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Environmental Assessment of Veterinary Medicinal Products in Denmark

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Content

Preface

The Danish Environmental Protection Agency and the Danish Medicines Agency have in-co-operation initiated the work described in this report due to spare available information on consumption, release, fate and effects of the veterinary medical product.

Members of the Steering Group

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- Tim Nis Corell, Plantedirektoratet
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- Per Spindler, The Danish Medicines Agency
- Henrik Tyle, The Danish Environmental Protection Agency
- Linda Bagge, The Danish Environmental Protection Agency

The authors terminated the editorial work in February 1998 and the conclusions were discussed at a Steering Group Meeting in March 1998. Due to delay of publishing the report, it was necessary to update the information of the consumption of the veterinary medical products.

The report was circulated to the members of the steering group and during this process the Danish Medicines Agency notified that they could not approve the conclusions in the report in spite of former agreements.

June 2000 an agreement was reached.

Summary

A Technical Guidance Document, EMEA/CVMP/055/96 outlines a detailed evaluation procedure to assess the environmental risk of new veterinary medical products used in husbandry. Despite a relative high usage of some veterinary medical product very little information on consumption, release, fate and effects of this group of substances is generally available. Therefore, the Danish Environmental Protection Agency and the Danish Medicines Agency in co-operation have initiated the work described in this report.

The main objectives of the report have been:

- to quantify the *use* of veterinary medicinal products in Denmark,
- to estimate the *environmental release* of active substances and residues,
- to identify groups of veterinary medicinal products that may pose a *risk to the environment*, and
- to discuss the *EU Technical Guidance Document* on risk assessment of veterinary medical products in relation to existing EU Technical Guidance Document on risk assessment of new and existing substances.

This report focuses primarily on the following therapeutic groups: antibiotics, antiparasitic compounds, and substances used for treatment of the alimentary tract, the central nerve system (CNS) and problems with metabolisms. These compounds are all used in significant quantities. Furthermore, hormones are included as they are a group of substances with a very high biological activity.

The use of antibiotics used therapeutically was 48.5 tons in 1997. Another 1.7 tons of antibiotics were used as feed administrated drugs in 1996 (no data available for 1997). Other therapeutically uses of veterinary medicinal products (VMP) included in 1997 14 tonnes of active substances for the alimentary tract and metabolism , 236 kg of antiparasitic compounds, 230 kg of for CNS-active products and 29 kg of active hormones.

In 1997 more than 105 tons of antibiotics were used non-therapeutically as antimicrobial growth promoters. The total quantity of antibiotics used thus exceeds 170 tons (including coccidiostatics). However, the data for the first six months of 1998, where only 28.9 tons were used, indicate a significant reduction in the annual use of antibiotics as feed additives in Denmark. No information is available on another widely used group of drugs, the antiparasitic compounds, as these are sold outside pharmaceutics without prescription.

The environmental release of a number of substances is estimated in realistic worst case scenarios. The main route of environmental exposure for all drugs is via manure or slurry. On the basis of the information on recommended doses of veterinary medical products and the normal application of manure in Denmark, Predicted Environmental Concentrations (PECs) was estimated. For antibiotics PECs were found in the range of 0.2 to 9 mg / kg soil depending on compound and application. For hormones PECs were found between 0.01 and 0.05 µg/kg soil and for substances used in connection with the alimentary tract PECs between 0.04 and 5.7 mg / kg soil were estimated. For substances used for CNS disorders in cows, PECs between 0.1 and 28 mg / kg soil were found. PECs are generally somewhat higher when soil is amended with cow manure than with pig manure, as the higher nitrogen

content in pig manure prescribes a lower load of manure according to the legislation that regulates the maximum load of nitrogen per hectare. In cases of direct deposition on the soil with no subsequent tilling, the concentration may locally be higher.

These results are all worst case scenarios, as no degradation during storage is incorporated in the calculations. Half-lives for antibiotics varies from a few days to years depending of the conditions, but information about the fate of these compounds during storage in the manure tank and in the soil is limited.

Very little is known about the ecotoxicological effects of most veterinary medicines. Best information is available for antibiotics and antiparasitic compounds. Even for these substances, it is not possible to conclude or exclude whether serious unwanted side-effects in the environment may occur as a result of normal use. The information is also too sparse to conclude whether specific types of substances are more harmful than others. However, it is generally assumed that the broad spectrum antibiotics and antiparasitic drugs may affect a broader range of organisms than more specific acting drugs.

It is not possible to conclude from the data available whether the current normal veterinary use of hormones or substances used for diseases associated to the CNS or the metabolism may have effects on the environment.

The risk assessment procedure for new and existing substances (chemicals) have briefly been reviewed and compared with the risk assessment of veterinary medicinal substances (drugs). The comparison reveals a number of differences in the evaluation procedure. Environmental concentrations of veterinary medicinal substances below certain cut-off values, e.g. 10 µg/kg in dung or soil, do not warrant an environmental risk assessment. The uncertainty of the predicted environmental concentration for veterinary medicinal products is significantly less than that of certain uses of existing chemicals. Furthermore in the calculation of the Predicted No Effect Concentrations (PNEC-values) for drugs, the recommended use of application (safety) factors on comparable data is often 10-100 times lower than for industrial chemicals. Finally, there is not, as in the case of existing chemicals, a national or international strategy of how and when to assess already existing VMP. On the basis of national priority list among the approximately 100,000 existing chemicals in the EU database EINECS and priority indicated by a European priority method (EURAM) a comprehensive risk assessment is produced by competent authorities in the European member states.

Dansk resumé

Én nylig udkommet Teknisk vejledning, EMEA/CVMP/055/96, beskriver i detaljer proceduren for at vurdere den miljømæssige risiko ved nye veterinærmedicinske produkter (VMP). På trods af et formodet relativ højt forbrug af visse VMP er kendskabet til forbrug, udslip, skæbne og effekter af denne gruppe af kemiske stoffer begrænset. Derfor har Miljøstyrelsen og Lægemiddelstyrelsen i samarbejde taget initiativ til denne rapport.

Hovedformålene med rapporten har været:

- at kvantificere forbruget af veterinærmedicin i Danmark
- at estimere udslippet af de aktive stoffer og deres metabolitter til miljøet
- at identificere grupper af veterinær medicinske produkter, som kan udgøre en risiko for miljøet
- at diskutere EU's Tekniske Vejledning for risikovurdering af VMP i relation til den eksisterende vejledning i EU for nye og eksisterende kemikalier, ofte kaldet industrikemikalier.

I denne rapport fokuseres primært på følgende terapeutiske grupper af veterinærmedicin: antibiotika, antiparasitære midler samt produkter, som anvendes i forbindelse med sygdomme relateret til fordøjelsessystemet, det centrale nervesystem (CNS) og til metabolismen. Produkter til bekæmpelse af disse sygdomme anvendes alle i relative store mængder. Derudover er hormoner medtaget i denne rapport, da de udgør en gruppe af stoffer med en meget høj biologisk aktivitet.

Til sygdomsbekæmpelse anvendes årligt (1997) 48,5 tons antibiotika, 14 tons medicin til fordøjelse- og metabolismeproblemer, 236 kg antiparasitære midler, 230 CNS-aktive produkter og 29 kg hormon-aktive produkter. Som medicintilskud i foder blev der i 1996 (ingen data fra 1997) anvendt 1,7 tons antibiotika.

I 1997 var forbruget af antibiotiske vækstfremmere mere end 105 tons. Det totale forbrug af antibiotika, inklusiv coccodiostatics, oversteg defor samlet 170 tons. Forbruget i det første halvår af 1998, 28,9 tons, peger dog på et kraftigt fald i anvendelse af antibakterielle vækstfremmere i Danmark. Syv antibiotiske vækstfremmere er nu forbudt i EU samtidig med landbrugsorganisationerne i Danmark har indgået en frivillig aftale med Fødevareministeren om at undlade at bruge antibiotiske vækstfremmere i produktionen af kyllinger, svin og kalve i fra fødsel til slagtning. Antiparasitære lægemidler, der tidligere kunne købes uden recept, er nu receptpligtige, hvorfor det må forventes at forbruget falder.

Udslippet af en række stoffer til miljøet er estimeret i såkaldte "worst case" scenarier. Den primære eksponeringsvej for alle stoffer er via husdyrgødning. De teoretiske koncentrationer i miljøet (PEC = Predicted Environmental Concentration) er udregnet på basis af informationer om den anbefalede veterinære dosis af stofferne og udbringning af husdyrgødning i henhold til den normale landbrugspraksis i Danmark. For anti-

biotika anvendt til sygdomsbekæmpelse er PEC udregnet til at ligge i størrelsesordenen 0,2-9 mg kg^{-1} afhængigt af stof og applikationsform. PEC i intervallerne 0,04-5,7 mg kg^{-1},0,1-28 mg kg^{-1} og 0,01-0,05 µg kg^{-1} er beregnet for henholdsvis fordøjelsesaktive stoffer, CNS-aktive stoffer og hormoner. I tilfælde af direkte udledning af ekskrementer på jorden kan værdierne lokalt være højere. PEC-værdierne er højere i jord, som har modtaget kvægmøg/gylle end i jord, der har modtaget svinemøg/gylle, da det højere kvælstofindhold i svinegylle iflg. lovgivningen foreskriver en lavere dosering.

Resultaterne bygger alle på såkaldte "worst case" scenarier, hvor bl.a. nedbrydningen af stofferne under opbevaring er sat til nul. Halveringstider for antibiotika varierer fra få dage til år og afhænger af stoffet og de fysisk/kemiske forhold. Kun meget begrænset viden om skæbnen af veterinære lægemidler i gylletankene og i jorden er tilgængelig.

De økotoksikologiske effekter af de fleste veterinære lægemidler er ukendte. Størst viden findes for antibiotika og de antiparasitære midler. Selv for disse stofgrupper er det ikke muligt at konkludere, hvorvidt uønskede effekter i miljøet kan opstå som følge af normal anvendelse. Datagrundlaget er også for sparsomt til at vurdere, hvorvidt én type af antibiotika er mindre skadelig end andre. Det formodes dog generelt, at de bredspektrede antibiotika og de antiparasitære midler, i modsætning til meget specifik virkende stoffer, kan påvirke en større vifte af organismer. Der kan ikke drages nogen konklusioner om miljøeffekterne af hormoner, CNS-aktive stoffer eller stoffer til behandling af metabolismen.

Risikovurderingen af veterinære medicinale produkter (VMP) er kort gennemgået og sammenlignet med den eksisterende risikovurdering i EU af nye og eksisterende stoffer, ofte kaldet industrikemikalier. Sammenligningen viser en række forskelle. Den vigtigste er måske, at VMP med en beregnet PEC under 10 µg kg^{-1} ikke nødvendigvis skal undergå en egentlig risikovurdering. En anden forskel er at usikkerheden for PEC beregningerne er væsentlig mindre for VMP end for visse typer af eksisterende stoffer.Desuden er den anbefalede brug af sikkerhedsfaktorer typisk en faktor 10-100 under de faktorer, som anvendes for industrikemikalier på det samme datagrundlag. Endelig findes der, i modsætning til allerede eksisterende industrikemikalier, ikke nationalt eller internationalt nogen strategi for, hvordan og hvornår allerede eksisterende VMP skal risikovurderes. For eksisterende stoffer har EU igangsat en proces hvor kompetente autoriteter i de enkelte medlemsstater udfærdige omfangsrige riskovurderingsdokumenter for stoffer, som de har udvalgt på baggrund af nationale prioriteringslister og prioritering indikeret af den europæiske prioriteringssystem EURAM.

1 Introduction

Veterinary medicinal products (VMP) are used in order to increase the health and well-fare of our animals. Veterinary medicinal products are authorised for use by regulatory authorities if they comply with scientific criteria on quality, efficacy, and safety. The authorities consider occupational health during production and handling and safety to the treated animal, to the consumer and to the environment. The environmental risk of veterinary medicinal products has recently become a matter of increasing public scrutiny and legal requirements. This report is focusing on the potential environmental risk of veterinary medicines. Other aspects of the use of veterinary medicinal products have been dealt with in different reports from the Danish authorities, e.g. the risk of increasing resistance of bacteria to antibiotics (Miljøstyrelsen 1997).

The environmental risk of veterinary medicines is assessed according to different regulations depending on whether the application is therapeutic or non-therapeutic. The legislation in the European Union (EU) on the environmental risk assessment of veterinary medicines is contained in Commission Directive 92/18/EEC. This directive outlines the basic requirements for conducting an environmental risk assessment of veterinary medical products. A detailed guidance on environmental risk assessment is given in a Technical Guidance Document, EMEA/CVMP/055/96. The procedure for environmental risk assessment of VMP only concerns products sold after the 1st of January 1998. There is currently no European initiative to assess environmental risk of already marketed veterinary medicinal products.

Directive No. 94/40/EEC lays down a guidance for assessing the effects of food additives (frequently used as medicinal products as well, e.g. antibiotics) to animals. This guidance is currently being revised by the Commission.

Residues of veterinary medicinal products may be deposited on arable land or pastures as a constituent of manure and slurry. The use of manure/slurry as fertilisers on arable land may thus involve a risk for the environment. On the other hand the use of manure/slurry is desirable to recycle nutrients and hence an important part of the concept of a self-sustainable agriculture.

The main objective of the report is to :

1) quantify the *use* of veterinary medicines in Denmark,
2) estimate the *environmental release* of active substances and residues,
3) identify groups of veterinary medicines that may pose a *risk to the environment*,
4) identify groups of veterinary medicines that are unlikely to cause environmental risk, and to

5) discuss the *EU Technical Guidance Document* on risk assessment of veterinary medicinal products (EMEA/CVMP/055/96) in relation to the existing EU Technical Guidance Document on risk assessment of new and existing substances.

Delimitation

A few hundred papers concerning the environmental fate and impact of veterinary medicines were found in databases such as Medline, Biological Abstract, Analytical Abstract, Agricultural Abstract and Toxline. Conclusions and recommendations of this report are based on these studies and data of

consumption of veterinary medicinal products in Denmark. No conclusions in the report have been related to specific products. Veterinary medicines used only for pets and horses are not considered in this report.

1.1 Grouping of veterinary medicinal products in Denmark

ATC and CAS code system

All veterinary medicines are internationally registered under ATC codes. The ATC code system is similar to the CAS code system. However, antimicrobial growth promoters are only registered under CAS codes as they are not considered to be medicines. *Appendix A* gives a list of the ATC and CAS codes for substances found in this report. *Box 1.1* gives examples on some of the definitions and terms used in this report.

Veterinary medicines may be divided into therapeutic groups as follows. Compounds used for diseases associated with the alimentary tract and metabolism (group QA), the cardiovascular system (QC). Compounds used as dermatological products (QD), as sex hormones (QG) or as systemic hormones (QH). Compounds for anti-inflammatory systemic uses (QJ01+QJ51). Compounds used for diseases associated with the musculo-skeletal system (QM) or the central nerveous system (QN). Compounds used as antiparasitics (QP) and a group compounds with miscellaneous uses (QV). The group codes refers to the ATC code system and has been used for analysing the consumption of veterinary medicines in Denmark (Veterinærmedicinsk Produktkatalog, 1997).

Labelling and dispensation of veterinary medicinal products (VMP)

Veterinary medicinal products have to be labelled with instructions concerning application, storage etc. in accordance with the laws. Veterinary Medicinal Products are dispensed as follows

A: May only be dispensed once with a prescription.
B: May only be dispensed once with a prescription unless number and intervals of dosing are indicated the prescription.
H: May be dispensed without prescription/requisition.
V: May be sold by other suppliers than pharmacies.

Following a special regulation dated the 15. December 1975, products sold as V-labelled medicine includes antiparasitic agents, iron and vitamins

VMP applied as feed additives are divided into three groups;

- A: Antibiotics (pigs, ruminants and poultry)
- D: Coccidiostatics and related drugs (poultry)
- J: Growth promoting substances (pigs).

The following products are used within each group :

A: Zinc Bacitrin, spiramycin, flavofosfolipol, tylosinfosfat, monensin natrium, salinomycin natrium and avilamycin.
D: Amprolium, amprolium-etophat, dinitolmid, dimetridazol, metik lorpindol, decoquinat, monensin natrium, robenidin, ronidazol, ipronidazol, metiklorpindol metylbenzoat, arprinocid, lasalocid natrium, halofuginon, narasin, salinomycin natrium, nicarbazin, nifursol, maduramincinammonium, and diclaruzil.
J: Carbadox and olaquindox

Premix

The sale of mixtures as feed administrated drugs, the so-called premix, may cause some minor inaccuracy in the data compilation. Double counting may occasionally have occurred between the data on antibiotics used therapeutically (*Table C.3*) and antibiotics used as feed administered medicine (*Table C.8*). As an example, according to the data from the DMA more than 600 kg of the tylosin containing premix "Tylan®Vet. 2%" was sold in 1996. Although the mixture "Tylan®Vet. 2%" is a medicinal feed additive, only 0.6 kg was registered for this used by the Plant Directorate in 1996. The same is true for other "premix" products.

Box 1.1. Definitions of terms as used in this report

Veterinary medicinal products
Veterinary medicinal products are defined as substances applied to animals to avoid, relieve, treat or cure diseases or symptoms or to stimulate functions of the organisms.

The use of veterinary medicinal products may be divided into the following categories:
- therapeutic use (treatment of disease)
- non-therapeutic use (to prevent diseases and/or to enhance growth).

Feed administrated drugs
Feed administrated drugs are defined as medicines. These may only be dispensed following prescription from a veterinarian. Feed administrated drugs are pre-mixtures of drugs mixed with one or more feeds. Feed administrated drugs are applied to pigs, cattle and fish. The following antibiotics are used in pre-mixtures registered in Denmark; chlortetracycline, enrofloxacin, oxilic acid, lincomycin, and sulfadiazin in combination with trimetroprim, and tylosin. The annual consumption of feed administrated drugs is registered by the Danish Plant Directorate.

Antimicrobial growth promoters
Antimicrobial growth promoters are defined as feed additives. The sale of Antimicrobial growth promoters is regulated by the Danish Plant Directorate. These substances may only be used as additives to the feed, if they have a beneficial effect, either on the animal performance or on the feed conversion ratio. The substances are used non-therapeutically only. The active compounds may, however, sometimes be uses as veterinary medicines also.

1.2 Danish legislation on veterinary medicinal products

Veterinary Medicinal Products used in Danish husbandry may be authorised for use according to laws and regulations issued by the Ministry of Health and the Ministry of Food, Agriculture and Fisheries, e.g. ‘Lægemiddelloven’, ‘Bekendtgørelse om lægemidler til veterinær brug’, ‘Bekendtgørelser om foderlægemidler til dyr og fisk’, ‘Bekendtgørelse om tilsætningsstoffer’. The regulations may overlap, depending on the application of the product as the identical active substance may be used both therapeutically and non-therapeutically, i.e. as a veterinary medicine and as growth promoter. The annual use of the identical active substance may therefore be recorded by different authorities. Due to a complex regulation a short overview of regulatory issues of particular interest to this report is given in *Appendix B*.

1.3 The structure of the report

The *first chapter* includes besides a short general *introduction* to the background and main objectives of the report a definitions of terms and a summary of the Danish regulation of veterinary medicines. In *chapter 2* a quantification, on single compound levels, of *the use* of veterinary medicines (A and B labelled), antimicrobial growth promoters, and feed administrated

drugs are presented. An overview is given on the anticipated environmental release and exposure routes of veterinary medicines.
Chapter 3 describes the *occurrence and fate* of veterinary medicinal substances in the environment, and includes calculations of the predicted environmental concentration of a few selected veterinary medicines. Fertilisation of arable land and pastures with manure/slurry is a major source to the environmental release of veterinary medicines. Therefore, a brief description of the relevant Danish legislation regulating the load of manure/slurry on agricultural soil is included in chapter 3. *Chapter 4* describes existing information dealing with *effects* of veterinary medicinal substances on organisms from aquatic and terrestrial environments. *Chapter 5* discusses the *Technical Guidance Gocument* on environmental risk assessment of veterinary medicinal substance in relation to the technical guidance document used for environmental risk assessment of industrial chemicals in the EU. At the end of each chapter a summary is given and conclusions drawn. Finally, *in chapter 6 and 7* the overall *conclusions and recommendations* are presented.

2 Use, consumption and environmental release of veterinary medicinal products in Denmark

Veterinary medicinal products sold in Denmark are authorised by the Danish Medicines Agency or the Danish Plant Directorate. The Danish Plant Directorate annually publish data on the consumption of antimicrobial growth promoters and feed administrated drugs. This report summarises both the data compiled by the Danish Medicines Agency and the Danish Plant Directorate .

2.1 Quantification of the use of single substances

This report focus primarily on the following therapeutic groups as they are used to a significant extent in the agricultural livestock production and hence are submitted for a potential environmental release;

- alimentary tract and metabolism (group QA),
- antibiotics (group QJ01 and QJ51),
- central nervous system (group QN)
- antiparasitic agents (group QP).

Furthermore,

- hormones (group QG and QH)

are included in the evaluation, as this group of substances have a high and specific biological activity and hence are potential hazardous if released to the environment.

Antimicrobial growth promoters and feed administrated drugs are also included in this report. The following therapeutically groups of medicines are not considered; Cardiovascular system (group QC), Dermatological products (group QD), Musculo-skeletal system (group QM), Miscellaneous (group QV), as their environmental release most likely is small.

Data from 1996 and 1997 concerning the amount of Veterinary Medicinal Products prescribed (A and B labelled) for therapeutic use were obtained from the Danish Medicines Agency. The estimate of the amount of active substance used in Denmark in 1996 and 1997 is based on amount of active substance sold as calculated from the number of recorded A- or B-labelled prescriptions.

The sale of V- and H-labelled products, e.g. ivermectin and hydrocortisone, has not been recorded, therefore it has not been possible to obtain information on the sale of these products. Data for single substances of feed administrated drugs, i.e. antimicrobial growth promoters, coccidiostatics, and antibiotics were obtained from the Danish Plant Directorate and used without further processing. The data for the selected therapeutic groups, for feed administrated drugs, coccidiostatics, and antimicrobial growth promoters are briefly reviewed below and presented in more detail in *Appendix C* (*Table*

C.1 to C.8).*Table C 9* in *Appendix C* shows an example of the calculation applied to the data received from the Danish Medicines Agency.

2.1.1 Drugs for the alimentary tract and metabolism (group QA), Table C.1

The total consumption of this group of drugs was in 1997 approximately 14 tons. Neomycin, an antibiotic, accounts for 3.9 tons and the majority of the remaining use is made up by Boric acid, calciumglyconat and magnesium-hypophosphit. All other substances are used in quantities less 1 tons.

2.1.2 Hormones (group QG and QH), Table C.2

Hormones are generally divided into two therapeutic groups; sex hormones (QG) and systemic hormones (QH). A total of ca. 30 kg of active hormones, divided on twelve different substances, were used in 1997. In the EU, as opposed to the US, hormones may not be used as growth promoters. This most likely explains the relatively small amount used in Denmark.

2.1.3 Antibiotics (group QJ01 and QJ51) , Table C.3

In 1997 a total of 48.5 tons of active substance was used therapeutically divided on more than 30 different antibiotics. Some pre-mix substances may also be used as feed administrated drugs. Hence, some data may be registered both by the Danish Medicines Agency and the Danish Plant Directorate. Double counting may therefore occur. Several compounds are used in quantities exceeding 1.0 tons, e.g. benzylpenicillin, lincomycin, amoxillin, ampicillin and the tetracyclines. Many antibiotics are used in quantities less than 100 kg. The use of some of the antibiotics, e.g. benzylpenicillin and spiramycin, were found by translating IE units to mg by use of conversion units found in Martindale (1996).

2.1.4 Drugs for the Central Nervous System (CNS) (group QN), Table C.4

The group consists of 14 substances all used in small amounts. Totally 230 kg active substance was used in 1997, dominated by the use of metamizol-natrium (177 kg).

2.1.5 Antiparasitic agents (group QP), Table C.5

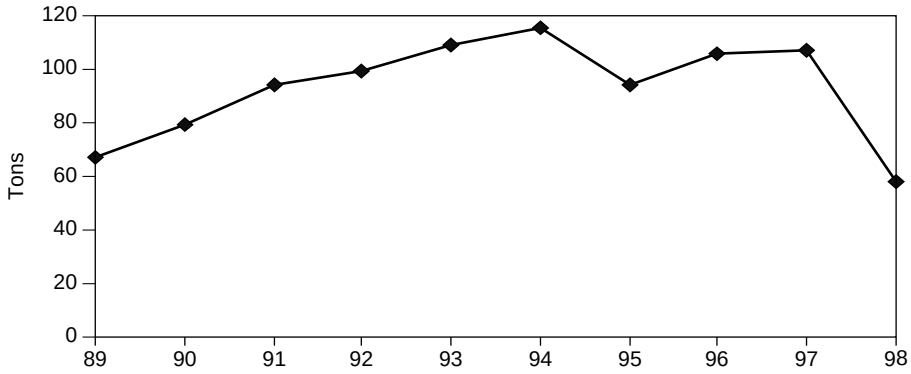
In 1997, an prescription of 236 kg of sulfaclozine was the only registered use of antiparasitic drugs. Antiparasitic drugs sold without prescription, e.g. ivermectin, are presumably used in relatively large amounts.

2.1.6 Antimicrobial growth promoters (non-therapeutic use), Table C.6

The use of growth promoters in Denmark from 1989-1998 is presented in *Figure 2.1*. Growth promoters are primarily used for production of pigs. The consumption of antimicrobial growth promoters according to animal species is presented in *Table C.9*. The use of microbial growth promoters has increased from about 65 tons in 1989 to around 120 tons in 1997. The data for the first six months of 1998, where only 28.9 tons were used, indicate a significant reduction in the annual use of antibiotics as feed additives in Denmark. Avoparcin was previous the most used antibiotic in Denmark. Due to the risk of inducing cross-resistance against antibiotics essential for treatment of serious human infectious diseases, the use of avoparcin was banned in 1995. In the following years, virginiamycin, tylosine, bacitracin, spiramycin, carbadox and olaquindox were banned as growth promoters in the EU. A voluntary agreement between the farmer organisations and the Ministry of Agriculture banned the use of antiobiotic growth promotors in the production of broilers, pigs and calves.

Figure 2.1.Annual use in tons of antimicrobial growth promoters during the period 1989-1998. (Data for 1998 is calculated on the basis of the use during the first six months). Source: Danish Plant Directorate.

Figur 2.1. Forbruget af antimikrobielle vækstfremmeer (tons pr. år) i perioden 1989 til 1998. (Tal for 1998 er baseret på de første seks måneders forbrug) Kilde: Plantedirektoratet.



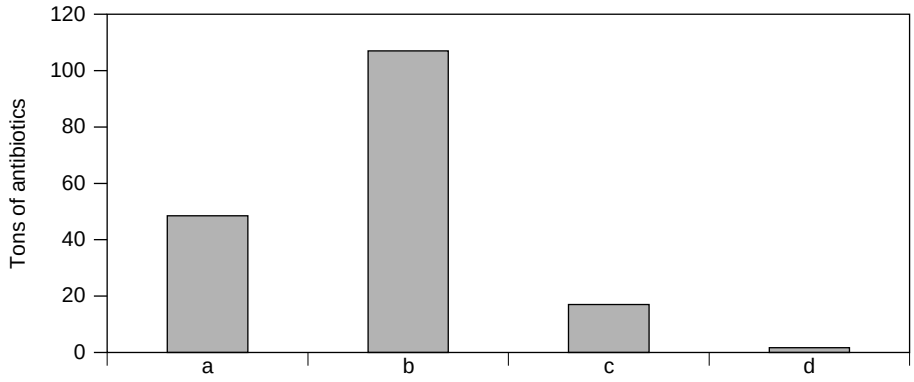
2.1.7 **Coccidiostatics used in poultry production, Table C.7**
The report includes data from the period 1989-1998. Approximately 17 tons coccidiostatics were used in 1997 and an estimated 16 tons have been used during 1998. In 1997 12 different substances have been used with Salomycin and Metichlorpindo/Methylbenzoquat taking up half of the total consumption.

2.1.8 **Veterinary medicine used as feed administrated drugs, Table C.8.**
The total use of feed administrated drugs was 1.720 kg in 1996, which was approximately a 30% decrease compared to 1995. Approximately 99 % of the total amount of feed administrated drugs are antibiotics. Approximately 20% of the total use in 1995 were used in poultry production. The remaining 80 % were used in fish farming, including 100 kilo of antibiotics (oxytetracycline and amoxcillin) that was used therapeutically.

2.1.9 **Summary of the use of veterinary medicinal products in Denmark.**
The use of Veterinary Medicinal Products in Denmark during 1997 is illustrated in *Figure 2.2*. Veterinary Medicinal Products prescribed by veterinarians constituted (in 1997) 14 tons of active substances used for the treatment of alimentary tract and metabolism, 29 kg of active hormones, 230 kg of CNS-active substances , and 236 kg of antiparasitic substances and 48.5 tons of antibiotics. 1.7 tons of antibiotics were used in 1996 as feed administrated drugs. Non-therapeutic use included more than 107 tons of antibiotics and 17 tons of active substances used as coccidiostatics in poultry production. The total therapeutic and non-therapeutic use of antibiotics exceeds 170 tons. Antibiotics are hence by far the dominating group of veterinary medicinal products used in Denmark. A significant reduction is, however, observed during the first half of 1998. No information about the sale of V- and H-labelled drugs was available. These groups include a number of commonly used antiparasitic substances, e.g. ivermectin, and are most likely also sold in large quantities.

Figure 2.2. Use of veterinary antibiotics in Denmark during 1997 as a) therapeutics, b) microbial growth promoters, c) coccidiostatics, and d) feed administrated drugs (1996 data).

Figur 2.2 Forbruget af veterinært anvendt antibiotica i Danmark i 1997 a) terapeutisk, b)som mikrobielle vækstfremmere, c) coccidiostatika, og d) foderlægemidler (1996 data).

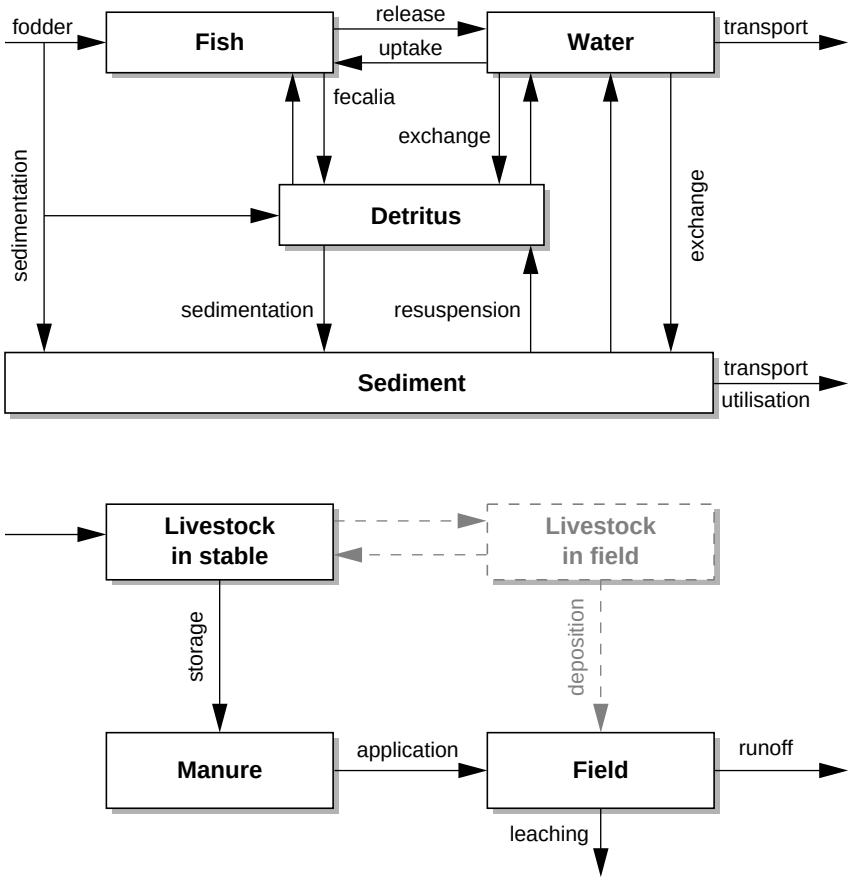


2.2 **Environmental Release**

Compared to industrial chemicals, the exposure routes of veterinary medicinal products to the environment is relatively easy to identify and related to specific field scenarios. Veterinary medicines may be spread to the environment, either directly when using the drugs or by subsequent excretion from the animals. The dominating pathway of environmental release in the terrestrial compartment is by amendment of arable soil with manure or slurry. In fish farms, an unknown part of food-pellets coated with or containing the medical compound (most often an antibacterial drug) will not be eaten hence reach the sediment unchanged. If taken up, the medication may be excreted as unchanged compounds or as metabolites and finally reaches the sediment. *Figure 2.3* shows the anticipated exposure routes for veterinary medicines to the environment.

Figure 2.3. Environmental release scenarios for veterinary medicinal products used in aquaculture (top) and in livestock production (bottom). The scenario for livestock covers all species of animals. However, not all routes of exposure is equal important. For example is the total environmental release from field going pigs and poultry insignificant in comparison to the release from animals in stables.

Figur 2.3. Eksponeringsveje af miljøet for veterinære lægemidler anvendt i aquakulturer (top) og i husdyrproduktionen (bund). Scenariet for husdyr dækker alle typer. Ikke alle eksponeringsveje er lige vigtige for alle dyr. For eksempel er det totale miljøudslip fra fritgående grise og høns minimalt i forhold til dyr i stalde.



Veterinary medicines are widely used for therapeutically treatment of all groups of animals. The group of antibiotics used non-therapeutic is used as growth promoters to especially pigs, but also cattle and poultry. Coccidiostatics is only used as growth promoters in poultry production. The medicinal mixtures sold as "premixed" drugs are used for therapeutic treatment of pigs, cattle and poultry. The approximately number of animals in Danish livestock production is shown in *Table 2.1*. A detailed list on single substance level divided in animal species is found for therapeutic and non-therapeutic uses of antibiotics in *Appendix D*, *Table D1* and *D2*. *Box 2.1* includes a short description of exposure scenarios for application of medicines to different types of animals.

No information is available on the fate of veterinary medicinal products during storage of manure/slurry. If the substance in question is hydrophilic it will be dissolved in the aqueous fraction of the manure/slurry. Opposite, if the substance is hydrophobic, it will mainly adsorb on the particulate matter. If the substances are used therapeutically for field animals, these substances, e.g. hormones, antibiotics or antiparasitic drugs, will be urinated or defecated directly on the field and the exposure might be of a high local concentration. Both spreading of manure/slurry and dropping of excreta by field animals may lead to a run-off of especially hydrophilic substances in cases of subsequent heavy rain. However, the legislation aims at reducing this risk by having restrictions on where, when and how to apply manure and slurry to the soil.

Veterinary medicinal products used in fish farms may enter adjacent ecosystems either directly by water flow or they may accumulate in sediments, which at a later stage may be spread on arable land for fertilising purposes.

Table 2.1. The approximate number of animals in Denmark in 1997. Data from Statistics Denmark 1997/1998.

Tabel 2.1. Det omtrentlige antal dyr i Danmark i 1997. Data fra Danmarks Statistik 1997/1998.

Species	Husbandry (1000)	Slaughtered animals (1000)
Chickens	18,156	116,676
Pigs	11,383	19,670
Cattle	2,004	703
Sheep	142	68
Horses	39	3

Box 2.1. Exposure scenarios following veterinarian drug application(s)

Six different scenarios for environmental release of veterinary drugs have been identified. These include release from: a) pigs, b) cattle, c)sheep, d) horses, e) poultry and f) fish in aquaculture

Within each group of animals the fate and environmental release of a particular drug vary with a number of parameters, including time of use relative to the production cycle of the animal(s) and excretion pathway. The excretion pathway is among other things depending on water solubility of the substance. Most antibiotics are water soluble, whereas the groups of aver-/ivermectins are more lipohilic.

Scenarios for cattle, sheep and horses
The scenarios of cows, sheep and horses are similar. (*Figure 2.3*.) Faeces and urine from stabled animals may be stored and used as fertiliser on arable land. Drugs released both by the urine and the faeces will be stored together and released simultaneous. Hence, the major exposure pathway and the excretion rates is less important. VMP administered to livestock will follow the manure/slurry ending up on arable land . Livestock on pastures will deposit their excreta directly to the fields. The drugs present in dung will, depending on its chemical properties, stay there during dung degradation or leach to the soil in cases of rain. Drugs released via the urine will immediately reach the soil and if water soluble leach further down to groundwater or adjacent water systems with the soil water. Most drugs excreted by the urine is by nature relatively water soluble, whereas drugs excreted via faeces in general is less soluble.

The quantity of manure/slurry produced by the three groups of animals differ, with cattle as the far most important, followed by sheep and horses.

Scenarios for pigs and poultry
The scenarios of pigs and poultry are similar (*Figure 2.3*). In both cases the animals in stables counts by far the highest numbers. Although the numbers of pigs and chickens kept on open land currently are increasing in Denmark, deposition by livestock directly on fields is insignificant in comparison to the amount produced by animals in stables.

Scenario for fish farms
Drugs are usually added with feeding tablets both for therapeutic and non-therapeutic use in fish farms. The drugs may be ingested by the fish or lost to the detritus or directly to the sediments. The non-ingested part may be as high as 80-90%. Depending on the solubility of the drug it will be associated with different pools. Water soluble VMPs are mainly associated with the the water phase, and generally have a high mobility in the system. Lipophilic compounds on the other hand are connected to the organic pools of the system, i.e. in the lipids of fish, in detritus or in sediments.

2.3 Summary and conclusions

- No public available database exists with information on the therapeutically use of veterinary medicines in Denmark. This report estimates the amount used based on data from 1996 and 1997 received by the Danish Medicines Agency (DMA).

- The Danish Plant Directorate (DPD) has since 1986 calculated the annual consumption of growth promoters and medicines for feed additives on single active substance level as kg active substance per year. This register is available for the public, but includes only the total quantity of growth promoters in Denmark. It would improve the usefulness if data was divided into species level and post boundary practise.

- Data for A and B labelled therapeutically used substances is included in this report. The sale of V labelled substances, such as antiparasitic drugs and H labelled compounds, such as acetylicaceticacid, are not registered. The quantity of antiparasitic drugs used is most likely significant.

- Data from the DMA showed the following use for therapeutic applications in 1997: 14 tonnes of active substances for diseases associated with the alimentary tract and metabolism, 29 kg of active hormones, 230 kg of CNS-active substances, 236 kg of antiparasitic drugs and 48.5 tons of antibiotics. .

- Data from the DPD showed that in 1997 more than 107 tons of antibiotics were used non-therapeutically. Another 1.7 tons were used as feed administrated drugs in 1997.

- 17 tons of active substance was used as coccidiostatic in the production of poultry.

- The total therapeutic and non-therapeutic application of antibiotics thus exceeded 170 tons in 1997. This amount also includes, although considered as a minor part, application for pets.

- The main environmental route of exposure for veterinary medicines entering the environment is through the faeces and urine of treated animals.

3 Environmental fate and occurrence of Veterinary Medicinal Products

Information about fate and occurrence of veterinary medical substance in the environment is found in a relatively small number of scientific papers. Far more information is available when it comes to the pharmacological fate of the veterinary medicine in the animals prior to excretion. All identified data concerning environmental fate and occurrence is presented in *Tables F1* and *F2* in *Appendix F*. Residues of veterinary medical substances have primarily been found in sediments as a result of the use of feed additives in fish farms or in cow dung as a result of treatment of grazing cattle with antiparasitics. In the following a few papers are reviewed in more details.

3.1 Pharmacological fate of veterinary medicines

Medicinal substances may be metabolised in the animal before entering the environment. This may have major importance for the risk assessment procedure. If the medical substance is metabolised to one or few major metabolites the environmental risk assessment has to be conducted on these major metabolites rather than on the parent substance.

Most medicines are metabolised to phase I or phase II metabolites before being eliminated in the urine and/or faeces. Phase I reactions usually consist of oxidation, reduction or hydrolysis Phase II reactions involve conjugation, which normally results in inactive compounds. Both phase I and phase II reactions changes the physical chemical behaviour of the substance because metabolisation always renders the metabolites more water soluble than the parent compounds. For a comprehensive introduction of drug metabolisation see Gibson and Skett (1986).

3.2 Fate in the environment

In the environment veterinary medicinal substances may be absorbed, transported, bioaccumulated or undergo transformations such as biotic and abiotic degradation or reactivation.

Reactivation

There are examples of breakdown products of veterinary medicinal products being converted back to their parent compounds in nature. Berger et al. (1986) showed that chloramphenicol glucoronide and N-4-acetylated sulphadimidine, both phase II metabolites, were converted to the parent compounds chloramphenicol and sulphadimidine in samples of liquid manure. Often metabolites are less toxic than the parent substance. However, if metabolites is reactivated in the environment it complicates the estimation of the environmental exposure and hence the risk assessment.

Abiotic degradation in water

Veterinary medicinal substances may undergo abiotic degradation in water by photolysis or hydrolysis. The importance of these degradation processes is relatively unknown. It is well known that dissolved furazolidone is photosensitive, e.g. Paul and Paul (1964). Aqueous solutions of oxytetracycline kept in dark for a period of two months seem very stable. If oxytetracycline solutions are illuminated, the situation dramatically changed. A half-life of

30 days in fresh water (pH = 7) and only 30 hours in sea water (pH = 8) was observed. Similar findings have been reported by Samuelsen (1989) and Lunestad and Goksøyr (1990). Oka et al. (1989) found seven metabolites of tetracycline after photodecomposition under conditions similar to natural waters in a fish culture pond. However, more field experiments should be carried out to evaluate the significance of photo-degradation under natural conditions.

Biodegradation in sediments

Whereas abiotic degradation dominate in the water, microbial degradation is the main degradation pathway in sediment. Antibiotics or their breakdown products may accumulate in the sediment of fish farms. The fate of drug residues in fish farm sediments is not completely clear. Factors like temperature, water flow, distance between cage and sediment, bacterial activity, chemical composition and depth of the sediment, will affect the decomposition and / or leaching of the drug.

The persistence of oxytetracycline (OTC), in bottom deposits from fish farms has been investigated by Jacobsen and Berglind (1988). It was found to be relatively persistent in anoxic sediments. OTC was found in concentrations varying from 0.1 to 4.9 mg/kg dry matter. A conservative estimation of a half-life of approximately 10 weeks was estimated through a pilot study. Coyne et al. (1994) investigated the concentration of OTC in the sediment of two cages at a fish farm site, and found half-lives of 16 and 13 days. Oxytetracycline, oxolinic acid, flumequine and sarafloxacine were all found to be very persistent in sediments (Hektonen et al. 1995). In the deeper layer of the sediment hardly any degradation had occurred after 180 days and a calculated half-life of more than 300 days was estimated. The residues in the top layer of the sediment disappeared more rapidly. The removal of these substances from the sediment is most probably due to leaching and redistribution rather than degradation. Quinolones were found to adsorb to the sediment. Sulfadiazine and Trimetroprim were less persistent than the quinolones. The concentration of Florfenicol decreased rapidly in the sediment with a calculated half-life of 4.5 days, and a metabolite, florfenicol amine, was identified in the sediment.

Samuelsen et al. (1994) showed that the toxicity of OTC to bacteria declined rapidly in sediments, although no degradation occurred. Binding to ions (Ca^{2+}, Mg^{2+}) and other substances were mentioned as possible explanation for the inactivation of oxytetracycline., The same study found that both oxolinic acid and flumequine sustained their antimicrobial activity over a six month period in sediment material.

Biodegradation in soils and manure

The degradation of ceftiofur sodium, a wide-spectrum cephalosporin antibiotic, was studied by Gilbertson et al. (1990) in different soils. Fortification of cattle faeces with [^{14}C]-ceftiofur showed that it was quickly degraded to non-degradable metabolites. Sterilisation of the cattle faeces inhibited the degradation of the substance which suggests that microorganisms may be responsible for the degradation. Halv-lifes of aerobic degradation of ceftiofur sodium in different soils were found in the range of 22.2 to 49.0. Gilbertson et al. also showed that the hydrolysis of ceftiofur was accelerated by increasing pH. The half-lives at pH 5, 7 and 9 was approximately 100, 8 and 4 days, respectively. Donoho (1984) found that monencin, an antibiotic applied as growth promoter for pigs, is degradable in manure and soil. The persistence of medicines from liquid manure throughout the food chains is outlined by Berger et al. (1986).

The antiparasitic compound ivermectin has been shown to be persistent in dung voided from treated cattle (Sommer *et al* 1992). It was not possible to detect any decrease in the ivermectin concentration in dung pats during the entire experiment (45 days) in the field under temperate conditions. Ivermectin is also persistent under tropical condition (Sommer and Nielsen 1992). These findings are in agreement with the insecticidal properties observed for aged dung (Madsen *et al.* 1990).

Binding properties to sediments and soils

Hektoen et al. (1995) reports that quinolones such as oxolinic acid, flumequine and sarafloxacin were found to adsorb to sediment of marine origin, and oxolinic acid retained its antibacterial activity throughout the experiment of 180 days. The latter is in accordance with Hansen et al. (1992). Björklund et al. (1991), however, found no antibacterial activity in sediment from fish farms 10 days after the addition of oxolinic acid.

The sorption of efromycin, an antibiotic, developed as a growth promoter for pigs, was investigated by Yeager and Halley (1990) in five soils of various properties. Sorption occurred within 7 hours. Sorption distribution constants ranged from 8 to 290. Classifying efromycin as immobile in most soils (Koc = 580 to 11000). Avermectin was also determined immobile in three different soils by Gruber et al. (1990).

3.2.1 **Bioaccumulation**

No information about bioaccumulation of veterinary medicinal substances has been found in the literature. However, based on the distribution coefficient of chemicals between n-octanol and water (Kow), which is commonly used as a good estimate of the potential for bioaccumulation in aquatic animals like fish, only a few veterinary medicinal products have high potential for bioaccumulation. Empirical calculations using the software ACD log P®, showed that only a few of the antibiotic substances covered in this report have log Kow values higher than 2 and hence are likely to bioaccumulate significantly in the environment if available as parent compounds. Antiparasitic substances are often more hydrophobic making them more bioaccumulative. Avermectins for example have log Kow values higher than 5. Antibiotics, being mono- or poly protic substances, are not always fitting the normal relationship between K_{ow} and the biocentration factor (BCF), which may complicate the estimation of bioaccumulation.

3.3 **Occurrence in the environment**

3.3.1 **Measured environmental concentrations**

Aquatic environment

Antibacterial agents have been detected near fish farms (Bjørklund et al. (1990; 1991); Lunestad, (1992); Ervik et al. (1994a,b); Coyne et al. (1994); Kerry et al. (1995b); Weston et al. (1994); Samuelsen et al. (1992). Cravedi et al. (1987) have shown that more than 90 % of orally administered oxytetracycline was excreted into the surrounding waters (without any biotransformation). Bjørklund et al. (1990) have shown that oxytetracycline may reach concentrations of zero to 16 µg/g sediment and that the compound conditions may be very stable in fish farm sediments at low temperatures and stagnant, anoxic conditions. Jacobsen and Berglind (1988) found also oxytretracycline in concentrations varying between 0.1 and 4.9 mg / kg dry matter in natural sediment samples.

Terrestrial environment

Warman and Thomas (1981) found chlortetracyclines in soil amended with chicken manure and Shore *et al.* (1988) found testosterone and es-

trogen in manure from American chickens. In the US it is, in contrariety to the EU, legal to treat chickens hormones as growth promoters.

Ivermectin

Ivermectin, an antiparasitic drug used for cattle, pigs, horses and sheep, is excreted almost entirely in faeces. Chiu *et al.* (1990) showed that 60-80% of an injection dose on 0.3 mg kg^{-1} was excreted in the faces over the first week and that more than 60% was excreted during the first three days. Less than 1% was excreted through the urine. Halley *et al.* (1989) found by using radio-labelled ivermectin that parent compounds consisted of approximately 40-45% of the total radioactivity in dung from steers, 60-70% and 40% in dung from sheep and pigs, respectively. Although not directly comparable, as the application form was not identical, this correspond to approximately 0.27, 0.67 and 0.22 mg parent ivermectin pr. kg dung. The remaining components were primarily polar metabolites. Sommer & Nielsen (1992c) found two days after injection with 0.2 mg kg^{-1} body weight a maximum concentration in dung from cattle of 3.8 mg kg^{-1} d.w. After 7 and 17 days the concentration dropped to 1.6 and 0.3 mg kg^{-1}, respectively. Sommer *et al.* (1992) detected from 0.4 to 9.0 mg kg^{-1} (d.w.) of ivermectin in cow dung. On the basis of the above studies it is concluded that the concentration of ivermectin in cow dung may occasionally reach 10 mg kg^{-1} (d.w.) shortly after application, but do not generally exceed 2 mg kg^{-1}.

Ivermectin and other antiparasitic drugs may be given to cattle and sheep by a so-called *sustained release boli*. These release the drug over an extended period of time, which most often encompasses the entire grazing season. This is of particular concern as it will prolong the time of exposure for dung and soil living organisms.

3.3.2 **Predicted Environmental Concentrations (PECs)**

For the environmental release scenarios described in *Box 2.1*, e.g. animals in stables, on grassland or in fish farms, it is possible to estimate the environmental concentrations (Predicted Environmental Concentrations - PECs) as a result of the release pathway in question. Important measures in this context is for an example, as described in *Box 2.1*, the use and consumption, interval of medical treatment, the metabolic rate, the excretion pathways and rate, the agricultural practise when collecting, storing and applying manure/slurry on the field etc. Not all of these issues are equally important for all release scenarios, e.g. is information on the excretion pathways and excretion rates of less importance for animals in stables, where the urine and faeces are collected and stored together in a period prior to application on the field. Information on excretion pathways and excretion rates is on the other hand very important if estimating the concentration of medicinal residues in dung or urine excreted directly on the soil by field going animals medicated with e.g. antiparasitics.

For the majority of veterinary medicines, including the antibiotics, the major environmental release is from animals in stables. Therefore, PEC calculations for a number of drugs used either for medical treatment or as growth promoters for animals in stables are presented in the following sections. The regulations controlling the application rate of manure and slurry are presented in *Box 3.1*. Relatively much information is available on the measured concentration of antiparasitics in dung from field going animals (see section above). Hence no attempt to calculate PEC values for this group of substances have been made.

Box 3.1. Regulations on the use of manure as fertiliser on arable land.

A certain fraction of the applied medicines will be recovered as parent compound and residues in the animal faeces or urine. Regulations dealing with fertilisation of arable land with manure and slurry is therefore important for quantifying the environmental release and hence the environmental concentration of veterinary drugs. Based on rates of manure incorporation into soil and the knowledge of the levels of drug residues in manure, the amount of drug residues in soil can be estimated from e.g. average or worst case scenarios. In most cases it will be the nitrogen content of manure which determines the application rate.

The regulation in EU ensure that arable land is not excessively fertilised with nitrogen or phosphorus. An amendment of the Statutory Order no 906 of October 14, 1996, ensures that the amount of nitrogen (N) applied to land does not exceed 230 kg N/ha annually. This is provided that more than 70% of the land available for manure application is cropped with beet, grass, or grass covered crops and that the level of nitrogen utilisation is 50%. Provided that land is cropped with less than 70 % of the above mentioned crops only 210 kg N / ha is allowed annually. This will be in force from December 1999. After December 2003, the quantity of manure applied to the land shall not exceed 210 kg N / ha annually, provided that 60-70% of the land is cropped as mentioned above and a maximum content of 190 kg N / ha annually, provided that 50 - 60 % of the land is cropped in the same way.

PEC estimates

On the basis of the information available about the use of prescribed drugs, the recommended dose of veterinary medicinal products and normal application of manure in Denmark, an attempt is here made to calculate the environmental concentration of veterinary medicinal products . The estimation is based on the recommendation on PEC calculations presented in a paper by Spaepen *et al.* (1997). It has only been attempted to calculate the PEC for soil, as the major environmental release of most veterinary medicines is associated to the terrestrial environment. Some relevant characteristics of the various livestock useful for PEC calculations are presented in *Table 3.1*. As an example of a worst case PEC calculation, the environmental concentration of the antibiotic tylosin is shown in *Box 3.2*. A soil concentration of approximately 1.5 mg kg^{-1} is predicted as a result of a single amendment with manure from animals receiving the recommended treatment of tylosin (*Box 3.2*).

Box 3.2. Predicted Environmental Concentration (PEC) scenario estimating tylosin and tylosin residues in soil after amendment with manure.

Fattening pigs are treated with Tylosin 2 % during a period of 21 days. I accordance with 'Veterinær medicinsk produktkatalog' (1997) the therapeutic dose to pigs is: 4 mg active compound per kg of body weight.
Total dose for the 21 days period: 8.4 gram tylosin.
The following data from *Table 2.2* is used for estimating the concentration in manure:
Body weight of fattening pig: 100 kg;
Manure produced during the 21 days: 1,764 kg/365*21 =101.5 kg.
Concentration of tylosin in manure: 82,7 mg / kg manure. It is assumed that no "dilution" is obtained by mixing the manure with medicine free manure.
It is assumed that no drug is degraded (abiotic or biotic) during storage and that no nitrogen is stripped from the manure tank before spreading, due to ammonia evaporation.
Following the Danish regulations for use of manure as fertiliser on arable land an average of up to 150 kg N/ ha land / year is permitted. In accordance with *Table 2.2*, 101,5 kg manure contains 0.55 kg N. To spread 0.55 kg N demands 36.6 m^2 arable land yielding a concentration of 230 mg / m^2 land.

The density of newly ploughed soil is estimated to 1500 kg m^{-3}. Ploughing depth = 10 cm (worst case).

Predicted Environmental Concentration (PEC) of tylosin in soil ; 1,5 mg tylosin and residues / kg soil.

A similar scenario for soil amended with cow manure from a dairy cow injected with 5-10 mg tylosin pr. kg body weight yields a PEC in soil in the range between 4,7 and 9,3 mg tylosin or residues of tylosin.

Table 3.1 Some relevant characteristics of the various types of husbandry. Data used to perform the exposure scenarios (PEC calculations). Source: Spaepen et. al. (1997).

Table 3.1 Relevante data for forskellige husdyr i landbruget. Data anvendt til eksponeringsscenarier (PEC beregninger). Kilde Spaepen et al. (1997).

Animal type	Body weight kg	Manure produced (excrata) (kg/animal/year)	Nitrogen content in manure (kg/animal/year)	Phosphorus content in manure (kg/animal/year)
Slaughter calf	160	4,660	10.9	5.28
Diary cow	500	20,391	77.29	39.08
Fattening Pig	100	1,764	9.59	8.32
Broiler Chicken	1.3	37.2	0.21	0.38

Similar calculations have been made for different types of veterinary medicinal products applied by different dosage form, e.g. premix, injection and growth promoter, to both pigs and diary cows. Detailed calculations are showed in *Appendix E*. For pigs and cows it was calculated, on basis of data received from the Danish Environmental Protection Agency, that pig and cow manure/slurry was dispersed on an average of 150 kg N /ha and 265 kg N / ha annually, respectively. A ploughing depth of 10 cm was used in the calculations. Results are presented in *Figure 3.1* and *3.2* and show that hormones (oxytocin and vasopressin) amended with pig or cow manure may be found in the range of 0.01-0.05 µg kg^{-1} soil. PECs for antibiotics used therapeutically were found in a range of 1 to 9 mg / kg soil. If the substance is given by injection it seems that the PEC value is slightly higher than when the same substance is given with the food. For antibacterial growth promoters used for pigs, PEC values are found at a much lower level, i.e. in the range of 0.2 to 1.3 mg / kg soil. For substances used in connection with the alimentary tract and metabolism, PECs were found in the range of 0.04 mg / kg soil (dexamethasoniconitat) to 5.7 mg / kg soil (menbuton). For CNS substances used to cows, PECs are found between 0.09 mg / kg soil (xylasin) and 28 mg / kg soil (metamizolnatrium).

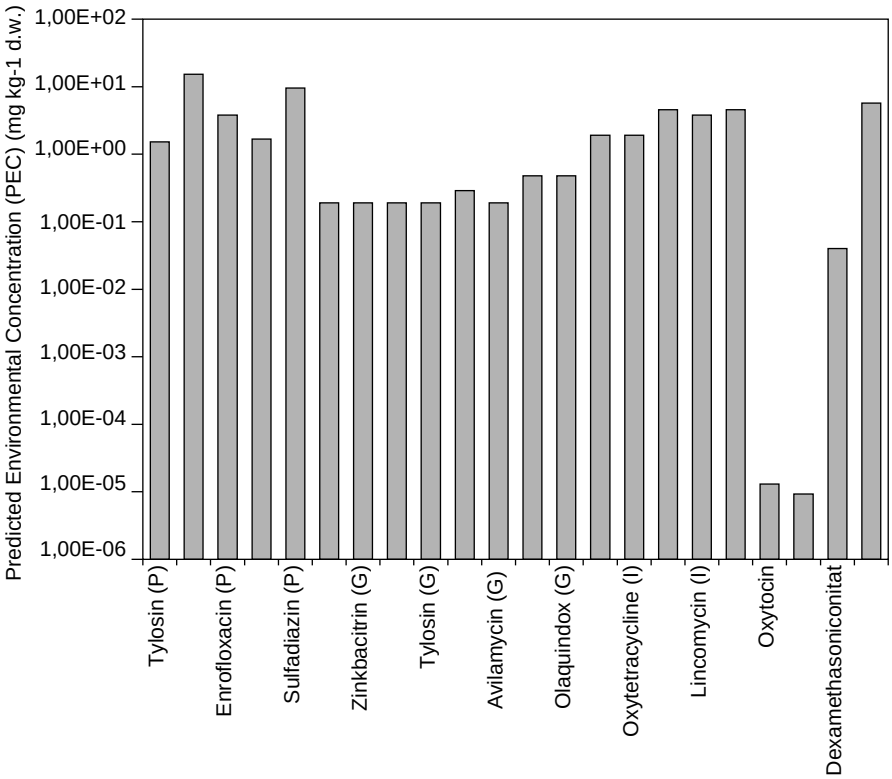


Figure 3.1 Estimated PEC values (mg kg^{-1} soil) for soil fertilised with pig manure containing various veterinary medicines. The substances may be ordinated in the feed as premix (P) or growth promoter (G) or it may given as injections (I).

Figure 3.1 Estimerede PEC-værdier (mg kg^{-1}) for jord gødet med svinegylle.Stofferne kan ordineres i foderet som præmix (P) eller som vækstfremmer (G) eller det kan gives ved injektion (I).

Due to higher N content in cow manure than in pig manure, PECs are generally somewhat higher when soil is amended with cow manure than with pig manure. All these PEC estimations are considered worst case situations and are submitted to a range of uncertainties. There is very little information available to confirm or resolve the calculations.

A paper published by Goll van (1993) estimates that if the total amount of growth promoters used in the Netherlands were spread over all the 2 million hectares of Dutch arable land, an annual average of 130 mg antibiotics and antibiotic metabolites per m^2 of arable land would be found. If this amount were located in the top ten cm of the field, a concentration of 0.87 mg/ kg of soil should be expected. These results falls in line with the estimated worst case PEC estimations presented above and in *Appendix E* of this report. None of the studies do, however, take fully or partly degradation into account. This may off course lead to major overestimation of the environmental concentrations. On the other hand it may be justified by the fact that very little is known about degradation during storage of manure and slurry and that some drug metabolites (e.g. glucuronides) excreted by medicated livestock are decomposed by bacterial action in the liquid manure and subsequently reconverted into the active drugs (Berger et al. 1986).

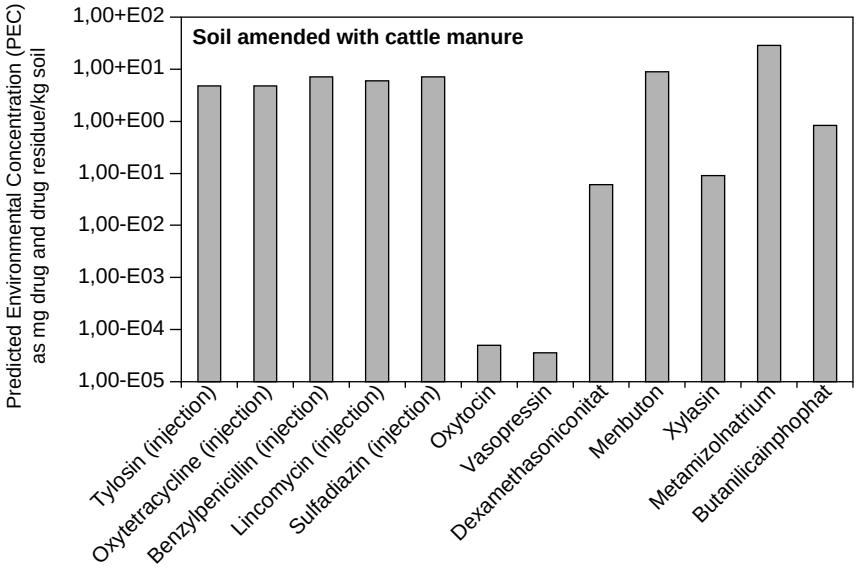


Figure 3.2 Estimated PEC values (mg kg^{-1}) for soil fertilised with cattle manure containing various veterinary medicines. The substances may be ordinated in the feed as præmix (P) or growth promoter (G) or it may given as injections (I).

Figur 3.2 Estimerede PEC værdier (mg kg^{-1}) for forskellige veterinære lægemidler i jord gødet med kvæggylle. Stofferne kan ordineres i foderet som præmix (P) eller som vækstfremmer (G) eller det kan gives ved injektion (I).

3.4 Summary and conclusions on fate and occurrence of veterinary medicines

- The environment may be exposed to residues of veterinary drugs in excreta, i.e. manure or slurry, from treated animals.

- Manure and slurry are normally substrates with a high biological activity. The fauna and microflora that help in the decomposition of organic matter and mineralisation of nutrients may be exposed to residues of veterinary medicines.

- Degradation data were only available for antibiotics and antiparasitic agents. Biodegradation data are found for 18 different antibiotics both applied therapeutically and non-therapeutically. Half-lives vary from a few days to years depending on characteristics of the substance and on environmental conditions such as temperature, humidity and pH.

- The antiparasitic drug ivermectin is only slowly degradable under normal storage conditions, and may hence maintain its antiparasitic properties in the dung for months.

- Data is scarce regarding fate of veterinary substances during storage of manure/slurry. It has, however, been shown that certain excretion metabolites can be reactivated and converted into the parent compounds in samples of liquid manure. This complicates the estimation of environmental release and exposure.

- Very few field measurements of veterinary medicines are found. The best information concerns antibacterial agents in the vicinity of fish farms and avermectines in dung dropped directly on the soil by animals on the field.

- The environmental release of veterinary medicines that are associated with fish farms is special, as the drugs are released directly and deliberately to the environment. Sediment concentration up to 11 mg oxytetracycline kg^{-1} have been observed in the vicinity of fish farms.

- Data on the environmental fate of the antiparasitic compound Ivermectin is well represented in the scientific literature. For cattle, dung concentrations of 1.6 mg / kg ivermectin was measured one week after injection with 0.2 mg ivermectin/kg body weight. After 17 days a reduction in dung concentration to 0.3 mg / kg dung was found.

Although submitted to a number of uncertainties, PEC_{soil} calculations was made for a number of substances used for medication of animals in stables. Worst case PEC estimations were made for hormones (0.01-0.05 µg kg^{-1}), antibiotics (0.2 to 9 mg kg^{-1}) and substances used for treatment of diseases associated with the alimentary tract and the metabolism, (0.04 - 5.7 mg kg^{-1}). PECs is generally somewhat higher when soil is amended with cow manure than with pig manure, as the higher nitrogen content in pig prescribes a lower load of manure/slurry according the regulations governing a maximum load of nitrogen pr hectare. In cases of direct deposition on the soil with no subsequent tilling, the concentration may locally be higher. For the estimation of PEC_{dung}/PEC_{soil} for animals in the field it is important to take into account the excretion rate and the pathway of excretion. If the drug is excreted primarily via the urine it may be very difficult to estimate the $PEC_{soil}/PEC_{groundwater}$.

4 Ecotoxicological effects of veterinary medicinal products

Assessments of environmental effects of veterinary medicinal products have only recently been required prior to the marketing of new products. This has contributed to the relatively low number of published studies dealing with environmental effects of veterinary medicinal products (van Gool, 1993 and Halling-Sørensen *et al.* 1998). Ecotoxicological effects of veterinary medicines are summarised in *Table G*, *Appendix G*, and briefly reviewed below.

Bacteria

One of the most widely used groups of veterinary medicinal products, the antibiotics, is specifically designed to control bacteria in animals. Obviously this makes them potentially hazardous to bacteria and other microorganisms in the environment (Warman 1980, Pursell *et al.* 1995). Antibiotics may have a broad spectrum of activity or designed to be either specifically active against gram negative or gram positive bacteria. Processes affected may therefore include more specific but environmentally important processes like the nitrification, which is driven solely by a few gram-negative bacteria species, or more general processes such as decomposition of organic matter, which is a co-operation between a conglomerate of different types of microorganisms. When evaluating the effect of antibiotics towards the microbial community it is hence important to keep in mind that the type of target organisms vary between antibiotics. The types of veterinary antibiotics used in Denmark and the target bacteria for each group of substances are given in *Box 4.1* and *Table 4.1*.

4.1 The aquatic environment

Phytoplankton

Streptomycin prevented growth of six blue-green algae species at concentrations from 0.09 to 0.86 mg/l (Harrass et al. 1985). The blue-green algae was generally more sensitive than the green algae tested. *Chlorella vulgaris*, *Scenedesmus obliquus* and *Ulothrix sp*. grew in active streptomycin concentrations less than 21 mg/l, while *Chlamydomonas reinhardtii* growth was prevented at concentrations of 0.66 mg/l. Algae growth in sublethal concentrations of streptomycin was slowed or delayed, and the maximum density attained by several species was decreased. Result published by Lanzky and Halling-Sørensen (1997) showed that *Chlorella sp*. are sensitive (EC_{10} = 2.03 mg/l and EC_{50} = 12.5 mg/l) to metronidazole (which is used in fish farms).

Crustaceans/copepods

The acute toxicity of furazolidone, 3-[(5-Nitrofurfurylidene)amino]-2-oxazolidinone, which are largely used in medicated fish feed, have been investigated by Macrì et al (1988). The authors found a significant toxicity of the compound to *Daphnia magna*, while *Artemia salina* proved to be the less sensitive. Migliore et al. (1997) showed a toxicity of several agricultural antibiotics to *Artemia*. Acute toxicity studies showed that the four antibiotics; aminosidine, bacitracin, erythromycin and lincomycin, all used as feed additive or mass therapy in intensive farming, were only slightly toxic to *Dapnia magna* (Dojmi di Delupis et al. 1992). EC_{50} values after 48 hours was found in the range of 30 mg/l to 500 mg/l with bacitracin as the mosttoxic.

Fish

Only very little information are outlined in the literature concerning the effects of medicines on fish species. Acute test on *Bradio rerio* with metroni-

dazole showed no effect on the survival (Lanzky and Halling-Sørensen 1997).

Box 4.1. Types of veterinary antibiotics used in Denmark.

The most important groups of veterinary antibiotics used therapeutically in Denmark belongs to the following types;

Aminoglycosides (gentamicin, neomycin and streptomycin),
Polypeptid antibiotica (bacitracin),
Betalactam antibiotics (benzylpenicillin, ampicillin and amoxillin),
Sulfonamides (sulfadiazin, sulfadoxin, sulfapyrazol, sulfatroxazol and sulfadimidine),
Macrolides (tylosin and spiramycin),
Lincosamides (lincomycin),
Quinolones (enrofloxacin and oxilinic acid),
Tetracyclines (oxotetracycline, chlortetracycline and tetracycline)
Di-aminopyridimine derivates (trimetroprim).

Veterinary antibiotics used as growth promoters used in Denmark belongs to the following types;

Oligosaccharides (avilamycin),
Polypeptid antibiotica (bacitracin),
Flavomycines (flavofosfolipol),
Quinoxalines (carbadox and olaquindox),
Streptogamines (virginamycin),
Ionophores (monensin and salinomycin)
Macrolides (tylosin and spiramycin).

The list is not complete, but covers the majority of veterinary antibiotics used in Denmark. The target bacteria for all groups of antibiotics is shown in *Table 4.1*.

Table 4.1. The target bacteria and the most common use of different groups of antibiotics in Denmark. Brackets () indicates that only a few substances within the group is affecting the indicated group of bacteria.

Tabel 4.1. Virkningsspekter for antibiotika anvendt i Danmark. Bakterietyper i parentes () betyder at det kun er et begrænset antal bakteriearter i gruppen som påvirkes.

Group of antibiotic	Therapeutic use	Growth promotor	Gram negative	Gram positive
Aminoglycosides	X		X	
Beta-lactam	X		(X)	X
Flavomycins		X		X
Ionophors		X		X
Lincosamides	X		(X)	X
Macrolides	X	X		X
Oligosaccharides		X		X
Polypeptides	X	X		X
Quinolones	X		X	(X)
Quinoxalines		X	X	
Sulfonamides	X		X	X
Tetracyclines	X		X	X

4.2 The terrestrial environment

Plants

Batchelder has tested the effects of the antibiotics chlortetracycline and oxytetracycline on plants when grown in both a nutrient solution media (Batchelder 1981) and in soils (Batchelder 1982). Two greenhouse studies were conducted to evaluate the effects on pinto bean plants (*Phaseolus vul-*

garis) grown in aerated nutrient media and in soil. Root growth and development were markedly decreased by both antibiotics as their concentrations were increased from 0 to 160 ppm in solution. Top dry weights were reduced 71 - 87 % by the antibiotic concentrations, and root dry weight were decreased 66 - 94%. Plant mortality increased as the antibiotic concentration were increased and all plants died at the 160 mg L^{-1} treatment level. The results showed that relatively low antibiotic concentrations can markedly affect pinto bean plant growth and development in nutrient solution. In the study using soil as growth media Batchelder (1982) found a large variation of the sensitivity among plant species. The most sensitive plant species was pinto beans when grown on sandy loam soil. Pinto beans were severely affected by antibiotics when the soil was watered with solution containing up to 160 mg tetracyclines L^{-1}. Phenothiazine has been implicated in deleterious changes in the botanical composition of pastures (Southcott 1988).

Insects

Since the 1970s is has been known that antiparasitic drugs excreted by animals could adversely affect the development and survival of non-target organisms, important in the process of dung degradation and nutrient cycling. Whereas drugs such as piperazine, thiabendazole and levamisole has little or no effect on beetles breeding in dung, formulations of coumaphos, dichlorvos and phenothiazine adversely affected their survival and reproduction for at least 4 to 5 days after treatment (Blume et al. 1976). Residues of dichlorvos has been shown to delay dung degradation (Lumaret 1986). The 1980´s saw the introduction of a new class of compounds known as macrocyclic lactones. This group, which includes avermectins (doramectin, abamectin and ivermectin) and milbemycins (moxidectin), are to a large extend excreted in the faeces of treated livestock as unaltered drug. Abamectin and ivermectin have been reported to have effects on a wide range of arthropods. In the late 80´s Wall and Strong (1987) discovered that residues of ivermectin in cattle dung had lethal effects on beneficial dung degrading insects and hence delayed the dung degradation. The environmental effects of ivermectin has later been investigated by others, e.g. Sommer C. (1992a; 1992b;1992c), Madsen *et al.* (1990) and Holter (1993). Research have shown that the duration of effects after treatment of ivermectin on dung degrading organisms is depended on the non-target species (beetles, flies, earthworms), form of drug application, and livestock species. By studying the number and development of immature dung beetles (*Onthophagus gazella*), Sommer & Nielsen (1992) showed a nearly 100% larvicidal effect of ivermectin (1.6 mg kg^{-1}, d.w.) in dung collected one week after treatment. In dung voided 17 days after treatment only half of the larvae were able to survive, although the ivermectin concentration had dropped to 0.3 mg kg^{-1} (d.w.). Madsen *et al.* (1990) observed the effects of ivermectin on two species of flies in dung from treated heifers. No chemical analysis of the dung was performed, but results from the laboratory studies showed that the face fly *Musca autumnalis* was more sensitive to ivermectin than the house fly *Musca domestica*, and that dung excreted 40 days after treatment still killed 50% of the fly larvae.

Mosquito Larvea

Macrì et al. (1988) showed that furazolidone had a significant toxic effect on the mosquito larvae *Culex pipiens*.

4.3 Summary and conclusions on environmental effects of veterinary medicines

- The literature primarily includes effect studies on acute toxicity of antibiotics in the aquatic environment and effects of antiparasitic agents on dung/soil fauna.

- Antibiotics are generally toxic to algae, but less toxic to crustaceans and fish. Especially the blue-green algae seems very sensitive to some antibiotics

- Many studies have shown that antiparasitic drugs may be lethal for beneficial insects living in dung voided several weeks after treatment of cattle. Only a single study has investigated effects of ivermectin on earthworms.

- Information is generally lacking on the long term ecotoxicological effects and on the ecological consequences of a continuous environmental exposure, e.g. in the cases of *boli* slowly releasing antiparasitic drugs during an entire grazing season.

- The environment will often be exposed to many drugs and/or other hazardous chemicals simultaneous. However, generally very little is known about the resulting total environmental hazard, risk and impact of simultaneous occurring chemicals, including veterinary drugs.

- Compared to industrial chemicals which often have a general narcotic mode of action, veterinary medicines generally have a specific mode of action. In the light of the limitations of the existing standardised test battery for evaluating the environmental hazard and risk of veterinary drugs, new and more appropriate test methods may be needed. Such new tests should, however, be validated and standardised before widely employed.

5 Legislation concerning environmental risk assessment of veterinary medicinal products

5.1 Introduction

This chapter includes a review of the current risk assessment of veterinary medicinal products and outlines the similarities and differences to the current risk assessment principle of new and existing chemicals in the EEC. Initially it should be stressed that this chapter only describes the risk assessment procedure for new veterinary medicinal products introduced to the EU market after January 1998. No legislation or risk assessment procedure for existing veterinary medicinal products exist. To the authors knowledge there is no initiatives for doing so in the future

Whereas legislation and regulation of domestic and industrial chemicals with respect to the protection of the environment have been implemented in Europe and North America for decades, such legislation of medicinal products was first initiated in the late 80´s in the USA (FDA 1985; 1987 and 1995) and in the beginning of the 90´s in Europe.

The European Commission first integrated a basic evaluation of the environmental impact of veterinary medicinal products in its Directive 81/852/EEC as amended by Directive 92/18/EEC. This Directive outlines basic requirements for conducting an environmental risk assessment of veterinary medicinal products. The 81/852/EEC and 92/18/EEC Directives was followed by a guidance document by the European Agency for the Evaluation of Medicinal Products (EMEA) - EMEA/CVMP/055/96, which provides a technical guidance for the assessment of environmental risk of new veterinary medicinal products. In addition, the European Commission has also laid down guidelines for an environmental risk assessment of animal food additives (94/40/EEC). These guidelines include a description of the basic requirements for assessing the fate and effects of the food additives in the environment, e.g. environmental fate and persistence; toxicity to algae, daphnia, fish in the aquatic environment and microbial processes and fauna in the terrestrial environment. At present, the Technical Directive for human medicinal products (75/318/EEC) does not include any reference to an assessment of their potential environmental risk or to ecotoxicology in general. However, a detailed technical draft guideline issued in 1994 (III/5504/94) indicates that the approach applicable for veterinary medicine also would apply for human medicinal products.

In the following sections a short review of the principles for assessment of environmental risk of new and existing chemicals in the EU is given (section 5.2) with the aim to compare these risk assessment methodologies with the risk assessment for veterinary medicinal products (section 5.3).

5.2 ERA for new and existing substances in the EEC region

The Council Directive 67/548/EEC (as amended by Directive 92/32/EEC) on the classification, packaging and labelling of dangerous substances requires the manufactures of new substances, if these are sold in quantities exceeding a certain limit (1 t per year per manufacturer or imposter , to notify relevant

national authorities in the country of manufacture or import. A notification includes a package of test data concerning various information of the physical-chemical, toxicological and ecotoxicological properties of the notified substance. With the base set of data, the competent authorities are then required to carry out an assessment of the risk of the substances to man and the environment in accordance with the principles of Commission Directive 93/67/EEC. For existing substances the Council Regulation (EEC) no. 793/93 requires an assessment of risk to man and environment of priority substances given in the Commission Regulation no. 1488/94 on risk assessment on existing substances. For existing chemicals an informal priority setting (EURAM-IPS) method is used for selecting chemicals among the 100,000 listed in the EINECS database (The European Inventory of Existing Commercial Chemical Substances). The purpose of IPS is to select chemicals for a detailed risk assessment among the EEC high production volume compounds, i.e. >1000 t/y/manufacture (HVPC, a subset of the EINECS database containing approx. 2700 chemicals). Data necessary for the IPS and an initial hazard assessment is called the HEDSET (European Commission 1993) and covers issues as environmental exposure, environmental effects, exposure of man and human health effects. The data are contained in the IUCLID database. On the basis of national priority lists of chemicals and priority indicated by the employed EURAM a comprehensive risk assessment documents is produced by competent authorities in the member states in collaboration with industry.

In support of the legislation a detailed Technical Guidance Document (TGD) and a software program (EUSES) have been produced, including extensive technical details for conducting hazard identification, dose-effect assessment, exposure assessment and risk characterisation in relation to man and environment. The risk assessment of new notified substances is based on the data submitted by the notifiers in accordance with Directive 67/548/EEC. The directive provides a scheme of step-wise, tonnage-related data requirements (*Figure 5.1*). The tests have to be carried out in accordance of the EU methods (Annex V to Directive 67/548/EEC). If no EU method is available or scientifically pertinent other international test guidelines, preferably OECD guidelines may be used. As a minimum requirement, the full base-set (FBS) testing required for new substances (Annex V, Directive 67/548/EEC) must be available for a risk assessment. Among the existing high production volume chemicals (>1000 tons/year/manufactor), the minimum data set for the high priority substances is the HEDSET, which encompasses the full base-set for new substances and a screening for reproductive toxicity (OECD 421). This ensures that for both new notified and for chemicals of priority data from studies on short term toxicity on algae, daphnia and fish and key data on the environmental fate, e.g. log Kow, biodegradability, vapour pressure and water solubility, are available for the risk assessment. For new substances further data are foreseen at level 1 and 2 of the risk assessment procedure. For existing substances information beyond the base-set is some time very limited other times very large and comprehensive. On the basis of the toxicity data available, an assessment factor ranging from 10-1000 is applied to the EC/LC/NOEC values to derive the Predicted No Effect Concentration (PNEC). The choice of assessment factor depends on the quantity and quality of toxicity data (*Table 5.1*).

Figure 5.1.The general principles of risk assessment of new and existing substances in the EU.

Figur 5.1. De generelle principper for riskovurderingen af nye og eksisterende kemiske stoffer i EU.

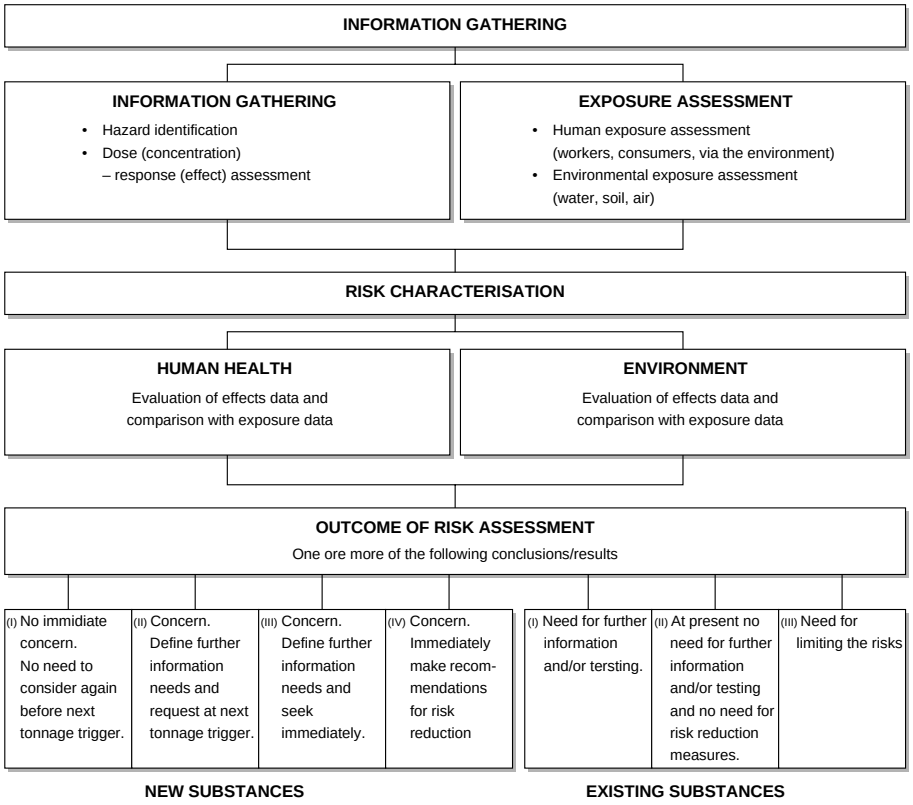


Table 5.1. Assessment factors to derive PNEC according to the Technical Guidance Document for new and existing substances. See TGD for detailed notification.

Tabel 5.1. Applikationsfaktorer for estimering af PNEC ifølge den tekniske vejledning for vurdering af nye og eksisterende stoffer.

	Assessment factor
At least one short-term L(E)C_{50} from each of three throphic levels of the base set (fish, Daphnia and algae)	1000
One long-term NOEC (either fish or Daphnia)	100
Two long-term NOECs from species representing two throphic levels (fish and/or Daphnia and/or a algae	50
Long-term NOECs from at least three species (normally fish, Daphnia and algae) representing three throphic levels	10
Field data or model ecosystems	case by case

The exposure assessment should be based on representative monitoring data and/or model calculations. However, for many chemicals it is very difficult to obtain representative monitoring data, as detailed monitoring studies only exist for a few organic substances. To calculate the exposure by e.g. models may be very complicated as detailed information about distribution, specific use and application may not be available. The primary manufactures of a chemical may not hold detailed information about the use of the chemical in products, i.e. "downstreeam uses". *Box 5.1* includes a brief discussion about uncertainties and limitations in the prediction of the environmental concentrations (PECs) .

An assessment of the risk of secondary poisoning to top predators has to be included in the risk assessment report if indicated by the available data, e.g. if the substance is bioaccumulative in fish and excert chronic or reproductive effects in mammals. At a base-set level the available physico-chemical and (eco)toxicological data can be used to decide whether or not there is any indications for a potential for bioaccumulation and/or indirect effects. The Technical Guidance Document provides detailed information on how to assess the risk of secondary poisoning

The risk assessment should be carried out for all relevant compartments (concern areas), i.e. for micro-organisms of sewage treatment plants, aquatic, sediment and soil dwelling species as well as for secondary poisoning of top predators of the food chains. A quantitative ERA is currently not possible for the air compartment. As the base set of effect data only include aquatic organisms and micro-organisms from sludge, and no base-set of tests currently exist for sediment and soil organisms, short-term toxicity tests on sediment and soil dwelling species are only required if a potential risk for the sediment or soil compartment have been identified on basis of a risk characterisation using the equilibrium partitioning method on the aquatic base-set, as outlined in the TGD. The OECD-tests on earthworm (207) and plants (208) are currently the only available internationally standardised tests for the soil environment. Standard tests on sediment species are currently being developed. Whereas, a quantitative evaluation of risk in the aquatic (especially the freshwater system) and the terrestrial environment should be possible for most substances, the risk assessment for air can only be carried out qualitatively, as no adequate biotic testing systems currently are available.

Box 5.1. Uncertainties and limitations in risk assessment

All kinds of risk assessment procedures are bound to have some degree of uncertainties and limitations. Many factors controlling exposure and toxicity has to be considered when performing a risk assessment, and as many of these vary from situation to situation, some margin of uncertainty has to be taken into account to reach the desired level of protection. This Box attempt to describe a few of the most important factors that have to be taken into account when performing a risk assessment.

PEC estimation:
Most of the variance between the ERA of drugs and chemicals as described above is due to the fact that veterinary substances to some extend is evaluated the same way as pesticides. This may be justified by the fact that exposure scenarios for veterinary medicines in the same manner as for pesticides often are far more reliable than for chemicals with a diverse use and environmental release. For new and existing chemicals PEC should be calculated on both a local and a regional spatial scale preferable from monitoring data If this information is not available estimates may be made from exposure models such as e.g. EUSES. One of the major challenges of estimating exposure of industrial chemicals is, however, to establish representative release estimates according to realistic worst case scenarios. The environmental release of an industrial compound may occur during the many steps from the initial producers via several consumers to its final dispose or incineration. Although the uses and recommended doses of veterinary medicines are relatively well known compared to industrial chemicals, less is known about metabolisms and excretion of the substances. Another major uncertainty in estimating the environmental concentration of veterinary drugs is a possible persistency in the excreta before and after the manure or slurry is applied as fertilisers to arable land. A uniform procedure to estimate PEC for veterinary medicinal products is suggested by Spaepen *et al.* (1997)

PNEC estimations:
Many uncertainties can be found in the estimations of predicted no environmental effect concentration (PNEC). The procedure of prediction a safe level for a complete ecosystem on the basis of laboratory short term tests on a few species is bound to include a high level of uncertainty. Of course this is the major reason for applying safety factors on the no effect concentration for single species. These assessment factors cover factors like inter- and intra species differences, acute to chronic exposure and extrapolation from laboratory to field, e.g. how to extrapolate effects observed in controlled single species tests to ecologically relevant effects in a unrestrained multispecies natural ecosystem. In addition to these a number of other uncertainties in PNEC calculations can be listed:

- The use of NOECs in the derivation of PNEC necessitate well planned test, and is depending on e.g. the choice of test concentrations.
- The choice of endpoint may have influence on the observed toxicity. The most sensitive endpoint may vary depending the mode of action of the chemical.
- It is assumed that by protecting the structure of an ecosystem, the function is protected as well.
- Coexistence of toxic chemicals may lead to synergistic, antagonistic or additive effects.
- Differences in bioavailability and/or degradation rates in field situation compared to controlled laboratory experiments.
- Multiple stresses such as temperature, humidity, shortage in food supply or predation may exist throughout the year in natural ecosystems. Large fluctuation in the environment may influence the sensitivity of chemicals compared to laboratory experiments performed under controlled and stable conditions

These parameters are not equally important for all chemicals or in all ecosystems, and do not necessarily draw in the same direction. However, they all represent a level of uncertainty in the present calculation procedure for PNEC and plead for the present use of assessment factors.

The requirements for the dossier to be submitted for the authorisation of a plant protection product is more extensive than the ones for new and existing substances (Annex VI, 91/414/EEC). In addition to the acute toxicity test with algae, daphnia and fish, chronic toxicity, bioaccumulation and sublethal studies on reproduction and growth is required for fish and daphnia. The applicant must furthermore provide data from toxicity studies on birds and other non- target organisms, e.g. honey bees and earthworms.

5.3 Comparison of ERA for veterinary medicinal products and food additives with ERA for new and existing substances in the EEC region

The European Commission has included the evaluation of the environmental impact of veterinary medicinal products in its Directive 81/852/EEC as amended by Directive 92/18/EEC. This Directive outlines in the basic requirements for conducting an environmental risk assessment of veterinary medicinal products. For details on the evaluation and a technical guidance, the document by the European Agency for the Evaluation of Medicinal Products (EMEA) - EMEA/CVMP/055/96, may be consulted.

The risk assessment as described in the EMEA guidance document is based on a tiered approach. Hence more detailed information is required as the risk assessment proceeds. The first level (phase 1) includes an identification of exposure routes and a first estimation of PECs. Phase 1 includes a number of cut-off values for further assessment. In the second step (phase 2 - Tier A) ecotoxicological effects are compared to the PECs, and a first risk assessment is made for the aquatic and the terrestrial environments. If further data are needed, Phase 2 - Tier B details additional toxicity tests and fate studies. The "Decision Trees" used for risk assessment in the EMEA-guideline is presented in *Figure 5.2-5.4*. In the following paragraphs this risk assessment will be compared to the one used for new and existing substances in the European Union. To facilitate the reading the term chemicals or industrial chemicals will be used as a collecting term for new and existing substances in this chapter, unless important differences in legislation exist for one of the two groups. The term drugs will regularly be used instead veterinary medicinal products.

Directive no. 94/40/EEC from the European Commission lays down a guideline for assessing the effects of food additives. According to the Directive applicants should review the physical-chemical, toxicological and ecotoxicological properties according to the Commission Directive 67/548 (latest amendment 93/105) or other international recognised methods. If other methods are used, such use must be justified. The guideline for assessing food additives, however, is currently being revised by the Commission. In addition the existing guideline is adopted from the risk assessment procedure for new chemicals, and no further discussion concerning food additives will therefore be presented in this report. Attention should however be drawn to the fact that many food additives, e.g. growth promoters, frequently are used as medicinal products as well.

Figure 5.2. Phase I in the EMEA procedure for assessing risk of veterinary medicinal products to the environment.

Figur 5.2. Fase I i EMEA-proceduren for miljørisikovurdering af veterinære medicinale produkter.

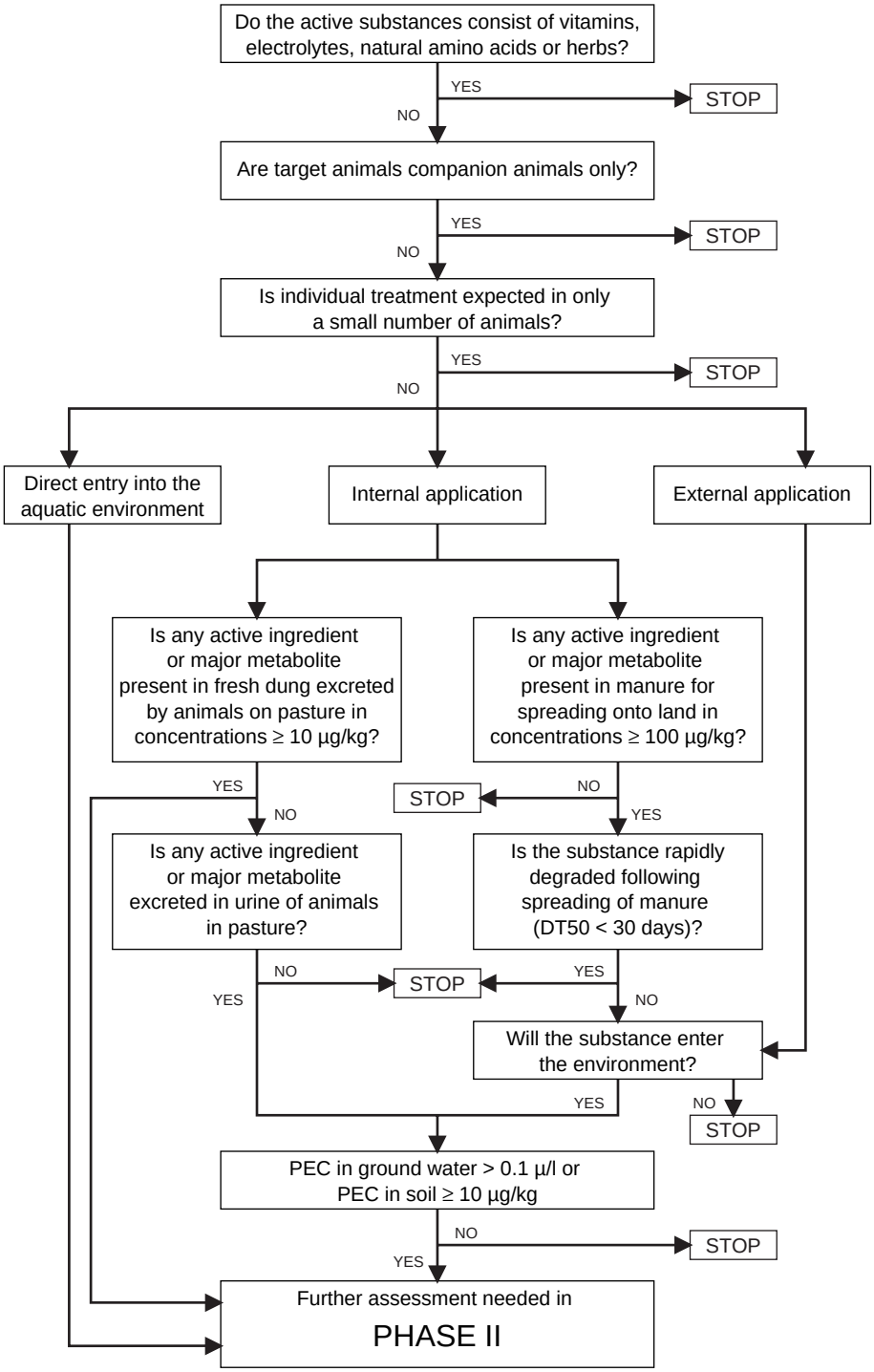


Figure 5.3. Phase II in the EMEA procedure for assessing risk of non fish medicines to the environment.

Figur 5.3. Fase II i EMEA-proceduren for miljørisikovurdering af de veterinære medicinale produkter, som ikke er fiskemedicin.

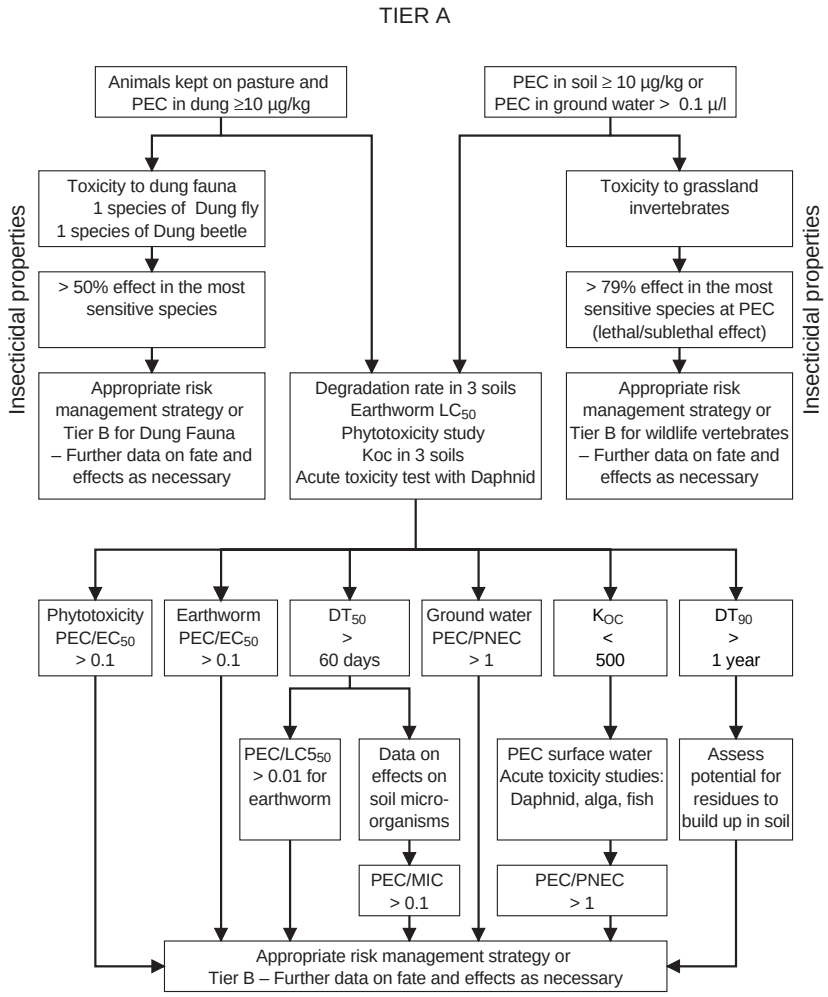
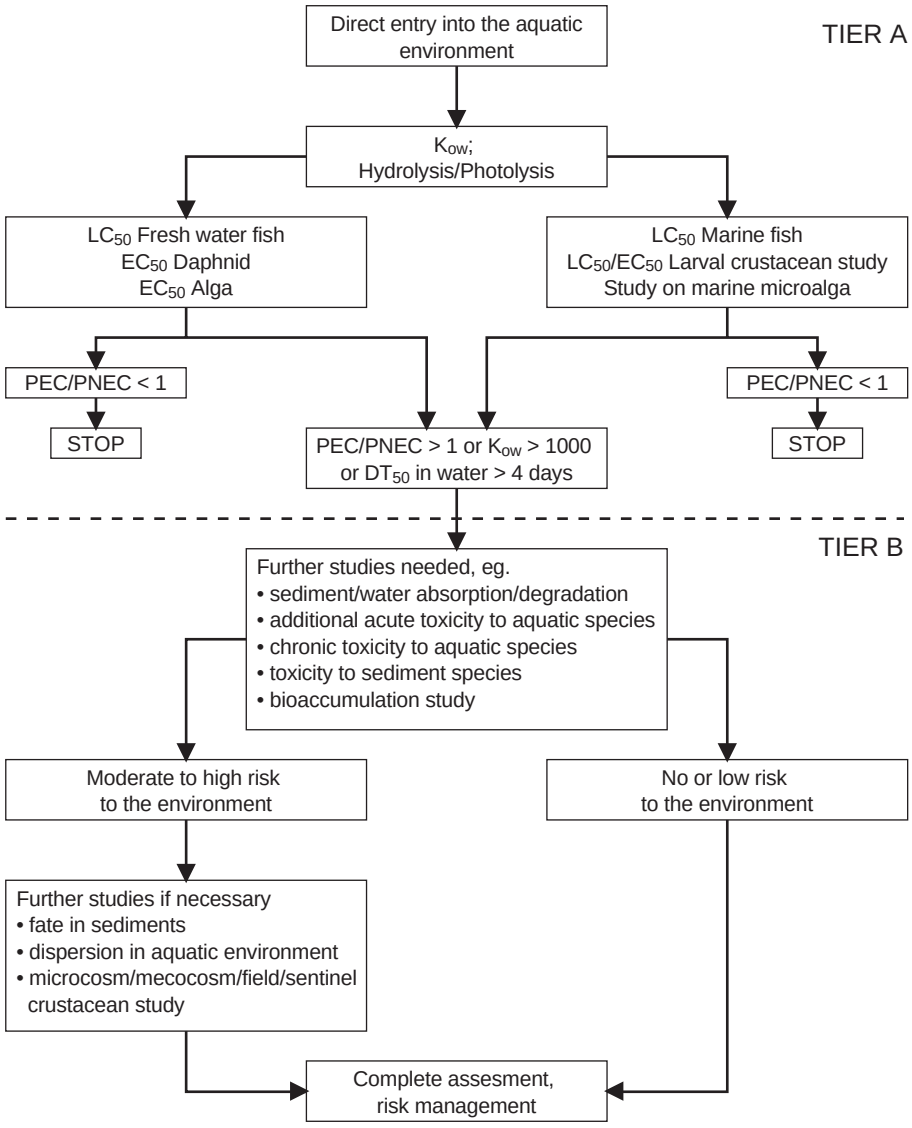


Figure 5.4. Phase II in the EMEA procedure for assessing risk of fish medicines to the environment.

Figur 5.4. Fase II i EMEA-proceduren formiljørisikovurdering af medicinske produkter for fisk.



5.4 **Phase 1**

The first step in Phase I of the EMEA guideline, is to identify the uses and the active ingredients, which is not assumed to lead to any significant environmental exposure or pose significant risk, e.g. vitamins, electrolyte or natural amino acids. The identification of substances which are presumably non-toxic or very low-toxic, aims at avoiding unnecessary and unjustified testing and assessment efforts. Although e.g. substances used for treatment of companion animals (pets) and individual treatment of domestic animals may be produced and used in relatively large quantities, the treatment is not expected to lead to any significant exposure of the environment. Similar to the use of human medicine, medicinal products for companion animals may, however, if washed out to the drains by rainwater, be found in municipal waste water treatment plants.

This differs from the evaluation of new chemical substances, where a certain quantity marketed triggers the requirement of experimental investigations, no matter assumed toxicity. For existing substances another priority is valid. This is briefly based on a first IPS screening for risk and national priority among the high production volume chemicals (see 5.2) and uses already available data.

After the first elimination of substances, the EMEA guideline provides three different assessment schemes, depending on the application to the animals or exposure to environment: Direct entry into the water; internal application or external application. If applied directly to water, the phase II evaluation gives a procedure for prediction of the environmental concentration (Predicted Environmental Concentration - PEC). The same applies for externally applied medicine when substances are likely to enter the environment, e.g. the soil. The guidance document does not lay down criteria for estimating if and how the substances may enter the environment by external application. In the case of internally applied medicine, a cut-off concentration in manure and fresh dung regulate the need for further assessment. If the concentration of the active compound or major metabolite is found in concentrations below 100 µg/kg in manure and in concentrations below 10 µg/kg in fresh dung and no active ingredient or major metabolite is excreted by animal urine on pasture, no further assessment is considered necessary. If substances are degraded with a half-life faster than 30 days after spreading of manure/slurry on land, no further assessment is necessary, even though the concentration of the substance exceed a concentration of 100 µg/kg in manure. After the evaluation of the possible exposure routes, with the above mentioned possibilities to stop further assessment, the EMEA document prescribe that further assessment is needed, as described in a phase II decision tree, only if the PEC in groundwater and soil exceed 0.1 µg/l or 10 µg/kg, respectively.

The ERA of new and existing substances differs in several aspects from the initial risk assessment of veterinary medicinal substances. First of all, regardless of substance category the fundamental steps for risk assessment, i.e. hazard identification, exposure and effect assessment, risk characterisation and possible risk management, as laid down in the TGD for new and existing substances, is not followed in the risk assessment procedure found in the EMEA guideline. In several instances during initial exposure assessment it is possible to stop further assessment Furthermore, in cases where PEC for groundwater is < 0,1 µg/l or PEC for soil is <10 µg/kg no further assessment is required. In such cases no PNEC is elaborated and risk characterisation, by a comparison of PNEC and PEC, not given.

5.5 **Phase II**

Phase II deals separately with fish medicines and other veterinary medicinal substances. Major differences exist between the risk assessment of these two groups of medicines, but the rationale behind this is not properly explained in the guideline. Although variation in the exposure situation in the aquatic and terrestrial environment is large, the same principle in risk characterisation could be applicable with a few amendments.

5.5.1 **Tier A**

The Phase I of the ERA of veterinary substances does not require a collection of toxicity data. Only in cases where the cut off values for PEC in dung (10 µg/kg), manure (100 µg/kg), soil (10 µg/kg) or groundwater (0.1 µg/L) are exceeded, do the assessor has continue to Tier A of phase II. . Here data on biological effects for the first time has to be conducted for both medicines applied on soil and medicines applied directly in the water.

Veterinary medicinal substances other than fish medicines

In phase II, a minimum of information about physical/chemical and ecotoxicological properties must be obtained for all drugs. For example, e.g. K_{oc} and degradation rate in 3 soils, toxicity test using plants, earthworms and if groundwater is subjected to risk also Daphnia. As indicated in the explanatory notes, but not described in the Decision Tree (*Figure 5.3*), the phase II - Tier A should include more detailed evaluation of the possible fate and effects of the drug and/or its major metabolites. Major metabolites are defined as metabolites with a PEC higher than 20% of the applied dose. For comparison, major metabolites of pesticides are defined as metabolites with a PEC higher than 10% of the applied dose . In phase II -Tier A, it should be considered whether there is indication of significant air exposure or exposure of freshwater systems by run-off. This is different from the ERA of chemicals, where all relevant compartments (areas of concern) in principle should be evaluated during the initial phase. Areas of concern for chemicals are: water, sediment, soil, air, secondary poisoning and the function of waste water treatment plants. Of these it is only the last that may be unimportant for veterinary drugs.

In cases where PEC in dung or soil exceed 10 µg/kg, K_{oc} and degradation rates in three different soil types have to be determined. Furthermore, a phytotoxicity tests and an acute toxicity tests with earthworm is required. For veterinary drugs with insecticidal properties additional toxicity tests on fauna are required. If the PEC in groundwater is estimated to be higher than 0.1 µg/l the acute toxicity test with Daphnia has to be included in the basic investigations in Tier A. The determination of Koc and degradation rates may lead to a demand of further studies. PEC for surface water and acute toxicity tests with algae, Daphnia and fish have to be include if the K_{oc} < 500. Data on the effects of the drug to soil micro-organisms is required if half-life of the parent compound is longer than 60 days. The recommendation of tests with plants and earthworms both refer to EU and OECD test methods, whereas an US-FDA microbial growth test is recommended.

The risk characterisation used in the EMEA guideline for veterinary medicinal substances differs from the ERA used for new and existing chemicals on the following issues.

1. The risk characterisation of veterinary drugs does not consist of a direct PEC and PNEC comparison, which is an essential principle of the risk

characterisation for new and existing chemicals. Instead effect values are compared directly with the PEC.

2. In cases were half-life has been estimated to be shorter than 60 days in three soils, the basic requirements for test species only includes two throphic levels (earthworms and plants), compared to three level in the risk assessment of new and existing chemicals. If DT_{50} > 60 days, a MIC for microorganisms is required.

The risk characterisation of veterinary drugs does not consist of a direct PEC and PNEC comparison. Instead are the effect values compared directly with the PEC. This is mainly an academic difference if the actual application of safety factor is the same in the risk estimation. However, this is not the case. For compounds with a DT_{50} < 60 days, the exposure-effect ratio (e.g. PEC/EC_{50}) has to exceed 0.1 to initiate an appropriate risk management strategy or a collection of further data on the fate effect (Tier B). Since PNECs normally are derived by the application of a assessment (safety) factor on the EC_{50}-value (or NOEC), this correspond in reality to an application of an assessment factor of only 10. In cases of DT_{50} > 60 days the risk quotient for earthworms has to exceed 0.01 to initiate further measures, which correspond to a safety factor of 100.

These assessment factors are not in general accordance with the ERA procedure for new and existing chemicals (c.f. Technical Guidance Document to Directive 93/67/EEC and Commission Regulation No. 1488/94), where a use of an assessment factor of 1000 is prescribed as a starting point on the lowest short-term effect data from studies of at least three throphic levels, e.g. algae, daphnia and fish or plants, earthworms and micro-organisms. An assessment factor of 10 should normally, according to the TDG, only be applied when long-term NOECs are available from at least three throphic levels. The same assessment factors are also used for the high priority existing chemicals that annually are evaluated in a detailed risk assessment. The number of existing chemicals evaluated in EU each year is presently around 20. As a consequence of this, the ERA procedure of veterinary drugs, not used as fish medicine, generally uses a 10-100 times lower margin of safety in the effect assessment than the procedure used for industrial chemicals. The absence of PNEC calculation for terrestrial organisms and the indirectly choice of lower safety factors is not discussed in details anywhere in the EMEA guideline. However, the principles for evaluation of the undesired effects of veterinary drugs do to some extent resemble the approach and principles that are used to assess the risk on non-target soil fauna by using pesticides on agricultural land, as laid down in Directive 91/414/EEC (Uniform Principles). Some countries, like Denmark, have expressed concern about whether the margin of safety applied in the Uniform Principles is sufficient to effectively protect non-target organisms towards unintended adverse effects of pesticides, and have therefore approved the use of higher assessment factors (*Box 5.2* for a discussion of risk management versus risk acceptance).

For veterinary substances, which have insecticidal properties, additional information on their toxicity to dung or grassland invertebrates is required if PEC exceed 10 µg/kg in dung or soil, respectively. Examples on evidence for insecticidal activities are given in Annex III of the EMEA guideline. The recommended test strategy for veterinary drugs with insecticidal properties follows guidelines given by the International Organisation of Biological Control (IOBC), which do not require a dose-respond calculation but rather gives cut-off values based on the effect level at relevant test concentrations. Concern for the environment is recognised in cases where more than 50 or 79% of the fauna is affected at PEC in dung and soil, respectively. If this is

the case an appropriate risk management strategy or collecting of additional information on fate and effects has to made in a Tier B.

For veterinary drugs a risk characterisation for the groundwater compartment has to be made if PEC > 0.1 µg/l. The PNEC is derived by an application of an assessment factor of 100 based on the result from the acute test with *Daphnia magna*. In comparison the groundwater scenario in the TGD for chemicals is solely based on a risk assessment of human health a result of exposure through groundwater. The PNEC for human health is based on long term mammalian toxicity tests.
For very persistent veterinary drugs with a DT_{90} exceeding one year an appropriate risk management strategy has to applied or the applicant has to deliver more detailed information about fate and effects of the substance as prescribed in Tier B.

Fish Medicine
The ERA of fish medicine or veterinary medicinal substances released directly into water systems, also include drugs used in rearing and production of crustacean etc. Basic requirements includes K_{ow}, an estimation of hydrolysis and/or photolysis, and toxicity studies with species covering three throphic levels, e.g. algae, daphnia (or other crustacean) and fish. Depending on the use of the medicine an evaluation of the fresh-water and/or marine system has to be made. On basis on the collected information a risk characterisation is performed (PEC/PNEC). A factor of 100 is used on the basic short term toxicity data set, based on the recommendation from a OECD workshop on extrapolation of laboratory toxicity data to the environment (OECD Monograph no. 33). Also in this respect the choice of assessment factor (100) is a factor of 10 below the one required for industrial chemicals on similar data.

Concern for the environment is recognised in cases where PEC/PNEC > 1 **or** K_{ow} > 1000 **or** the half-life in water exceed four days. In such cases further assessment in a Tier B is required.

5.5.2 Tier B

If any of the trigger values are exceeded further assessment according to Tier B is required. At this stage the guidelines suggested in the EMEA document should only be considered as recommendations to the applicant, as many of the suggested tests, especially for the terrestrial environment is not standardised or even well developed. Therefore it is recommended to include the regulatory authorities already at the planning stage of tests required in a Tier B approach.

Veterinary medicinal substances other than fish medicines
As it was the case in Tier A, the ERA for the terrestrial environment (not presented in a decision tree) is mainly based on the ERA for pesticides, and includes e.g. identification of metabolites from the transformation pathway; effect on the microbial activity in the carbon and nitrogen mineralisation processes; sublethal effects on earthworms; further laboratory studies on the effects on plants and invertebrates found in dung and grassland or finally field studies on possible adverse effects caused by the substance on relevant plant and animal species.

An assessment of poisoning of terrestrial vertebrate wildlife following use or disposal of veterinary medicinal products has to be made for the first time in Tier B. This includes both direct effects and biomagnification effects. An arbitrary classification system of toxicity to wildlife as recommended by the US-EPA is used to indicate whether there is need for further assessment. The

classification is based on avian single-dose oral LD_{50}, and an avian dietary LC_{50} test which group the substances into 5 different classes ranging from practically non-toxic to very highly toxic. If the substance is found highly or very highly toxic further assessment is needed, whereas for "practically non-toxic" substances no further assessment is needed. For "slightly" and "moderately" toxic substances the EMEA guideline states that the need of assessment depends on whether the level of exposure is sufficiently high (not specified). The risk assessment procedure for veterinary substances on vertebrate wildlife differs on a few minor issues compared with the ERA for chemicals.
Whether there is a need for assessing secondary poisoning is made at a later stage than for chemicals, as the consideration of whether the substance has any indication of bioaccumulation potential has to be made in the initial phase for chemicals. If the compound has a potential for bioaccumulation in fish **and** belongs to a certain classification (T+/T/Xn and R48/R60-R64) a risk characterisation has to be made. The EMEA document is less specific on the criteria for bioaccumulation, but refer to information collected as part of the residue package, i.e. information concerning the use of substances in domestic animals and pharmacokinetic studies in fish. For toxic compounds used for dips a warning printed on the package warning users of the need to keep wildlife away from the dip. For toxic compounds with a likely exposure of wildlife through feeding on terrestrial invertebrates or aquatic species such as fish, a procedure for risk assessment is outlined in the EMEA guideline. A collection of sufficient toxicity data is recommended if available in the literature. The EMEA guideline indicates that an assessment factor of 10 should be applied to toxicity data on species likely to be exposed or to a wider range of toxicity data.

Fish medicine
In cases where PEC/PNEC > 1 or DT_{50} in water is longer than 4 days, further aquatic studies have to be included in the final ERA of fish medicines. These may include additional acute toxicity studies on aquatic species; long-term toxicity studies to aquatic species; toxicity studies on sediment species and bioaccumulation studies, depending on estimations on the environmental fate of the substance. If degradation studies indicate a short exposure period, the EMEA guideline prescribes that the PEC should be considered as a short-term PEC, to which it is only necessary to compare short-term effects on aquatic species. This leads to a recommendation of adjusting the assessment factor used on the basic acute toxicity set down to only 10, as it is no longer considered necessary to take long-term effects into account. It is not explicit in the guideline when exposure is considered to be acute or long term. This procedure of veterinary medicinal products is somehow different from the procedure used for chemicals, which do not take biodegradation into account when recommending assessment factors. For chemicals only a change in the set of toxicity data, i.e. changes in the number of test species or by going from acute to chronic exposure, may alter the use of assessment factors, whereas the biodegradation data is used when estimating PECs..

In cases where application is repeated, which is not unusual in many fish farms, the exposure may be more or less chronic although the substance is broken down relatively fast. Therefore, reduction in the assessment factor due to degradation properties should only be accepted if repeated exposure does not occurs or occurs at very long intervals like it is discussed in the section concerning intermittent releases of industrial chemicals in the TGD.

In cases where chronic exposure is likely, long-term studies on the most sensitive group of the acute toxicity base-set should be conducted. In deriving the PNEC on the basis of this test, the guideline prescribe the use of an

assessment factor of 10. For chemicals a safety factor of 10 is only prescribed in cases of long-term studies of at least three species or on data from long-term studies on the most sensitive species in the short term tests, supplemented by at least one other NOEC.

In Tier B of Phase II, a guideline for assessing the fate and effects of veterinary substances in the sediment compartment is given for the first time. This is at a later stage than for chemicals, where a risk characterisation for all relevant media is an essential part of the basic RA. The EMEA guideline suggests an initial assessment of PEC based on an estimate of the partitioning between sediment and water as extrapolated on basis of the physical/chemical properties of the test substances. The estimated interstitial water concentration should be compared to the PNEC derived for water column species as a first screening for risk. If the PEC/PNEC ratio is of concern or if the compounds, due to e.g. very lipophilic properties, is unsuited for such extrapolation, test using spiked sediment must be carried out. The EMEA guideline recommend the same assessment factors for deriving PNEC for the sediment fauna as for the assessment of the pelagic PNEC. These are again one order of magnitude lower than in the ERA of chemicals.

As studies using spiked sediment is time consuming and expensive it makes sense to recommend a first risk characterisation based on extrapolated values. This is very much in line with the prescription in the TDG for chemicals. However, as this initial ERA of sediment is not associated with extra costs, it should be considered to employ this assessment already in Tier A, on equal terms with fresh and marine waters, for all veterinary substances released directly into the aquatic environment. When concern for the benthic fauna is identified by such an initial risk characterisation or if the K_{ow} is higher than 1000, additional information using tests with spiked sediment is required in Tier B.

A parallel to the discussion above on secondary poisoning for the terrestrial environment can be made for the aquatic environment. It is not in line with the ERA of chemicals, not to have at least a first risk assessment of secondary poisoning in Tier A. Accumulation studies have to be carried out if the pharmacokinetic studies in fish for veterinary drugs indicate a potential for bioaccumulation. From the concentration of residues that are considered likely to occur in fish, on basis of the bioaccumulation studies, secondary poisoning is assessed by assuming a worst case situation where a 2 kg heron eats 500 g of contaminated fish pr. day. The risk characterisation follows the one for wildlife in the terrestrial environment, using the same assessment factors.

On basis of the initial studies in Tier B, the assessor has to estimate whether the substances pose a moderate to high risk to the environment or only pose a low or no risk at all to the environment. The EMEA guideline do not provide any criteria for deciding the level of acceptable risk, leading to a completion of the ERA, or a non acceptable risk leading to further studies, e.g. fate studies in sediment or aquatic field or mesocosm studies.

Box 5.2 Risk management and risk acceptance

The risk characterisation and the following risk classification of new and existing chemicals may necessitate a reduction of risk. For new and existing chemicals the process of developing strategies for risk reduction are given in a special Risk Reduction Guidance . For priority substances the repporteur shall suggest a strategy for limiting risk of the substance for man and environment, including control measures and/or surveillance programmes. Risk reduction can take many forms and many risk management decisions is often made on voluntary basis. Whether risk should be reduced or not is depending on the result of a detailed risk-benefit analysis. The overall objective of such a process is to identify risks and benefits by a single substances and a comparison of these to the risk and benefit of alternative substitutes. Economy is an integrated part of such a risk-benefit analysis. A substitution or ban may generate major economical impact. Protection of man and environment may hence be very cost-intensive and the highest possible protection should therefore be achieved by the money lost. The loss represent in this way the price society is willing to pay to reduce risk (risk acceptance).

The guidance document on risk assessment of new veterinary medicinal products do somehow accept a lower margin of safety compared to industrial chemicals. . This may also in some circumstances be the case for pesticides. An argument often presented for accepting higher environmental risk by pesticides, is that they are released to the environment with the purpose to destroy specific harmful target organisms and hence application is associated with large beneficial result for the growth of crops. This may also be the case for fish medicine released directly into the water, but not for most veterinary substances released by dung or sewage sludge to the terrestrial environment. Although the use of veterinary substances without any doubt is associated with large benefit for the producers of domestic animals, the benefit is not a result of the release to the environment.

In principle risk management should not influence the processes and assumptions made in the risk assessment. Therefore both veterinary substances and pesticides should be evaluated on the same basis as other chemicals If regulators or society, due to obvious beneficial results, are willing to accept a higher degree of environmental risk this could be realised through changes in the risk management and risk reduction strategies.

5.6 **Summary and conclusions**

The risk assessment procedure for new and existing substances (chemicals) has briefly been reviewed and compared to the risk assessment of veterinary medicinal substances (drugs). A comparison reveal a number of differences of more less important character. The most important being:

- No guidance exist on how and when to assess the environmental risk of already existing veterinary drugs, and apparently there is no current national or international regulatory strategy on how to deal with environmental risk of existing veterinary drugs.

- The estimation of PEC for drugs is different from that of new and existing chemicals and plant protection products. The major environmental release is from treated animals, and is influenced by e.g. treatment intervals, dosages, formulation of drugs, metabolisation of drugs and by the species of animal treated.

- The ERA procedure of drugs not used as fish medicine may stop further assessment on the basis of arbitrary cut-off values for the environmental concentration, i.e. a risk characterisation for drugs found in concentrations below 10 µg/kg in dung or soil can be left out because no risk is assumed. A half-life shorter than 30 days in soil of the parent compound may also stop the need for further assessment.

- When making the risk characterisation by comparing the exposure level with the effect level of drugs (risk quotient), this is not done by directly

estimating the PEC/PNEC ratio, but rather as a direct comparison of effect data with the PEC (e.g. PEC/LC_{50}). The required assessment factors used for deriving PNEC for the terrestrial environment is at least a factor of 10 lower than the assessment factors prescribed for assessing chemicals, and in cases of compounds with half-life shorter than 60 days it is a factor of 100 lower. In the ERA of fish medicine the assessment factor is generally a factor of 10 lower than that for chemicals.

- When conducting the initial risk characterisation for VMP, risk to the sediment and risk for secondary poisoning is not considered. Such a risk characterisation will only be made if the compounds are submitted for a further assessment in a Tier B of Phase II.

6 Conclusions

This report presents the amount of both therapeutically and non-therapeutically veterinary medicinal products used in Denmark. On the basis of the information collected on use, fate and effects of veterinary medicinal products is it possible to draw a number of overall conclusions

- No public available database exists on the therapeutically use of veterinary medicines in Denmark. Based on data from Danish Medicines Agency, this report present the amount used in 1996 and 1997.

- The Danish Plant Directorate calculates annually the total consumption of growth promoters and medicines for feed additives on single active substance level. Data from the period 1986-1998 is presented in this report.

- Data for selected A and B labelled therapeutically used veterinary medicines is included in this report. The sale of V and H labelled substances, such as antiparasitic drugs and, and H labelled compounds, such as acetylicaceticacid, are not registered

- Data showed the following use of VMPs in 1997:
  - ◊ 14 tons for the alimentary tract and metabolism
  - ◊ 29 kg of hormones
  - ◊ 230 kg of substances for CNS-active products
  - ◊ 236 kg of antiparasitic drugs
  - ◊ 48.5 tons of antibiotics used for therapeutic applications

- The quantity of antiparasitic drugs sold as V-labelled products is most likely significant.

- More than 105 tons of antibiotics were used in 1997 non-therapeutically as growth promoters.

- 17 tons of active substance was used as coccidiostatic in the production of poultry.

- The main environmental release of veterinary medicines is through the faeces and urine of treated animals. Very little information is available about the fate of veterinary medicinal products when stored in manure and slurry tanks. It has, however, been shown that certain excretion metabolites can be reactivated and converted into the parent compounds in samples of liquid manure.
- Biodegradation data showed that half-lives varies from a few days to years depending on characteristics of the substance, the matrix (e.g. soil, water or manure) and on environmental conditions such as temperature, humidity and pH.

- Although submitted to a number of uncertainties, PEC_{soil} calculations was made for a number of substances. Worst case PEC_{soil} estimation was made for hormones (0.01-0.05 µg kg^{-1}), antibiotics (0.2 to 9 mg kg^{-1}) and substances used for treatment of diseases associated with the alimentary tract and the metabolism, (0.04 - 5.7 mg kg^{-1}).

- Very few field measurements are available to verify PEC calculations. Oxytetracycline reach concentrations between 0.1 and 11 µg/kg in sediments located in the vicinity of fish farms For cattle, dung concentrations of 1.6 mg / kg ivermectin was measured one week after injection with 0.2 mg ivermectin/kg body weight. After 17 days a reduction in dung concentration to 0.3 mg / kg dung was found.

- Antibiotics are shown toxic to algae, crustaceans and fish. Especially blue-green algaes seem very sensitive to some antibiotics

- No information is available on effects of VMPs on soil living organisms or soil functions

- Many studies have shown that antiparasitic drugs may be lethal for beneficial insects living in dung voided several weeks after treatment of cattle.

- Information is lacking on the ecological consequences of a continuous environmental exposure, e.g. in the cases of *boli* slowly releasing antiparasitic drugs during an entire grazing season, and on the fact that the environment often will be exposed to many drugs and/or other hazardous chemicals simultaneous.

- A Guidance Document on how to assess environmental risk of new (i.e. post 1.1.1997) veterinary medicinal products have been published by the EMEA. A guidance on how to assess the environmental risk of already existing VMPs. Apparently there is currently no national or international regulatory strategy on how to deal with environmental risk of existing VMPs.

- A number of deviations exist between the risk assessment procedure of veterinary drugs and the procedure used for new and existing chemicals and plant protection products.

- The procedure of environmental risk assessment (ERA) of drugs may stop further assessment on the basis of arbitrary cut-off values for the environmental concentration, i.e. a risk characterisation for drugs found in concentrations below 10 µg/kg in dung or soil can be left out. A half-life shorter than 30 days in soil of the parent compound may also stop the need for further assessment.

- The required assessment factors used for deriving PNEC for VMPs in the terrestrial environment is at least a factor of 10 lower than the assessment factors prescribed for assessing chemicals.

7 Recommendations

On the basis of the overall conclusions drawn above and the identification of gabs in our knowledge on consumption, release, fate and effects of veterinary medicinal products a number of recommendation is made. The recommendations do not represent the opinion of the individual academia or regulatory authority organisations represented in the advisory group but reflects the opinion of the experts of the group.

The aim of the recommendations is to stimulate regulatory, academia and industry efforts to improve the current understanding and assessment of environmental risks associated with the use of veterinary medicinal products. It is by no means the aim of the recommendations to preclude the use of veterinary medicines in animal health treatment or to hinder a rational medicinal management of livestock in Denmark.

The report identifies a considerable lack of basic knowledge on the environmental release, fate and effects of veterinary medicinal products. Therefore, it is recommended that research in environmental fate and ecotoxicological effects of veterinary medicinal products is stimulated.

The report has furthermore identified certain limitations regarding the assessment of environmental exposure and effects of veterinary medicinal products. In order to improve such assessments, the following recommendations should be considered:

- It should be considered to register the use of both prescription and non-prescription veterinary medicinal products. This would provide an overview of all veterinary medicinal products used in Denmark. At present prescription veterinary medicinal products (A- and B-labelled products) are registered, but non-prescription veterinary medicinal products (H- and V-labelled products) are not.

Antimicrobial active substances and antiparasitic substances constitute the greater part of veterinary medicines used in Denmark. Harmful effects on non-target fauna, flora and microbial organisms have been reported for these types of veterinary medicines. Furthermore, the environmental release of these substances and/or their metabolites may be of significance. Nevertheless, the actual impact on the environment of these products is not known. Therefore, it is recommended to consider an evaluation of individual existing high-use substances from these product classes. Such an evaluation could include present concepts and principles in environmental risk assessment procedures for veterinary medicine, and where relevant those employed for other use categories of substances.

Other therapeutic products used in lower quantities (e.g. hormones), which may be suspected of having potential environmental impact may also be considered for further evaluation.

Actions according to the recommendations given above should be considered being made known to other regulatory and scientific bodies in the EU member states for stimulating further improvement of the current EU methodology for environmental risk assessment of veterinary medicinal products.

The advisory group has discussed the current methodologies of environmental risk assessment of veterinary medicinal products in the European Union. *In lieu* of the recommended use of arbitrary exposure related cut-of-values in the evaluation of veterinary substances, in addition, it may be considered to substitute the use arbitrary cut-of-values in soil and manure with triggers based on biological effects with relevant test organisms. Another alternative is to conduct an actual risk assessment of VMPs, i.e. a comparison of exposure and effects for target environments/environments following the same principles as these for new and existing chemicals.

Furthermore, it may be useful to include new endpoints for the evaluation of some veterinary medicines. Compared to industrial chemicals which often have a general narcotic mode of action, veterinary medicines generally have a specific mode of action. In the light of the current doubt about the existing standardised test battery being sufficient to evaluate e.g. the risk of endocrine disrupters, new and more appropriate test methods for evaluating the risk of certain veterinary medicines could be considered. Prior to the implementation of such new tests, they need, however, to be developed, validated and standardised.

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9 Appendix A - List of CAS and ATC codes for substances quantified.

Drug	CAS nr	ATC code
Neomycinsulfat	1405-10-3	QA07AA01
Dihydrostreptomycin	128-46-1	QA07AA90
Colistinsulfat	1264-72-8	QA07AA10
Benzetimidchlorid	5633-14-7	QA03AB90
Alfa-tocopherolacetat	-	QA11JA
Boric acid	10043-35-3	QA12AX
Menbuton	-	QA05AX90
Calciumglyconat	299-28-5	QA12AX
Oxytocin	50-56-6	QH01BB02
Medroxyprogesteronacatat	71-58-9	QG03DA02
Methylprednisolonacetat	53-36-1	QH01BB02
Dinoprost	551-11-1	QG03DA02
Gonadorelin	33515-09-2	QH01CA01
Choriongonadotropin	9002-61-3	QG03GA99
Dexamethasonisoniconitat	55812-90-3	QH02AB02
Serumgonadotropin	9002-70-4	QG03GA99
Flumetason	2135-17-3	QH02AB90
Proligeston	23873-85-0	QG03DB90
Vasopressin (arginin form)	113-79-1	QH01BB02
Vasopressin lysine form)	50-57-7	QH01BB02
Luprostiol	67110-79-6	QG02AD91
Buserelinacetat	68630-75-1	QH01CA90
Sulfadiazin	68-35-9	QJ01EW10
Clindamycin	18323-44-9	QJ01FF01
Sulfatroxazol	23256-23-7	QJ01EW14
Tetracyclin	60-54-8	QJ01AA07
Chlortetracyklin	57-62-5	QJ01AA03
Sulfadoxin	2447-57-6	QJ01EW13
Benzylpenicillin	61-33-6	QJ01CE01
Sulfapyrazol	852-19-7	QJ01EQ01
Spiramycin	8025-81-8	QJ01FA02
Metronidazol	443-48-1	QJ01RA90
Penethamathydroiodid	808-71-9	QJ51CE90
Baquiloprim	102280-35-3	QJ01EW11
Tiamulinhydrogenfumarat	55297-96-6	QJ01XX92
Cloxacillinnatrium	642-78-4	QJ51RC01
Gentamicinsulfat	1405-41-0	QJ01GB03

Bacitracin	1405-87-4	QJ51BC
Spectionomycin	1695-77-8	QJ01FF02
Neomycin	1404-04-2	QJ51RC23
Sulfadimidin	57-68-1	QJ01EW11
Enrofloxacin	93106-60-6	QJ01MA90
Oxytetracyklin	79-57-2	QJ01AA06
Trimetroprim	738-70-5	QJ01EW10
Amoxicllin	26787-78-0	QJ01CA04
Ampicillin	69-53-4	QJ01CA01
Florfenicol	76639-94-6	QJ01BA90
Oxilinc acid	14698-29-4	QJ01MB91
Tylosin	1401-69-0	QJ01FA90
Doxycyklin	17086-28-1	QJ01AA02
Lincomycin	154-21-2	QJ01FF02
Medetomidinhydrochlorid	86347-15-1	QN05CM91
Propionnylpromazinphos-phat	-	QN05CM06
Xylasin	7361-61-7	QN05CM92
Amperozid	75558-90-6	QN05AX90
Propofol	2078-54-8	QN01AX10
Diprenorphin	14357-78-9	QN02AF99
Ketaminhydrochlorid	1867-66-9	QN01AX03
Etorphin	14521-96-1	QN02AF99
Detomidinhydrochlorid	90038-01-0	QN05CM90
Acepromazinmelat	3598-37-6	QN02AF99
Azeperon	-	QN05AD90
Tiletaminhydrochlorid	14176-50-2	QN01AX99
Metamizolnatrium	5907-38-0	QN02BB02
Butanilicainphosphat	3785-21-5	QN01BB05
Sulfaclozinnatrium	27890-59-1	QP51AG04
Spiramycin	8025-81-8	-
Virginamycin	11006-76-1	-
Flavofosfoliol	-	-
Monesinnatrium	17090-79-8	-
Salinomycin-natrium	53003-10-4	-
Avilamycin	11051-71-1	-
Carbadox	6804-07-5	-
Olaquindox	23696-28-8	-
Amprolium/Ethopabat	121-25-5	-

10 Appendix B - Danish legislation

The following laws and regulations regulates the therapeutic and non-therapeutic application of veterinary medical substances in Denmark

1) **The medicinal law, "Lægemiddelloven**[1], regulates all aspects concerning the application of medicine including veterinary medicine and medicinal feed additives.
This includes the V-marked substances such as antiparacetic agents, e.g. ivermectin, iron and vitamins used in livestock production and that can be purchased without a prescription or requisition. The consumption of V-marked substances are not quantified in any official register. Antiparacetic agents also differs from other veterinary medicines in the way that they may be sold from other sources than from pharmacies even though they are registered as a medical substance. Veterinary vaccines are primarily sold as indicated in "lægemiddelloven" through the Danish Veterinary Laboratory.
The import of feed administered drugs[2] applicable to animals and fish are regulated by the Danish Medicines Agency. Information concerning the production and sales of medicinal substances on single product level may be purchased by the Ministry of Health from companies or trade organisations, if necessary.

2)"Bekendtgørelsen om foderlægemidler til dyr[3]**",** regulates the import, export, production, control and sale of feed administered drugs for livestock. The term medical feed additive is applied to all mixtures of medicinal compounds (pre-mixtures) with one or more feed ingredients. The product is pre-mixtured before sale with the intention to either have actual treatment of diseases (therapeutic use) or help in precautions treatment (non-therapeutic use). Feed administered drugs may only be prepared with registered medicinal compounds. These preparations of medical substances used in feed named "premix" may be sold under the supervision of the Danish Medicines Agency by both specially authorised feed companies and pharmacies. Data have to be collected, on a daily basis, by the company producing feed administered drugs, on the amount sold of active substance, the name and address of the livestock owner and the prescribing veterinarian. The Danish Plant Directorate issues a list of the applied amounts of feed administred drugs to livestock on a yearly basis.

Only companies registered by the Danish Medicines Agency may produce medicinal feed additives. The permission is given in co-

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1 Lov nr. 327 af 26 juni 1975 om lægemidler (jf. lovbekendgørelse nr. 656 af 28 juli 1995), som senest er ændret ved lov nr. 1228 af 27 december 1996

2 Jf. Sundhedsstyrelsens bekendgørelse nr. 947 af 23. november 1994 om indførelse af visse foderlægemidler til dyr og fisk.

3 Sundhedsministeriets bekendgørelse nr 1140 af 15. december 1992 om fremstilling og forhandling m.v. af foderlægemidler til dyr.

operation with the Danish Plant Directorate. These companies are mutually supervised by the Danish Medicines Agency and Danish Plant Directorate.

3) "Bekendtgørelsen om foderlægemidler til fisk[4] ", regulates the use of feed administred drugs to fish. This regulation is similar as the one for animals[2] on main issues and is Also issued annually by the Danish Plant Directorate.

4) "Bekendgørelse om tilsætningsstoffer[5]" regulates the preparation, sales and application of feed additives used non-therapeutic such as antibiotics, cocciostatics and growth promoters. For each single feed additives including medicinal products the Danish Medicines Agency issues detailed regulations, for individual types of livestock, regarding withdrawal times, maximum applicable quantities for certain livestocks and maximum concentration of drug in the feed. Also here the Danish Plant Directorate supervises that issued regulations are kept and inspects from time to time the companies that are licensed allowed to prepare feed additives.

5) "Apotekerloven[6]". is the law regulating pharmacies. This law regulates all sales of medical products and determents all practise in pharmacies. The Danish Medicines Agency guides and controls the pharmacies. The Ministry of Health may decide, if it is considered necessary, that companies and trade organisations provides the ministry with information concerning the production and sales of single medicinal products.
For all veterinary medicinal products sold on prescription through pharmacies information on the veterinarians name, address, medicinal formulation, amount prescribed and date of issue is kept by the pharmacy. These informations may be coded for eventual registration. The amount of veterinary medical substances sold without prescription (H or V marked) are also registered by the pharmacies but less detailed.

6) "Husdyrsygdomsloven[7]" , is the law regulating treatment of diseases in livestock. The law primarily ensure that diseases do not spread due to unexpected side effects due to the use of veterinary medicine. For certain veterinary medical products, e.g. vaccines, immunological products and antibiotics certain withdrawal times may be introduced before for example slaughtering or milk production. In fish farms

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4 Sundhedsministeriets bekendgørelse nr 574 af 5. december 1995 om fremstilling og forhandling m.v. af foderlægemidler til fisk m.m.

5 Jf. Plantedirektoratets bekendgørelse nr. 862 af 10. oktober 1994 om tilsætningsstoffer til foderstoffer (med senere ændringer).

6 Lov nr 279 af 6 juni 1984 om apoteksvirksomhed, (jf lovbekendgørelse nr 657 af 28 juli 1995), som senest er ændret ved lov nr 1228 af 27. december 1996.

7 Jf. lovbekendtgørelse nr 381 af 7. juni 1993 om husdyrsygdomme.

withdrawal times are also introduced after antibiotic application. The Danish Medicines Agency regulates these withdrawal times for a substance in concern in connection with the registration of the compound. The Danish Veterinary and Food Administration inform all veterinarians on withdrawal times for new substances.

7) Dyrelægeloven[8], is the law concerned with veterinarian praxis. The law guides the veterinarian with the application, prescription and handling of veterinary drugs. A veterinarian may only prescribe and hand over veterinary medicinal product in connection with personal treatment of the livestock.

Hormones[9] and hormone mimicking substances may not be used in livestock production with the purpose of growth promotion. Sulfadimidin may not be used for pigs.

Treatment of livestock with antibiotics is specially regulated. Antibiotics may only be used as therapeutics to livestock when an infection is diagnosed.

8 Jf. lovbekændelse nr. 492 af 28 juni 1990 om veterinærvæsnet samt om udøvelse af dyrlægegerning, som ændret ved lov nr. 171 af 16. marts 1994.

9 Jf. Veterinærdirektoratets bekendgørelse nr. 303 af 11. maj 1995 om lægemidler til veterinær brug.

11 Appendix C. Quantification of the use of single substances.

Tabel C 1. Forbruget af lægemiddelstoffer (tons aktivt stof i) 1996 og 1997, anvendt terapeutisk til fordøjelse og metabolisme sygdomme (gruppe QA).
Table C 1. Consumption (tons active substance) in 1996 and 1997 used therapeutically for the alimentary tract and metabolism (gruop QA).

	1996	**1997**		**1996**	**1997**
Substance	**Tons**	**Tons**	**Substance**	**Tons**	**Tons**
Neomycinsulfat	3.366	3.926	Benzetimidchlorid	<0.001	-
(antibiotic)	-	-	-	-	-
Colistinsulfat	0.208	0.193	Boric acid	1.879	1.593
Alfa-tocopherolacetat	0.106	0.077	Calciumglyconat	7.629	7.748
Menbuton	0.073	0.062	Magnesiumhypophosphit	0.858	1.054
Dihydrostreptomycin	0.091	0.089	Selen	<0.001	<0.001
(antibiotic)	-		**Total**	**14.212**	**14.743**

*Tabel C 2. Forbruget af lægemiddelstoffer (kg aktiv stof) til hormon behandling i 1996 og 1997 (gruppe QH og QG). * Antibiotika givet sammen med hormoner.*
Table C 2. Consumption (kg active substance) in 1996 and 1997 of hormones (gruop QH and QG).
** Antibiotics in connection with hormones.*

-	**1996**	**1997**		**1996**	**1997**
Substance	**kg**	**kg**	**Substance**	**kg**	**kg**
Oxytocin	0.085	0.103	Choriongonadotropin	0.022	0.030
prednisolonacetat (methyl)	8.333	10.728	Serumgonadotropin	0.056	0.102
Gonadorelin	0.001	<0.001	Proligeston	1.160	0.848
Dexamethasoniconitat	2.035	1.965	Luprostiol	0.330	0.356
Flumetason	0.056	0.041	Cloprostenolnatrium	0.040	0.040
Vasopressin	0.003	-	**Total Hormones**	**27.301**	**28.673**
Buserelinacetat	<0.001	<0.001	Oxytetracyklin*	13.020	14.150
Medroxyprogesteronacatat	14.916	14.149	Sulfadimidin*	1156.0	1267.8
Dinoprost	0.303	0.349	Benzylpenicillin*	43.665	38.692
-	-	-	Streptocillin*	145.55	128.98

Tabel C 3. Forbruget af antibiotika (tons aktiv stof) anvendt terapeutisk i 1996 og 1997 (gruppe QJ01 og QJ51).
Table C 3. Consumption (tons active substance) in 1996 and 1997 of antibiotics for therapeutical treatment (gruop QJ01+QJ51).

	1996	**1997**		**1996**	**1997**
Substance	**Tons**	**Tons**	**Substance**	**Tons**	**Tons**
Sulfadiazin	3.414	4.476	Sulfapyrazol	0.969	0.397
Dihydrostreptomycin	2.557	2.806	Metronidazol	0.014	0.015
Tetracyclin/Chlortetracyklin	9.869	11.090	Baquiloprim	0.010	0.007
Benzylpenicillin	6.321	7.336	Cloxacillinnatrium	0.067	0.084
Spiramycin	0.543	0.621	Bacitracin	0.021	0.012
Penethamathydroiodid	0.637	0.686	Neomycin	0.063	0.063
Tiamulinhydrogenfumarat	2.573	2.948	Enrofloxacin	0.391	0.442
Gentamicinsulfat	0.003	0.003	Trimethoprim	0.963	1.204
Spectionomycin	0.845	0.853	Ampicillin	1.075	0.603
Sulfadimidin	0.074	0.060	Oxilinc acid	0.778	0.726
Oxytetracyklin	2.600	2.662	Doxycyklin	0.009	0.009
Amoxicillin	3.874	6.281	Danofloxacin	<0.001	0.004
Florfenicol	0.039	0.047	Cefadroxil	0.001	0.036
Tylosin	1.380	1.084	Ceftiofur	0.022	0.033
Lincomycin	2.570	2.323	Cefquinom	<0.001	<0.001
Clinadamycin	0.018	0.019	Cefaperazon	-	0.002
Sulfatroxazol	0.457	0.563	**Total**	**42.968**	**48.530**
Sulfadoxin	0.809	1.034			

Tabel C 4. Forbruget af anæstetika (tons aktiv stof) i 1996 og 1997.
Table C 4. Consumption (tons active substance) in 1996 and 1997 of Central Nervous System (CNS) active substances.

	1996	**1997**		**1996**	**1997**
Substance	**Tons**	**Tons**	**Substance**	**Tons**	**Tons**
Medetomidinhydrochlorid	<0.001	<0.001	Amperozid	<0.001	<0.001
Xylasin	0.006	0.006	Diprenorphin	<0.001	<0.001
Propofol	<0.001	0.002	Etorphin	<0.001	<0.001
Ketaminhydrochlorid	0.004	0.004	Acepromazinmelat	<0.001	<0.001
Detomidinhydrochlorid	<0.001	<0.001	Tiletaminhydrochlorid	<0.001	<0.001
Azeperon	0.002	-	Butanilicainphosphat	0.027	0.031
Metamizolnatrium	0.198	0.177	Romifidin	-	<0.001
Propionnylpromazinphosphat	<0.001	<0.001	Zolazepam	<0.001	<0.001
			Total	**0.237**	**0.230**

Tabel C 5. Forbruget af antiparasit midler (tons aktiv stof) (A eller B udlevering) i 1996 og 1997(gruppe QP).
Table C 5. Consumption of antiparacetic agents (tons active substance) in 1996 and 1997(A or B labelled substances) (gruop QP).

	1996	**1997**
Substance	**Tons**	**Tons**
Sulfaclozinnatrium	0.242	0.236
Total A and B labelled	**0.242**	**0.236**

Tabel C 6. Forbruget af antibiotika (tons aktiv stof) som antimicrobielle vækst fremmere i Danmark i perioden 1989-97 samt første halvår af 1998. Kilde: Plantedirektoratet.
Table C 6. Consumption of Antibiotics (tons active substance) used as Antimicrobial Growth Promoters in Denmark 1989-97 and for the first six months of 1998. Source: The Danish Plant Directorate.

Substance	**1989**	**1990**	**1991**	**1992**	**1993**	**1994**	**1995**	**1996**	**1997**	**1998§**
Zinkbacitracin	4.573	3.983	3.675	5.657	10.636	13.689	7.910	6.399	8.544	2.939
Siramycin	0.506	-	-	-	-	0.095	0.507	0.015	0.003	-
Virginamycin***	2.266	3.837	3.704	15.537	12.262	2.801	2.590	5.055	10.644	0.828
Flavofosfoliol	0.075	0.494	1.021	1.299	0.415	0.077	0.048	0.018	0.093	0.002
Tylosinfosfat	40.356	42.632	33.189	26.980	27.986	37.111	52.275	68.350	62.009	8.379
Monesinnatrium*	1.015	2.381	2.532	3.700	4.451	4.755	5.007	4.741	3.008	0.894
Avoparcin**	13.644	13.718	23.153	17.210	19.572	24.117	5.690	-	-	-
Salinomycin natrium*-	1.397	0.012	-	-	1.224	0.213	0.850	0.759	0.460	0.079
Avilamycin	-	0.010	0.180	0.853	0.132	0.433	1.665	2.740	0.670	0.002
Carbadox	0.296	0.850	3.135	7.221	15.536	10.012	1.181	1.985	4.153	1.189
Olaquindox	3.162	11.391	23.665	21.193	16.871	22.483	16.213	13.486	17.595	14.608
Total	67.290	79.308	94.254	99.650	109.085	115.786	93.936	105.548	107.179	28.920

* Used both as growth promoters in livestock production and as cocciostatica. Some uncertainty may therefore occur concerning the figures.
** Avoparcin has been banned in Denmark since 20. May 1995 and in EU since 1. April 1997.
*** Virginamycin has been banned in Denmark in January 1998.
§ used during the first six months of 1998.

Tabel C 7. Forbruget af Coccidiostatika (tons aktiv stof) i Danmark i 1989-1997 samt de første 6 måneder af 1998. Kilde: Platedirektoratet.
Table C 7. Consumption of Coccidiostatics (tons active substance) in Denmark in 1989-1997 and first six months of 1998. Source: The Danish Plant Directorate

Substance	**1989**	**1990**	**1991**	**1992**	**1993**	**1994**	**1995**	**1996**	**1997**	**1998§**
Amprolium/Ethopabat	3.042	3.562	3.475	2.716	2.805	2.342	1.257	1.339	0.488	0.195
Diemetrizol		-	-	-	-	-	-	0.038	0.542	
DOT	3.063			-	-	0.300	-		0.352	-
Monesin natrium	4.679	-	0.140	0.108	0.192	1.016	2.463	3.405	3.690	0.370
Robenidin	0.003	0.033	0.033	0.295	0.020	0.858	1.286	0.293	0.672	0.340
Ronidazol*		0.036	0.078		-	-	-	-	-	-
Metichlorpindol /Methylbenzoquat		0.089	2.799	1.503	1.210	3.360	3.409	4.857	3.928	0.757
Lasalocid natrium	0.485	0.075	0.090	-	-	0.005	0.011	0.773	1.359	0.795
Halofuginon	0.004	-	-	-	-	0.019	0.069	0.008	0.007	-
Narasin	0.358	1.588	1.588	5.157	5.209	6.370	4.359	3.905	1.777	2.008
Salomycin natrium	7.774	7.783	8.282	10.298	11.598	6.018	3.758	4.531	4.197	3.743
Nicarbazin		-	-	-	-	-	-	0.115	0.004	0.021
Nifurasol			-	0.395	0.246	-	0.001	0.146		0.101
Diclazuril			-	-	0.001	0.018	0.015	0.034	0.021	0.003
Nitrovin*	1.567	0.403	0.297			-	-	-	-	-
Total	**20.975**	**13.569**	**16.782**	**20.472**	**21.281**	**20.306**	**16.628**	**19.444**	**17.037**	**8.333**

* Banded

§ used during the first six months of 1998.

Tabel C 8. Opgørelse over forbruget af forderlægemidler (kg aktiv stof) i Danmark i 1995 og 1996. Kilde: Plantedirektoratet. Table C 8. Consumption of Feed Administrated Drugs (kg active substance) used in poultry. fish farms and livestock. Data from 1995 and 1996. Source: The Danish Plant Directorate.

	1995	**1996**
Medicin in feed additive (foderlægemidler) (kg active substance)	**-**	**-**
Antibiotics	-	-
Sulfadiazin/trimetroprim	1489	1013.8
Oxilinc acid	906	510.8
Eurofloxacin	17.0	0
Tiamutin	22.0	45.3
Lincomycin	1.5	3.73
Tylosin	1.9	0.6
Chlortetracycline	34.0	131.9
Trimazin	1.8	0
Substances against worms	-	-
Flubendazol. Flubenol	6.9	7.0
Thiophanat. Nemefax	11.0	7.4
Ivomectin	0	0.18
Total	**2.491.1**	**1720.7**

Tabel C 9 Forbruget af antimicrobielle vækstfremmer (tons aktiv stof) i 1995 for forskellige dyre typer. Kilde: Plantedirektoratet. Fordeling på dyrearter er baseret på skøn.
Table C 9. Consumption of antimicrobial growth promoters (tons active substance) during 1995 distributed on differet animal species, Source: The Danish Plant Directorate. Distribution amoung animal species is estimated.

Antimicrobial growth promoter	Total Tons	Pigs Tons	Cattle Tons	Poultry Tons
Flavomycin	0.048	-	-	0.048
Avoparcin	5.690	2.500	2.090	1.100
Monensin natrium	5.007	-	5.007	(2.463)*
Salinomycin	0.850	0.850	-	(3.758)*
Spiramycin	0.507	-	-	0.507
Tylosin	52.275	52.275	-	-
Avilamycin	1.665	0.265	-	1.400
Zinkbacitracin**	7.910	6.000	-	0.610
Carbadox	1.181	1.181	-	-
Olaquindox	16.213	16.213	-	-
Virginamycin	2.590	1.500	-	-

* Used as coccidiostatics.
** Approximately 1.3 Tons used in mink.
Table C 10

Calculation example showing how the data recveied from the Dansh Medical Agency was translated from packet numbers in to weight of active compound

Tiamulinhydrogenfumerat

ATC code	Number in recived data list	Official Number in Agency list	Number of packets sold in 1996	Amount of active substan-ce in gram in packet size	Total amount of compound in gram	
QJ01XX92	35	´036525	129	400	51600	premix
	36	´036533	1713	500	856500	premix
	226	´426759	120	2000	240000	
	247	´442970	59	500	29500	
	248	´453456	7	600	4200	
	319	´529453	2626	31.25	82062.5	
	320	´529461	3070	312.5	959375	
				SUM	**2223238**	**gram**
					2.22	
					tons	

12 Appendix D

D1- Detailed list on therapeutically used single substances

Drug	Pigs (fattening pigs)	Cattles (Calves)	Sheeps	Horses	Fish	Poultry
Sulfonamide						
Sulfadiazin	X	X		X	X	
Sulfatroxazol	X	X				
Sulfadoxin	X	X	X	X		
Sulfapyrazol	X	X		X		
Sulfadimidin		X	X	X		
Tetracyclines						
Tetracycline		X				
Chlortetra-cycline	X					
Oxytetra-cykline	X	X	X	X		
Beta-lactam antibiotics						
Benzylpeni-cillin	X	X		X		
Amoxilin	X	X	X			
Ampicillin	X	X	X			
Quinolones						
Enrofloxacin	X	X				X
Oxilinc acid					X	
Macrolider						
Tylosin	X	X				X
Spiramycin	X	X				X
Aminoglyco-side						
Dihydro-streptomycin	X	X	X	X		X
Gentamicin-sulphate	X					
Neomycin	X	X				
Micellaneous						
Spectiono-mycin	X					X
Penethamat-hydroiodid						
Baquiloprim		X				
Tiamulinhy-drogenfumarat	X					X
Cloxacillin-natrium	X					X
Bacitracin		X				
Trimetroprim	X	X	X	X	X	
Florfenicol					X	
Lincomycin	X					

D2- Detailed list on non- therapeutically used single substances

Drug	Pigs (fattening pigs)	Cattle (Calves)	Sheeps	Horses	Fish	Poultry
Antibiotics group A						
Zinkbacitracin	X					X
Spiramycin	X					X
Virginamycin	X	X				X
Flavofosfolipol	X	X				X
Tylosinfosfat	X					
Monensin natrium		X		X*		
Avoparcin	X	X				X
Salinomycin natrium	X					
Avilamycin	X					X
Coccidiostatics and other drúgs group D						
Amprolium						X
Amprolium-Etopabat						X
Dinitolmid						X
Dimetridazol						X
Metiklorpindol						X
Decoquinat						X
Monensin natrium						X
Robenidin						X
Ronidazol						X
Ipronidazol						X
Metiklorpindol						X
Arprinocid						X
Lasalocid natrium						X
Halofuginon						X
Narasin						X
Salinomycin natrium						X
Nicabazin						X
Nifursol						X
Maduramicin ammonium						X
Diclazuril						X
Narasin/nicarbasin						X
Growth promoting substances group J						
Carbadox	X					
Olaquindox	X					

* Source: Veterinærmedicinsk produktkatalog

13 Appendix E

Worst case PEC calculations for veterinary medical substances in Denmark

References to daily doses: Veterinærmedicinsk Produktkatalog 1997

I. Scenario applying manure from fattening pigs on arable land. 150 kg N / ha / year

Antibiotics. Feed administrated drug - premix

Active compound	Weight of livestock	Animal dose	Daily dose	Production of manure	Production of manure	Area needed	Area dose	Concentration in top 10 cm
	kg	mg/kg	g/day	kg/pig/day	kg N/pig/day	m^2	g/m^2	mg/kg
Tylosin	100	4	0,4	4,8328767	0,026274	1,7515982	0,2283629	**1,52**
Chlor-tetracycline	100	40	4	4,8328767	0,026274	1,7515982	2,2836288	**15,22**
Enrofloxasin	100	10	1	4,8328767	0,026274	1,7515982	0,5709072	**3,81**
Lincomycin	100	4,4	0,44	4,8328767	0,026274	1,7515982	0,2511992	**1,67**
Sulfadiazin	100	25	2,5	4,8328767	0,026274	1,7515982	1,427268	**9,52**

Antibiotics. Antimicrobial growth promoters. Fattening pigs: 2.5 kg daily feed (Personal communication H. Damgaard Petersen, DJF, Faulum)

Active compound	Weight of livestock	Animal dose	Daily dose	Production of manure	Production of manure	Area needed	Area dose	Concentration in top 10 cm
	kg	mg/kg	g/day	kg/pig/day	kg N/pig/day	m^2	g/m^2	mg/kg
Spiramycin	100	20 mg/kg feed	0,05	4,8328767	0,026274	1,7515982	0,0285454	**0,19**
Zinkbacitrin	100	20 mg/kg feed	0,05	4,8328767	0,026274	1,7515982	0,0285454	**0,19**
Virginamycin	100	140 mg/kg feed	0,35	4,8328767	0,026274	1,7515982	0,1998175	**1,33**
Flavofosfo-lipol	100	20 mg/kg feed	0,05	4,8328767	0,026274	1,7515982	0,0285454	**0,19**
Tylosinphos-phat	100	20 mg/kg feed	0,05	4,8328767	0,026274	1,7515982	0,0285454	**0,19**
Salinomycin	100	30 mg/kg feed	0,075	4,8328767	0,026274	1,7515982	0,042818	**0,29**
Avilamycin	100	20 mg/kg feed	0,05	4,8328767	0,026274	1,7515982	0,0285454	**0,19**
Carbadox	100	50 mg/kg feed	0,125	4,8328767	0,026274	1,7515982	0,0713634	**0,48**
Olaquindox	100	50 mg/kg feed	0,125	4,8328767	0,026274	1,7515982	0,0713634	**0,48**

Antibiotics. Administred by injections.

Active compound	Weight of livestock	Animal dose	Daily dose	Production of manure	Production of manure	Area needed	Area dose	Concentration in top 10 cm
	kg	mg/kg	g/day	kg/pig/day	kg N/pig/day	m^2	g/m^2	mg/kg
Tylosin	100	0,005	0,5	4,8328767	0,026274	1,7515982	0,2854536	**1,90**
Tylosin	100	0,01	1	4,8328767	0,026274	1,7515982	0,5709072	**3,81**
Oxytetracycline	100	0,005	0,5	4,8328767	0,026274	1,7515982	0,2854536	**1,90**
Oxytetracycline	100	0,01	1	4,8328767	0,026274	1,7515982	0,5709072	**3,81**
Benzylpenicillin*	100		1,2	4,8328767	0,026274	1,7515982	0,6850886	**4,57**
Lincomycin	100		1	4,8328767	0,026274	1,7515982	0,5709072	**3,81**
Sulfadiazin	100		1,2	4,8328767	0,026274	1,7515982	0,6850886	**4,57**

1E6 IE = 600 mg (Martindale 1996)

Hormones

Active compound	Weight of livestock	Animal dose	Daily dose	Production of manure	Production of manure	Area needed	Area dose	Concentration in top 10 cm
	kg	mg/kg	g/day	kg/pig/day	kg N/pig/day	m^2	g/m^2	mg/kg
Oxytocin*	100	0.5-2.0IE	3,424E-06	4,8328767	0,026274	1,7515982	1,955E-06	**1,30E-05**
Vasopressin**	100	1 IE	2,439E-06	4,8328767	0,026274	1,7515982	1,392E-06	**9,28E-06**

*12.5IE=21.4myg
**8.2IE = 20myg

Alimentary tract and metabolisms

Active compound	Weight of livestock	Animal dose	Daily dose	Production of manure	Production of manure	Area needed	Area dose	Concentration in top 10 cm
	kg	mg/kg	g/day	kg/pig/day	kg N/pig/day	m^2	g/m^2	mg/kg
Dexamethasoniconitat	100	2 mg pr. 20 kg	0,01	4,8328767	0,026274	1,7515982	0,0057091	**0,04**
Menbuton	100	1-2 gram pr. animal	1,5	4,8328767	0,026274	1,7515982	0,8563608	**5,71**

II. Scenario applying manure from slaughter calf on arable land. 265 kg N / ha / year
Antibiotics administred by injection.

Active compound	Weight of livestock	Animal dose	Daily dose	Production of manure	Production of manure	Area needed	Area dose	Concentration in top 10 cm
	kg	mg/kg	g/day	kg/pig/day	kg N/pig/day	m^2	g/m^2	mg/kg
Tylosin	160	0,005	0,8	12,767123	0,029863	1,1269062	0,7099083	**4,73**
Tylosin	160	0,01	1,6	12,767123	0,029863	1,1269062	1,4198165	**9,47**
Oxytetracycline	160	0,005	0,8	12,767123	0,029863	1,1269062	0,7099083	**4,73**
Oxytetracycline	160	0,01	1,6	12,767123	0,029863	1,1269062	1,4198165	**9,47**
Benzylpenicillin*	160	0.6x2	1,2	12,767123	0,029863	1,1269062	1,0648624	**7,10**
Lincomycin	160	1-2 ml/10kg	1	12,767123	0,029863	1,1269062	0,8873853	**5,92**
Sulfadiazin	160	3 ml/100kg	1,2	12,767123	0,029863	1,1269062	1,0648624	**7,10**

Hormones

Active compound	Weight of livestock	Animal dose	Daily dose	Production of manure	Production of manure	Area needed	Area dose	Concentration in top 10 cm
	kg	mg/kg	g/day	kg/pig/day	kg N/pig/day	m^2	g/m^2	mg/kg
Oxytocin*	160	2-10IE(5IE)	8,56E-06	12,767123	0,029863	1,1269062	7,596E-06	**5,06E-05**
Vasopressin**	160	2.5 IE	6,098E-06	12,767123	0,029863	1,1269062	5,411E-06	**3,61E-05**

*12.5IE=21.4myg
**8.2IE = 20myg

Alimentary tract and metabolisms

Active compound	Weight of livestock	Animal dose	Daily dose	Production of manure	Production of manure	Area needed	Area dose	Concentration in top 10 cm
	kg	mg/kg	g/day	kg/pig/day	kg N/pig/day	m^2	g/m^2	mg/kg
Dexamethasoniconitat	160	2 mg per 20 kg	0,01	12,767123	0,029863	1,1269062	0,0088739	**0,06**
Menbuton	160	1-2 gram animal	1,5	12,767123	0,029863	1,1269062	1,331078	**8,87**

CNS substances

Active compound	Weight of livestock	Animal dose	Daily dose	Production of manure	Production of manure	Area needed	Area dose	Concentration in top 10 cm
	kg	mg/kg	g/day	kg/pig/day	kg N/pig/day	m^2	g/m^2	mg/kg
Xylasin	160	0.1 mg/kg	0,016	12,767123	0,029863	1,1269062	0,0141982	**0,09**
Metamizol-natrium	160	10-40mg/kg	4,8	12,767123	0,029863	1,1269062	4,2594495	**28,40**
Butanilicainphosphat	160	100-140mg/dyr	0,14	12,767123	0,029863	1,1269062	0,1242339	**0,83**

14 Appendix F

Table F2. Fate of medical compounds in the environment.

Medical compound or residue	Therapeutic use	Process	Fate	Sphere / conditions	Reference
Veterinary threatment					
Avermectin B_1a	Antiparacetic agent	Biodegradation	$T_{½}$ = 14-28 days	Conc. 0.1-1ppm, different soils in lab. test. (Lufkin fine sandy loam and Huston clay)	Bull et al. (1984)
			$T_{½}$ = 28-56 days	Conc. 50 ppm, different soils in lab.test as above.	
		Leaching	No leaching potential		
		Plant uptake	Minor uptake of residues		
		Mobility	Koc = $4.76*10^3$	Immobile in different soils	Gruber et al. (1990)
Bacitracin	Antibiotic – growth promoter	Biodegradation	$T_{½}$ = 22.5 days (20°C)	Feces (5%) / soil matrix	Gavalchin and Katz (1994)
			$T_{½}$ = 12 days (30°C)		
Bambermycin	Antibiotic – growth promoter	Biodegradation	Persistance < 25 days (over 20°C)	Feces (5%) /soil matrix	Gavalchin and Katz (1994)
Carbadox	Antibiotic – growth promoter	Biodegradation	No informations		Goll van (1993) excist.
Ceftiofur sodium	Antibiotic	Biodegradation	$T_{½}$ = 22.2 days (pH = 5)	Aerobic degradation in soils; clay loam, sand and silty clay loam.	Gilbertson et al. (1990)
			$T_{½}$ = 49 days (pH = 7)		
			$T_{½}$ = 41.1 days (pH = 9)		
		Photodegradation	Minimal	Water	Gilbertson et al. (1990)
		Hydrolyse	$T_{½}$ = 100.3 days (pH = 5)		
			$T_{½}$ = 8.0 days (pH = 7)		
			$T_{½}$ = 4.2 days (pH = 9)		
Chloramphenicol	Antibiotic	Primary degradation		In liquid manure the main metabolite chloramphenicol glucoronide is craked by bacterial to chloramphenicol. Thus reactivating the parent drug.	Berger et al. (1986)
Chlortetracycline	Antibiotic – growth promoter	Biodegradation	After 30 days at 30°C 44% of added com pound remaind. At 20°C and 4°C, 88% and 100% were per sistent after 30 days.	Feces (5%) / soil matrix	Gavalchin and Katz (1994)

Table F2 (continued). Fate of medical compounds in the environment.

Medical compound or residue	Therapeutic use	Process	Fate	Sphere / conditions	Reference
Veterinary threatment					
Efromycin	Antibiotic - growth	Sorption	Koc = 580-11000	Different soils	Yeager and Halley (1990)
		Desorption	Only 50% of sorbed dose was desorped even with organic solvents.		
Enrofloxacin	Antibiotic	Excretion from 10 % unmetabolised animals			Vancutsum et al. (1990)
Erytromycin	Antibiotic - growth promoter	Biodegradation	$T_{½}$ = 11.5 days (20°C). At 4°C, 97% of the activity remained during 30 days	Feces (5%) / soil matrix	Gavalchin and Katz (1994)
Florfenicol	Antibiotic - feed additive in fishfarms	Biodegradation	$T_{½}$ = 4-5 days	Degrades to persistent amine metabolite at all sediment dephts	Lunestad et al. (1992a, 1992b)
Flumequine	Antibiotic - feed additive	Biodegradation	$T_{½}$ = 150 days	Surface sediment	Lunestad et al. (1992a, 1992b)
Furazolidone	Antibiotic	Biodegradation	$T_{½}$ = 50 hrs to 2 months		Roij de and Vries de (1982)
	Antibiotic - feed additive in fish farms	Biodegradation	Readily degraded to inactive metabolite	Surface sediment	Ervik (1993)
Ivermectin	Antiparasitic agent	Biodegradation	Persistence in dung < 6 days	End of spring, field conditions (spain)	Lumaret et al. (1993)
		Biodegradation	$T_{½}$ = 93 - 240 days	Laboratory, dark, 22°C in soil / feces mix	Halley et al. (1989)
		Biodegradation	$T_{½}$ = 1 - 2 weeks	Outdoor, summer soil / feces mixture	Halley et al. (1989)
		Photodegradation	$T_{½}$ = 3 hours	Outdoor, thin dry film on glass, sunlight	Halley et al. (1989)
Monensin	Antibiotic – growth promoter	Biodegradation	Persistent under an- aerobic conditions. (after 10 weeks 60-70% unchanged). More degradable under aerobic conditions.	Lab. experiments with feces	Donoho (1984)
		Biodegradation	Primary degradation within 33 days with or without manure.	Field experiments, soils	Donoho (1984)
Neomycin	Antibiotic	Excretion	Efter oral intake 97% will be excreated via lifestock feces.		
Oxolinic acid	Antibiotic - feed additive in fish farms	Biodegradation	$T_{½}$ = 150 to 1000 days	Supeficial sediment at various sediment deepts (up to 7 cm)	Ilektone et al. (1993) Samuelsen et al. (1992b)
Oxytetracycline	Antibiotic - feed additive in fish farms	Binding to sediment	$T_{½}$ = 30 to 142 days	Surface sediment at different conditions	Samuelsen (1989) Poliquen et al. (1992;1993) Ervik (1993)
		Biodegradation	$T_{½}$ = 9 days and 419 days	At two different locations with anoxic cond	Björklund et al. (1990)

Table F2 (continued). Fate of medical compounds in the environment.

Medical compound or residue	Therapeutic use	Process	Fate	Sphere / conditions	Reference
Veterinary threatment					
		Wash-out from sediment	At a sediment conc. of 285 µg / g sediment, the maximum water conc. was predicted to 0.11 µg/l. was predicted to 0.11 µg/l. At sediment conc. of 10.9 µg/g sediment a similar water conc. of 0.016 µg/l was estimated	Sediment	Smith and Samuelsen (1996)
Penicillin	Antibiotic	Biodegradation	Inactivation due to c ombinati- on of micro- bial induced enzyma- tic and chemical hydrolyse	Feces (5%) / soil matrix	Gavalchin and Katz (1994)
Streptomycin	Antibiotic	Biodegradation	Complete adsorption to clay fraction of the soil.	Feces (5%) / soil matrix	Gavalchin and Katz (1994)
Sulphadimidine	Antibiotic	Primary degradation		In liquid manure the main metabolite N-4-acetylated sulphadimidine is craked by bacterial to sulphadimidine. Thus reacti- vating the parent drug	Berger et al. (1986)
Sulphatrimetroprim Antibiotic	Biodegradation	Within one year 75 % undegrated		Surface water	Goll van (1993)
Tetracycline	Antibiotic Fish pond	Photodecomposition		Seven tetracycline metabolites were found under conditions similar conditi- ons as fish pond cultures	Oka et al. (1989)
Tylosin	Antibiotic – growth promoter	Biodegradation	At 4°C, 40% of the compound remained unchanged after 30 days of incubation. At 20°C and 30°C degradation occured rapidly	Feces (5%) / soil matrix	Gavalchin and Katz (1994)

15 Appendix G

Table G. Toxic effects of medical compounds on the environment.

Medical compound or residue Veteranry treatment	Therapeutic use	Test organisms	Toxicity	Sphere / conditions	Reference
Ampicillin	Antibiotic	Sediment bacteria *Vibrio harveyi*	Antibiotic resistance > 100 mg/l	growth rate	Sandaa et al. (1992) Thomulka et al. (1993)
Amitriptylin	Psycopharmaca	*Daphnia magna*	EC50= 5.0 mg/l		Lilius et al. (1995)
Amprolium	Antiprotozoal	Nitrifying bacteria	No effect at 204µg / g manure of amprolium		Warman (1980)
Aureomycin	Antibiotic	Nitrifying bacteria	No effect at 22.5 µg / g manure of auromycin		Warman (1980)
Avermectin B1	Antiparasetic agent	*Musca vetustissima* (Bushfly) *Onthophagus binodis* (dung beetle)	No bush flies survived from egg to adult following cattle injectioon of 200µg/kg Not affected in dung of cattle treated with avermectin B1		Ridsdill-Smith (1988) Ridsdill-Smith (1988)
Avilamycin	Antibiotic – growth promoter	Anaerobic digestion	No overall effects on gas production		Sutton et al. (1989)
Bacitracin	Antibiotic – growth promotor	*Daphnia magna* *Artemia salina* *Daphnia Magna* Plants	LC_{50} (24 hours) = 126 mg/l LC_{50} (48 hours) = 30 mg/l LC_{50} (24 hours) = 34 mg/l LC_{50} (48 hours) = 21.8 mg/l LOEC = 5 mg/l EC50 (24 hours) = 126 mg/l EC50 (48 hours) = 30 mg/l	 Effects on plants mean root weight and mean stalk Leaves weight dramatically reduced.	Brambilla et al. (1994) Migliore et al. (1997) Di Delupis et al. (1992) Brambilla et al. (1994)
Bromocyclen	Antiparacitic agent	Algae *Daphina magna*	EC10 > 100 mg/l EC50 > 100 mg/l LC10 = 0.4 mg/l LC 50 = 0.7 mg/l NOEC = 0.1 mg/l LC10 = 0.064 mg/l LC 50 = 0.353 mg/l	 Acute test Reproduction test	Kopf (1995)
Carbadox	Antibiotic – growth promoter	Mutagensity test	No effect on overall manure composition		Cihak et al. (1983) Kreuzer (1994)
Chloramphenicol	Antibiotic	*Vibrio harveyi*	EC50 = 0.16 mg/l	Biolumicens test	Thomulka et al. (1993)

Table G. (continued)Toxic effects of medical compounds on the environment.

Medical compound or residue	Therapeutic use	Test organisms	Toxicity	Sphere / conditions	Reference
Chlortetracycline	Antibiotic	*Phaseolus vulgaris* (Pinto bean plants)	At conc. up to 160 ppm in solution top dry weight were reduced 71-87% and root dry weight were reduc- ed 66-94 %. All plants died at 160 ppm treatment level.	liquid lab. testsolution	Batchelder (1981)
		Raphanus sativus L. (Edible radish) *Triticum aestivum* L. (wheat) *Zea mays* L. (corn)	At sub. conc. up to 160 ppm stimulation of growth and N uptake was observed in a sand loam soil.		Batchelder (1982)
		Phaseolus vulgaris (Pinto bean plants)	At sub. conc. up to 160 ppm decrease of plant hights, top and root dry weight was obser- ved		
		Soil biological activity	no effect	soil ammended with antibiotic contaning poultry manure	Warman and Thomas (1981)
		Methane production	18 mg/l antibiotic inhibited mesophilic anaerobic dig- estion of swine manure		Fedler and Day (1985)
Flumequine	Antibiotic	*Artemia salina*	LC_{50} (24 hours) = 477 mg/l LC_{50} (48 hours) = 308 mg/l LC_{50} (72 hours) = 96 mg/l		Brambilla et al. (1994) Migliore et al. (1997)
		Aeromonas salmonicida Bacteria	MIC (24 hours) = 4µg/ml (Tryptone soya broth) MIC (24 hours) = 128 µg/ml (Tryptone plus sea water ion) MIC (72 hours) = 16 µg/ml (Tryptone soya broth) MIC (72 hours) = 256 µg/ml (Tryptone plus sea water ion) MBC (24 hours) = 16 µg/ml (Tryptone soya broth) MIC (24 hours) = 2048 µg/ml (Tryptone plus sea water ion) MIC (72 hours) = 32 µg/ml (Tryptone soya broth) MIC (72 hours) = 256 µg/ml (Tryptone plus sea water ion)	MIC = minimum inhibitory concentrati- on MBC = minimum bactericidal concen- tration	Pursell et al. (1995)

Table G. (continued)Toxic effects of medical compounds on the environment.

Medical compound or residue	Therapeutic use	Test organisms	Toxicity	Sphere / conditions	Reference
		Plants		Effects on plants mean root weight and mean stalk Leaves weight dramatically reduced.	Brambilla et al. (1994)
Furazolidone	Antibiotic feed-additive fish farm	Sediment bacteria *Chlorella pyrenoidosa* (algae) *Dapnia magna* *Salmo gairdneri* *Lebistes reticulatus* (guppy)	Antibiotic resistance EC50= 1.3 mg/l LC50 = > 30 mg/l LC50 = > 30 mg/l LC50 = 25 mg/l	Algal toxicity test	Nygaard et al. (1992) Canton and Van Esch (1976)
Ivermectin residues	Antiparasitic agent	*Neomyia cornicina* (dung-dwelling Diptera) *Euroniticellus fulvus* (dung beetle)	No larvel development at 0.16 mg / kg dung after injection with 200 µg / kg body weight (steers) Slight delayed development at 0.06 mg / kg dung after injection with 200 µg / kg body weight (steers)		Lumaret et al. (1993)
Ivermectin H_2B_{1a}	Antiparasitic agent	*Daphnia magna*	LC_{50} (48 hours) = 0.025 ppb NOEC (48 hours) = 0.01 ppb		Halley et al. (1989)
Ivermectin monosaccharide	Antiparasitic agent	*Daphnia magna*	LC_{50} (48 hours) = 0.4 ppb NOEC (48 hours) = 0.1 ppb		
Ivermectin H_2B_{1a} - aglycone	Antiparasitic agent	*Daphnia magna*	LC_{50} (48 hours) > 17 ppb NOEC (48 hours) > 9 ppb		
Ivermectin	Antiparasitic agent	*Daphnia magna* *Salmo gairdneri* (Rainbow trout) *Lepomis macrochines* (Bluegill sunfish) *Eisemia foetid* (Earthworm) *Chlorella pyrenoidosa* (green algae)	Toxic to some extent LC_{50} (96 hours) = 3.0 mg/l NOEC (96 hours) = 0.9 mg/l LC_{50} (96 hours) = 4.8 mg/l LC_{50} (28 days) = 18-100 mg/kg soil NEL > 9.1 mg /l		Nessel et al. (1989) Halley et al. (1989) Halley et al. (1989)
Ivermectin residue	Antiparasitic agent	*Musca vetustissima* (Bush fly) *Musca domestica* (house fly)	Inhibited larvel development for 7 to 14 days after animal treatment Inhibited larvel development for 7 to 14 days after animal treatment.		Wardhaug et al. (1996)

Table G. (continued)Toxic effects of medical compounds on the environment.

Medical compound or residue	Therapeutic use	Test organisms	Toxicity	Sphere / conditions	Reference
Ivermectin	Antiparasitic agent	*Haematobia irratans*	56 days 63 days 42 days	Sensitivity of dipteran larvae, indicated by days post-treatment until adult emergence from dung equalled that of control	Miller et al. (1981) Fincher (1992) Schmidt (1983)
		Musca autumnalis	14 days	Sensitivity of dipteran larvae, indicated by days post-treatment until adult emergence from dung equalled that of control	Mayer et al. (1980)
		Neomyia cornicina	32 days 17 days	Sensitivity of dipteran larvae, indicated by days post-treatment until adult emergence from dung equalled that of control	Wardhauge et al. (1988) Lumaret et al. (1993)
		Stomoxys calcitrans	14 days	Sensitivity of dipteran larvae, indicated by days post-treatment until adult emergence from dung equalled that of control	Schmidt (1983)
		Cyclorrapha	> 30 days 42 days	Sensitivity of dipteran larvae, indicated by days post-treatment until adult emergence from dung equalled that of control	Madsen et al. (1990) Sommer et al. (1992b)
		Nematocera	20 days 0 days	Sensitivity of dipteran larvae, indicated by days post-treatment until adult emergence from dung equalled that of control	Madsen et al. (1990) Sommer et al. (1992b)
		Ontophagus gazella	17 days 21 days	Sensitivity of coleopteran larvae, indicated by days post-treatment until adult emergence from dung equalled that of control	Sommer and Nielsen (1992c) Fincher (1992)
		Aphodius spp.	10 days 13-14 days 14 days	Sensitivity of coleopteran larvae, indicated by days post-treatment until adult emergence from dung equalled that of control	Madsen et al. (1990) Sommer et al. (1992b) Strong and Wall (1994)
		Copris hispanus	16 days	Sensitivity of coleopteran larvae, indicated by days post-treatment until adult emergence from dung equalled that of control	Wardhaugh et al. (1988)
		Euoniticellus fulvus	10 days	Sensitivity of coleopteran larvae, indicated by days post-treatment until adult emergence from dung equalled that of control	Lumaret et al. (1988)
Kanamycin	Antibiotic	Sediment bacteria	Antibiotic resistance		Sandaa et al. (1992)

Table G. (continued)Toxic effects of medical compounds on the environment.

Medical compound or residue	Therapeutic use	Test organisms	Toxicity	Sphere / conditions	Reference

Moxidectin residues	Antiparasitic agent	*Musca vetustissima* (Bush fly) *Musca domestica* (House fly)	No effect on larvea survival, but delayed development was observed. No effect on larvea survival, but delayed development was observed.		Wardhaug et al. (1996)
Nitrofurazone		*S. capricornutum* (algae) *Daphia magna*	EC50 = 1.45 mg/l LC50 = 28.67 mg/l	algal toxicity test	Macri and Sbardella (1984)
Novobicin	Antibiotic	Sediment bacteria *Vibrio harveyi*	Antibiotic resistance LC50 = 0.08 mg/l	 Biolumisens test	Sandaa et al. (1992) Thomulka et al. (1993)
Oxolinic acid	Antibiotic feed additives, fish farm	Sediment bacteria	Antibiotic resistance		Nygaard et al. (1992)
Oxytetracycline	Antibiotic	*Phaseolus vulgaris* (Pinto bean plants) *Raphanus sativus* L. (Edible radish) *Triticum aestivum* L. (wheat) *Zea mays* L. (corn) *Phaseolus vulgaris* (Pinto bean plants) Sediment bacteria	At conc. up to 160 ppm in solution top dry weight were reduced 71-87% and root dry weight were reduced 66-94 %. All plants died at 160 ppm treatment level At sub. conc. up to 160 ppm stimulation of growth and N uptake was observed in a sand loam soil. At sub. conc. up to 160 ppm decrease of plant hights, top and root dry weight was observed Antibiotic resistance	liquid lab. testsolution	Batchelder (1981) Batchelder (1982) Husevåg et al. (1991) Sandaa et al. (1992) Samuelsen (1992a) Nygaard et al. (1992) Kerry (1995a) Kerry et al. (1995b)
Streptomycin	Antibiotic	*Vibrio harveyi* Blue green algae *Chlorella vulgaris* *Scenedesmus obliquus* *Chlamydomonas reinhardtii*	LC50 = 19 mg/l growth prevented at 0.09 to 0.86 mg/l growth prevented at 21 mg/l growth prevented at 0.66 mg/l	Biolumicens test	Thomulka et al. (1993) Harrass et al. (1985)

Table G. (continued)Toxic effects of medical compounds on the environment.

Medical compound or residue	Therapeutic use	Test organisms	Toxicity	Sphere / conditions	Reference
Sulphadimethoxine	Antibiotic	*Artemia salina*	LC_{50} (24 hours) = 1.8 g/l		Brambilla et al. (1994)

		Plants	LC_{50} (48 hours) = 0.9 g/l LC_{50} (72 hours) = 0.5 g/l LC_{50} (96 hours) = 19 mg/l Effects on plants mean root weight and mean stalk . Leaves weight dramatically reduced.		Brambilla et al. (1994)

16 Appendix H - Terminology and abbreviations

Acute toxicity test	In tests for acute toxicity the exposure time and the observed period are comparatively short in relation to the life cycle of the test organisms used (usually max. 96 hours).
Adsorption	Enrichment of one or more components in an interfacial layer.
Adverse effect	Change in morphology, physiology, growth, development or lifespan of an organism.
BCF	Bioconcentration factor showing the ratio between the concentration in an organism and the concentration of a substance in the ambient environment (typically water).
Bioaccumulation	the net result of the uptake, distribution, and elimination of a substance due to all routes of exposure.
Bioconcentration	the net result of the uptake, distribution, and elimination of a substance due to water-borne exposure.
Biodegradation	see degradation
Biotransformation	see transformation
BOD	Biological Oxygen Demand.
Chronic toxicity test	Toxicity determined in tests covering the life cycle of an organism or at least the most sensitive life stages.
Degradation	conversion of a molecule to smaller molecules by biological (micro-organisms) or chemical action.
Degradation rate	the rate at which a chemical compound ca be degraded. Often expressed as a DT_{50} (time at which 50% of the parent compound has disappeared from soil or water by transformation or degradation.
Dissipation	disappearance of the parent compound from a compartment (water or soil) in which various processes such as conversion, evaporation leaching, etc. can participate.
DO	Dissolved Oxygen
DOC	Dissolved Organic Compound
Dose-response	the estimation of the relationship between a dose or concentration and the assessment incidence and severity of an effect.
DT_{50}	time in which 50% of the parent compound has disappeared from soil or water by transformation or degradation.
Dung	faeces from grazing animals.
EC_{50}	Effect concentration resulting in 50% effects (e.g. growth inhibition) in a group of laboratory organisms after a given exposure time.
Effect assessment	concerns the hazard identification and dose-response assessment.
Environmental hazard	The EU system for the classification of chemical substances on the basis classification of their environmental properties.
Exposure assessment	the determination of the emission, pathways and rates of movement of a

	substance and its transformation or degradation products in order to estimate the concentrations/doses to which ecological systems and populations are or may be exposed.
EUSES	European Uniform System for the Evaluation of Substances, a decision-support system, including models for calculation of exposure and hazard in environmental compartments.
Guideline	an official Guideline (i.e. authorised by national or international institutions. e.g. EPA, NEN, BBA, OECD) for the protocol and the report of a test.
Hazard	the inherent potential of a substance to cause adverse effects
Hazard assessment	the process designed to estimate the incidence and severity of the adverse effects likely to occur in an environmental compartment due to actual or predicted exposure.
Hydrolysis	a chemical reaction of a substance with water in which a part of the molecule of the reacting substance is replaced by an OH group.
LC_{50}	Effect concentration involving 50% mortality in a group of laboratory organisms after a given exposure period.
LD_{50}	Effect dose involving 50% mortality in a group of laboratory organisms after a given exposure period.
Leaching	transfer of a chemical from the top layer of soil to the subsoil.
log Kow	The logarithm of the octanol-water partition coefficient.
Manure	mixture of faeces and urine produced by housed animals. When mixed with dirty water, the mixture is denoted slurry.
Metabolite	substance formed from the parent compound by chemical transformation.
Mineralisation	degradation of a substance into inorganic end products; it is usually estimated in terms of CO_2 production.
NOEC	No-Observed-Effect-Concentration; the highest test substance concentration without adverse effects.
PEC	Predicted Environmental Concentration; the expected concentration in an environmental compartment.
Photochemical	breakdown of a compound as a result of irradiation by light. transformation
Risk	Probability of a substance to cause adverse effects.
Risk estimation	the quantitative estimation of the probabilities of clearly described effects by including uncertainty analysis.
TOC	Total Organic Carbon.
transformation	conversion of a molecule to larger or smaller molecules by (micro)biological or chemical action.