik van waarden. Het model levert dan een verdeling van uitkomsten op, die inzicht geeft in het spectrum van mogelijke gevolgen. Welke modelbenadering ook gevolgd wordt, het is belangrijk dat bij het gebruik van modeluitkomsten duidelijk is hoe valide deze zijn in het licht van het oorspronkelijke doel.

De uitdaging is om die waarde te kiezen, in samenhang met de keuze die voor andere waarden gemaakt moet worden, waardoor het model een voorspelling levert die het doel dient. De verzameling van waarden voor de landbouw- en milieukundige grootheden die samen een relevante modelsituatie vormen, noemen we een scenario. Afhankelijk van de onzekerheid in de waarden, de variabiliteit in de werkelijkheid en de complexiteit van de modellen, representeert de combinatie van een scenario en een model een deel van de werkelijkheid, en bevatten de uitkomsten een (on)zekere onnauwkeurigheid.

Voor de blootstellingbeoordeling van oppervlaktewater door viskwekerijen, van mest van grazers en van bodem en water door het uitrijden van gier met daarin resten van diergeneesmiddelen, heb ik een aantal modellen ontwikkeld en deze samen met bestaande modellen vergeleken en gevalideerd.

De kweek van vis in Nederland vindt voornamelijk plaats in bedrijven met kweekbakken. Van geneesmiddelen die toegevoegd worden aan het water of aan het voer, kunnen resten het milieu bereiken via het afvalwater. Concentraties in het milieu worden daarom bepaald door de concentratie in het afvalwater, het volume van het afvalwater, de verdunning door uitstroom in het ontvangende oppervlaktewater, en de aan- of afwezigheid van waterzuivering. De concentratie in het afvalwater hangt af van de dosering, de opname en uitscheiding van de stoffen door de vissen, de afbraak in de kweekbakken, de wijze waarop de kweekbakken ververst worden en de wijze waarop het afvalwater behandeld wordt. Afhankelijk van de eigenschappen van het geneesmiddel speelt de aanwezigheid van bezinkers, filters en rioolwaterzuivering een grote rol.

Een bezinker is bedoeld om organische resten en slibvlokken af te vangen, waardoor het water hergebruikt kan worden en het afvalwater minder belastend voor het milieu is. In het model is de retentie van opgeloste diergeneesmiddelen in de bezinker gebaseerd op een interpretatie van gegevens van bestrijdingsmiddelen die in de champignonkweek en de bloembollenteelt gebruikt worden. Bij nadere beschouwing van deze gegevens is gebleken dat de interpretatie dat de helft van de opgeloste bestrijdingsmiddelen in de bezinker achterbleef, onjuist was. Daarnaast is het bezinksel in de viskweek, dat voornamelijk uit organisch materiaal bestaat, niet vergelijkbaar met bezinksel uit de champignon- of bloembollenteelt, dat voornamelijk uit minerale delen bestaat.

Afhankelijk van de stofeigenschappen kunnen concentraties van een diergeneesmiddel in het oppervlaktewater met een factor 30 verlaagd worden door afvoer via een rioolwaterzuivering.

Tenslotte is de menging tussen afvalwater en ontvangend oppervlaktewater van belang. Indien er weinig stroming is, kan er weinig verdunning optreden. Meetgegevens bij een tropische viskwekerij tonen aan dat er sprake is van een verdunning met slechts een factor 2.

Deze gegevens in aanmerking nemend, heb ik moeten concluderen dat het door mij voorgestelde model niet verantwoord is. Als een eerste stap in de beoordeling moet de concentratie in het milieu gelijkgesteld worden aan de (therapeutische) concentratie in de kweekbakken. Voor een verdere beoordeling is het noodzakelijk dat het blootstellingmodel wordt gebaseerd op een representatieve procesbeschrijving.

Het tweede voorbeeld betreft residuen van diergeneesmiddelen in mest van grazende dieren. Dergelijke residuen kunnen grote invloed hebben op de mestfauna. Er zijn ongeveer 250 soorten geleedpotigen bekend die afhankelijk zijn van mest, als voedselbron of als verblijfplaats van prooidieren. Daarnaast is er een scala aan wormen, nematoden, schimmels en bacteriën aangewezen op mest, en diverse vogels en zoogdieren zijn deels aangewezen op de insecten die aangetrokken worden door en voortkomen uit de mest[37]. Voor de concentratie in de mest was in de eerste leidraad een drempelwaarde van 10 microgram per kilogram voorgesteld voor de verdere risicobeoordeling. De berekening van deze concentratie was daarvoor cruciaal. Bij een vergelijking met veldwaarnemingen bleek dat de voorgestelde rekenwijze veel te hoge concentraties gaf, waardoor deze in ieder geval beschermend was. Met behulp van een eenvoudig model waarin de uitscheiding als functie van de tijd berekend werd, kon ik voorspellingen doen van concentraties in de mest op gegeven momenten, die redelijk nauwkeurig overeenstemden met de meetgegevens.

Het derde voorbeeld betreft de verspreiding van diergeneesmiddelen met het inwerken van gier in de bodem. Deze emissieroute van de gier van de intensieve veehouderij via de bodem naar water is onmiskenbaar een belangrijke route, maar ook een ingewikkelde. Gezien de vele soorten doeldieren, mest, opslag, en bemestingsschema's, stel ik voor gebruik te maken van een model met gekozen waarden zonder volledige kansverdelingen, met scenario's toegesneden op verschillende doeldieren. De belangrijkste argumenten voor deze keuze zijn de inzichtelijkheid van deze scenario's, naast het gebrek aan beschikbare informatie om zinvol met kansverdelingen te rekenen.

Uit een vergelijking van de beschikbare modellen is gebleken dat de keuze van het aantal momenten dat gier uitgereden wordt een grote invloed heeft op de concentratie van het geneesmiddel in de bodem. Afhankelijk van de afbreekbaarheid van het diergeneesmiddel zijn verschillen in voorspelde concentraties in de bodem van een factor 40 te verwachten. Vergeleken met gemeten concentraties in mestkelders bleken de voorspellingen van het

[37] (Putman, 1983; De Bok, 1997; Jagers op Akkerhuis and Siepel, 2001; Anonymous, 2001; ARKive, 2001)

model van de leidraad niet toereikend. De modellen die ik heb voorgesteld slaagden hier beter in, maar niet in alle gevallen. De afspraken over de hoeveelheid mest die op het land gebracht wordt, blijken van groot belang te zijn. Als er veel tijd is tussen het moment van toedienen van het diergeneesmiddel en het moment van uitrijden, is het residu sterk verdund, of verregaand afgebroken. In de modellen mag de tijd tussen toediening en uitrijden daarom niet te groot gekozen worden. Ook is aan het licht gekomen dat de omstandigheden voor afbraak van een diergeneesmiddel in een opslagbekken een bijzonder grote variatie kennen. De verschillen in temperatuur, zuurgraad, en gehalte aan zuurstof, vocht en voedingsstoffen binnen opslagsystemen en tussen opslagsystemen zijn groot, en de invloed op de omzetting hiervan is onbekend. Het gevolg is dat het onduidelijk is hoe afbraak in de gier gemodelleerd moet worden, en welke situatie als realistische 'worst case' zou moeten worden aangemerkt.

Vergelijkingen tussen gemeten concentraties van een diergeneesmiddel in de bodem lieten zien, dat concentraties in de bodem met wel een factor 30 kunnen variëren. Deze variatie is niet te vermijden. De berekende concentraties gaan echter uit van een gelijkmatige verdeling, die in de helft van het areaal 1 tot 10 keer lager kan zijn dan de werkelijke concentratie. Door te rekenen met een gemiddelde concentratie kunnen risico's onderschat worden. Het risico ten gevolge van een hogere concentratie op de ene plek wordt immers niet gecompenseerd door de lagere concentratie op een andere plek.

Meetresultaten van concentraties diergeneesmiddelen in drainagewater en bodemwater, nadat deze met gier op het land gebracht waren, bleken niet voorspelbaar te zijn met de eenvoudige modellen die beschikbaar waren. Daarom heb ik voorgesteld gebruik te maken van het modelinstrumentarium voor de beoordeling van gewasbeschermingsmiddelen; niet alleen beschrijven deze de transportprocessen in groter detail, maar deze modellen beschikken ook over scenario's voor combinaties van bodemgesteldheid en klimaat, die relevant zijn voor stoffen die over akkers verspreid worden.

Ten behoeve van de beoordeling op Europees niveau, heb ik voorgesteld de blootstellingberekening voor de bodem na toepassing van diergeneesmiddelen bij gestalde dieren te beperken tot de verspreiding van een eenmalige dosis van het diergeneesmiddel, zonder afbraak, in de totale mestproductie van 30 dagen met een vastgesteld gehalte aan stikstof, over een bodemlaag van 5 centimeter landbouwgrond. Als 'worst case' aanname wordt een hoeveelheid gier gelijk aan 600 kilogram stikstof per hectare uitgereden. Indien de berekende concentratie in de bodem beneden de drempelwaarde is, dan is het gebruik van het diergeneesmiddel aanvaardbaar in alle EU landen. Is dat niet zo, dan wordt de berekening herhaald met een verdeling over een bodemlaag van 20 centimeter en een vracht van 170 kilogram stikstof per hectare. Indien de drempelwaarde nog steeds wordt overschreden, is een verdere risicobeoordeling noodzakelijk voor alle EU landen, zo niet, dan zal op het niveau van de lidstaat de beoordeling uitgevoerd moeten worden.

Opmerkelijk is dat het quotiënt van de jaarconsumptie van antibiotica (circa 400 ton) en het totale areaal aan landbouwgrond in Nederland, ongeveer dezelfde bodemconcentratie oplevert als het model van de leidraad. In Nederland wordt méér mest geproduceerd wordt dan er feitelijk plaatsingsruimte is op het land. In deze situatie, waarbij het voorkomen van diergeneesmiddelen in mest blijkbaar net zo algemeen is als het voorkomen van stikstof of

bacteriën, zou de risicobeoordeling voor de grotere watergangen op regionaal niveau gedaan kunnen worden. Het voordeel hiervan is dat gebruik gemaakt kan worden van modellen die de blootstelling van oppervlaktewater in stroomgebieden nabootsen.

Wanneer een risico voor het milieu is vastgesteld, staan er twee mogelijkheden open voor de besluitvormer: de toelating afwijzen, of maatregelen treffen die het risico verminderen tot een aanvaardbaar niveau. De laatste optie bestaat in de praktijk uit het opnemen van speciale aanwijzingen op de bijsluiter en verpakking van het middel. Diegene die het middel gebruikt is verplicht deze voorschriften te volgen, mits deze persoon daar redelijkerwijs toe in staat is. Voorschriften betreffende de behandelde dieren of verontreinigde mest missen hun uitwerking indien de veearts het middel toedient, omdat deze noch het lot van de dieren noch dat van de mest bepaalt. Daardoor blijft de veearts formeel buiten schot. Als de eigenaar van de dieren of de mest de middelen toedient, is deze wel gehouden aan de betreffende voorschriften. Als de eigenaar de dieren of de mest verkoopt, is de derde partij echter opnieuw tot niets verplicht. Zodra een van deze situaties aan de orde is, is het voorschrift geen effectieve maatregel, omdat in dat geval het milieu onverminderd blootgesteld wordt.

Een andere beperking van voorschriften op een bijsluiter betreft de verplichting dat alle informatie op de bijsluiter moet worden gestaafd door het dossier. Maatregelen waarvan het effect niet aangetoond kan worden door de modelberekeningen, zijn niet in overeenstemming met de bepalingen in de wet. Zo kan het effect van de mogelijke afbraak van een diergeneesmiddel in de mest door een verplichting de mest tijdelijk op te slaan, niet worden uitgerekend, omdat in de scenario's geen waarden voorhanden zijn voor de parameters die de afbraak controleren. Evenmin kan het effect van een verplichting de mest niet binnen een afstand van 10 meter van de slootkant uit te rijden niet aangetoond worden, omdat geen van de beschikbare modellen dit kan uitrekenen. Bij het opstellen van een risicobeoordeling en het voorstellen van voorschriften dient dus rekening gehouden te worden met deze haken en ogen, om te voorkomen dat oneigenlijke maatregelen opgenomen worden of dat technisch zinvolle maatregelen hun uitwerking missen.

De rol van de wetenschap in het proces van registratie

Wetenschap en beleid zijn met elkaar verbonden door een groot aantal geschreven en ongeschreven spelregels. Voor zover het de Europese registratie van diergeneesmiddelen betreft, zijn beleidsmatige, uitvoerende en wetenschappelijke verantwoordelijkheden gescheiden. De Europese Commissie, de Raad, en het Parlement zijn verantwoordelijk voor beleid en regelgeving. Het Europese Geneesmiddelenbureau behandelt de aanvraag en is daarmee verantwoordelijk voor de uitvoering. Het Comité voor geneesmiddelen voor diergeneeskundig gebruik levert de wetenschappelijke opinie. Ten behoeve van de beoordeling van het milieurisico heeft het Comité, zoals eerder opgemerkt, leidraden uitgevaardigd. Deze leidraden zijn cruciaal in het proces van registratie, omdat ze uitwerking geven aan de vereisten van de wetgeving en aan de beschermdoelen die niet benoemd zijn in

de wetgeving zelf. In dat verband is het opmerkelijk dat voor veevoederadditieven, voor bestrijdingsmiddelen, en voor biociden, waaronder stoffen vallen die ook als diergeneesmiddel gebruikt worden, de beschermdoelen benoemd en gekwantificeerd zijn in de wetgeving, onder supervisie van het beleid, en niet in leidraden. Volgens het Europees Sociaal Comité is vanuit beleidsmatig oogpunt voor het vaststellen van Europese beschermdoelen en methodologie een samenwerking van beleidsmakers, uitvoerders, wetenschappers en belanghebbenden gewenst. Vervolgens is evenwel, volgens het Europese Hof van Justitie, het opstellen van wetenschappelijke en technische beoordelingen waarop de rechtmatigheid van maatregelen steunt, voorbehouden aan de wetenschappelijke comités. Naar mijn mening vallen zowel de leidraden voor de milieubeoordelingen, als de beoordelingen zelf, onder de verantwoordelijkheid van de wetenschap. Het vaststellen van beschermdoelen is echter niet een zaak van wetenschappers alleen. De meest recente leidraad is opgesteld door een groep wetenschappers afkomstig uit overheden en industrie. Deze groep kan als een wetenschappelijk comité worden aangemerkt, maar kent geen vertegenwoordiging van alle betrokken partijen.

In de leidraad die door de wetenschappelijke werkgroep van de Veterinaire Internationale Conferentie voor de Harmonisatie (VICH) is opgesteld, is het risicomodel veranderd ten opzichte van de oudere leidraad van het Comité voor geneesmiddelen voor diergeneeskundig gebruik. De drempelwaarde voor de concentratie in de bodem ten gevolge van verspreiding van mest van gestalde dieren is bovendien verhoogd van 10 naar 100 microgram per kilogram bodem. Deze verandering was gemotiveerd met een wetenschappelijke beoordeling van beschikbare effectgegevens door de Amerikaanse branchevereniging van de industrie. Deze motivering heb ik gevalideerd. De gebruikte gegevens bleken afkomstig te zijn uit de registratieprocedure in de Verenigde Staten. Wanneer alleen deze gegevens van deze stoffen in beschouwing genomen worden, betekent dat, dat indien overschrijding van de drempelwaarde leidt tot het produceren en beoordelen van deze gegevens, de drempelwaarde inderdaad beschermend zal blijken voor deze stoffen. De beoordeling zal immers exact dezelfde informatie genereren. Voor andere stoffen, en voor andere risicomodellen, is deze wederkerigheid echter afwezig. De verzameling gegevens was zodanig beperkt, zowel voor wat betreft het aantal stoffen, als voor wat betreft het aantal soorten en effecten, dat hij niet kan dienen om het risico van nog onbekende stoffen af te wegen. De gegeven argumenten voor het afzien van veiligheidsfactoren (die dienen om de onzekerheid in effecten, en de vergelijkbaarheid met andere soorten, in acht te nemen) voldeden niet aan de leidraden die de Europese Commissie heeft uitgevaardigd. De argumenten stroken echter evenmin met de beoordelingswijze van de effectgegevens die uitgevoerd moet worden bij overschrijding van de drempelwaarde. Als gevolg hiervan kan de effectwaarde in de risicobeoordeling lager zijn dan de drempelwaarde. De drempelwaarde is daardoor zelfs niet valide voor de stoffen waarop de drempelwaarde gebaseerd is. Gebaseerd op deze gegevens, met behulp van gangbare methodieken voor effectbeoordeling, voorspel ik dat 80% van de diergeneesmiddelen bij de drempelwaarde schade kunnen veroorzaken aan de microbiële levensgemeenschap in de bodem. Wanneer overige vrij beschikbare informatie in ogenschouw wordt genomen kan worden beredeneerd dat de drempelwaarde niet op 100,

maar op 0,1 microgram per kilogram vastgesteld moet worden. Zou nog in aanmerking worden genomen, dat de concentratie in de bodem nooit mag leiden tot een overschrijding van een drempelwaarde in oppervlaktewater, dan zou de drempelwaarde voor bodem met nog een factor 8 moeten dalen. Deze enorme variatie in uitkomsten is zorgelijk, aangezien het de indruk wekt dat wetenschap alle gewenste antwoorden kan formuleren.

Tenslotte

Ik heb diverse technische en principiële tekortkomingen in het gebruik de risicobeoordeling in het proces van registratie aangeroerd. Als belangrijkste merk ik op dat het vastleggen van beschermdoelen in de leidraad niet bindend is. Dat is in deze situatie wellicht een voordeel, aangezien de meest recente leidraad niet op alle Europese milieucriteria aanstuurt (in het bijzonder ontbreken grondwater en water), en de risicobeoordeling afhangt van een onvoldoende onderbouwde drempelwaarde voor de bodem. De modellen die in eerste instantie gebruikt worden om de blootstelling van het milieu in te schatten blijken niet altijd beschermend te zijn tengevolge van het ontbreken van gestandaardiseerde scenario's voor doeldieren en mest. Sommige gebruiksvoorschriften wekken ten onrechte de indruk dat het milieurisico tot een aanvaardbaar niveau teruggebracht wordt, terwijl daar geen wettelijke basis voor aanwezig is. Als gevolg van deze tekortkomingen kunnen milieugevaarlijke diergeneesmiddelen geregistreerd worden, waardoor de regionale of lokale overheid, die verantwoordelijk is voor de plaatselijke milieukwaliteit, gedwongen is het gebruik van diergeneesmiddelen, het uitrijden van mest, of het weiden van vee, middels vergunningen te reguleren. Dit zal in de praktijk een uitermate omvangrijke administratieve last betekenen.

Over de wijze waarop de ontwikkeling van resistentie tegen antibiotica betrokken moet worden in de beoordeling is nog onvoldoende kennis ontwikkeld.

De beschreven tekortkomingen ondermijnen in meer of mindere mate de rechtmatigheid van het registratieproces en van de wetenschap. De recente Richtlijn 2004/28/EC, die voor 30 oktober 2005 in nationale wetgeving omgezet moet worden, gaat voor de beoordeling van een registratie uit van de verhouding voordelen/risico's. Met relatief weinig inspanning zouden gemeenschappelijke besluitvormingscriteria vastgesteld kunnen worden, waartoe in dit proefschrift een eerste aanzet gegeven is. Het belangrijke werk van wetenschappers moet gefundeerd worden op heldere besluiten van beleidsmakers, en verankerd in een uniform en inzichtelijk besluitvormingsysteem. Een verdergaande uitwerking van scenario's betreffende de Europese landbouw- en milieukundige omstandigheden, van beleidsmatige en wettelijke beperkingen, en van een integraal risicomodel voor deze kosten/baten analyse, is daarvoor noodzakelijk.

Curriculum Vitae

Mark Henricus Maria Marcellinus Montforts werd op 8 november 1967 in Geleen geboren. In 1986 behaalde hij zijn gymnasium diploma aan het St. Michiel te Geleen en startte met zijn studie Biologie aan de Rijksuniversiteit Utrecht. In 1991 rondde hij de kopstudie Milieukunde af aan de Interfacultaire Vakgroep Milieukunde in Utrecht, gevolgd in 1992 door een doctoraal in de Biologie bij de vakgroep Natuurwetenschap en Samenleving.

Van 1991 tot 1993 was hij (vrijwillig of betaald) werkzaam bij de Wetenschapswinkel Biologie. In 1992-1993 werkte hij als toegevoegd docent bij de vakgroep Natuurwetenschap en Samenleving. Vanaf 1993 is hij werkzaam bij het Rijksinstituut voor Volksgezondheid en Milieu (RIVM). In 1999 werd hij opgenomen in het register van toxicologen van de Nederlandse Vereniging voor Toxicologie. In de loop der jaren hebben zijn werkzaamheden zich verplaatst van het samenvatten van studierapporten naar het beheren van projecten, het ontwikkelen en valideren van risicobeoordelingsmethodologie, en het formuleren van wetenschappelijke adviezen voor normstelling en beleid op het gebied van de milieurisicobeoordeling van gewasbeschermingsmiddelen, biociden, diergeneesmiddelen en geneesmiddelen, in nationale en internationale kaders. Vanuit deze context en met ondersteuning van het RIVM is dit promotieonderzoek uitgevoerd.

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