NATIONAL UNIVERSITY OF SCIENCE AND TECHNOLOGY BUCHAREST POLYTECHNIC
FACULTY OF BIOTECHNICAL SYSTEMS ENGINEERING
DOCTORAL SCHOOL: BIOTECHNICAL SYSTEMS ENGINEERING

PHD THESIS SUMMARY

RESEARCH ON THE PERFORMANCE OF A METHOD ENVIRONMENTAL IMPACT ASSESSMENT A VETERINARY MEDICINAL PRODUCTS

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CONTENT

* The content follows the pagination in the doctoral thesis. Also, the numbers of the figures and tables in this summary correspond to those in the doctoral thesis.

KEYWORDS

Environmental risk assessment, veterinary drugs, software method for environmental risk assessment, monitoring antibiotics in surface waters, SPE-online-UHPLC-MS/MS method.

INTRODUCTION

The continued expansion of the human population has led to a proliferation of the amount and diversity of veterinary medicinal products consumed and subsequently excreted into the environment.

In this PhD study, the compounds of interest are antibiotics, active ingredients in veterinary drugs that have become an integral component in maintaining animal health but at the same time, a major concern due to their potential effects on the environment. The prevention of antimicrobial resistance, a growing concern for public health, requires a rigorous control of their consumption, monitoring of residues, evolution and elimination from the environment [1-9].

In the environmental risk assessments of veterinary pharmaceuticals required by the U.S. Food and Drug Administration (FDA) since 1980 and in the European Union since 1997, the effects of veterinary drugs on the environment are analyzed. The results of the studies carried out in these assessments are accessible in numerous publications and provide information on the environmental impact of veterinary medicinal products [10-13]. All the assessments presented in this thesis help to develop the potential ecological risk of these veterinary drugs in order to develop management strategies to reduce them in the environment. Antibiotics are not regulated by current European water quality standards, which must concern their widespread contamination of the environment. The results of these studies provide information on residues present in soil, water, sediment and the environmental risk of individual substances to target and non-target organisms. [14-19].

The major importance presented by the environmental impact assessment studies of veterinary medicinal products in order to obtain authorization for their commercialization, was one of the reasons for choosing the theme of the present doctoral theses. The second argument that motivated the choice of the theme, is to support the evaluators by creating a software method that allows them to perform particularly thorough environmental risk analyzes in short evaluation periods. The topic of the doctoral thesis is in line with the European Union's ongoing concern for increasing the quality of life by reducing pollution. Environmental risk assessments were performed in accordance with the prescriptions and recommendations of the European Medicines Agency (EMEA) guideline.

The target pollutants of this doctoral thesis are the analyzed veterinary drugs:

- LEVASOL 10%, oral solution for cattle, sheep, goats, pigs and birds containing 100 mg/ml levamisole hydrochloride.
-TIASOL 10% - oral solution, indicated for pigs, rabbits and birds (chickens, broilers, turkeys, pigeons, pheasants, guinea fowls) containing 100mg/ml tiamulin hydrogen fumarate.
- FLORFENICOL FP 10% - oral solution for chickens (broilers, replacement youth) and pigs containing 100 mg/ml florfenicol.
- LIN – SPE – MIX 880 - water-soluble powder for pigs and chickens containing 293mg/g lincomycin and 293mg/g spectinomycin.
- AMPROLIUM FP 25% premix for broilers, chickens, turkeys, containing 250 mg/g amprolium.
- BENZYLPENICILLIN POTASSIUM PASTEUR 25% - oral powder for piglets, chickens, broilers and turkeys containing 250mg/g benzylpenicillin potassium (Penicillin G).

The purpose and objectives of the doctoral thesis

Through this doctoral study, it was aimed to make contributions regarding the development of assessments to determine the environmental risk of veterinary medicinal products. As part of the doctoral thesis, a procedure was developed that aimed to determine the content of antibiotics in surface water

samples using the SPE-online-UHPLC-MS/MS technique. The main objective of this doctoral thesis is the development and application of a methodology for an environmental risk assessment of veterinary medicinal products, for the application of analysis software created on the basis of a proprietary agorithm. To achieve this objective, three main research directions have been developed, as follows:
1. Studies on the environmental risk assessment of the veterinary medicines LEVASOL 10%, TIASOL 10%, FLORFENICOL FP 10% and LIN – SPE – MIX 880, based on an analytical analysis according to the provisions of the EMEA guide.
2. Studies on the environmental risk assessment of the veterinary medicines BENZYLPENICILLIN POTASSICA PASTEUR 25% and AMPROLIUM FP 25% based on a software analysis method created on the basis of a proprietary agorithm that complies with the provisions of the EMEA guide.
3. Studies on the development of procedures aimed at determining the content of antibiotics in surface water samples (penicillin G and trimethoprim), using the SPE-online-UHPLC-MS/MS technique.

The structure of the doctoral thesis

The doctoral thesis is structured in 7 chapters.
Introduction of the presentation of aspects related to the impact of the risk of veterinary medicinal products on the environment and the need for environmental assessment. In this section, the main objective of the thesis, the directions followed to achieve this objective and the research activities are presented.
Chapter 1 presented the procedure for assessing the environmental impact of veterinary medicinal products according to European legislation and a summary of the current state of research on the environmental impact assessment of veterinary medicinal products.
Chapter 2 describes in detail our own environmental assessment research for 4 veterinary antibiotics, which was applied in this study to assess the safety risk requirement of veterinary medicines containing these antibiotics.
Chapters 3 and 4 original contributions of this doctoral thesis that consist in the development of a software method for the analysis of environmental risk created on the basis of a proprietary calculation algorithm and the advantages of applying these methods.
Chapters 5 and 6 describe the development of a procedure that aimed to determine the content of antibiotics in surface water and waste water samples, using the SPE-online-UHPLC-MS/MS technique, and present the following monitoring of penicillin G, trimethoprim in surface water . by SPE-online-UHPLC-MS/MS.
The last chapter presents the **general conclusions** of this study, followed by the presentation of the references used in the preparation of the doctoral thesis, the list of papers published in ISI-rated international specialized journals and the list of papers presented at national and international conferences.
The results obtained as a result of the research carried out during the doctoral study were communicated at scientific events and also published/accepted/sent for publication in specialized magazines (Scientific Activity).

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CHAPTER 1.
CURRENT STATE OF RESEARCH ON THE ENVIRONMENTAL IMPACT ASSESSMENT OF VETERINARY MEDICINES

1.1. European legislation on environmental impact assessment and veterinary medicinal products

Environmental risk assessment (ERA) research aims to identify environmental risks as well as the need for specific risk mitigation measures.

The ERA takes into account the administration of the drug, the physicochemical and ecotoxicological properties of its active substances. The result of the ERA is summarized in the evaluation report of the Committee for Medicinal Products for Veterinary Use (CVMP) and is available in the European Public Assessment Report (EPAR) of the medicine.

VICH a trilateral program (EU-Japan-USA) aimed at harmonizing the technical requirements for the registration of veterinary products (Veterinary International Conference on Harmonization). In the EU, scientific guidelines and regulations on ERA are developed and adopted by the (CVMP) of the European Medicines Agency (EMEA), with the support of experts from the Environmental Risk Assessment Working Group (ERAWP) of the CVMP. Prior to approval by the Committee, any draft guidance is published for consultation to allow interested parties to provide input.

Environmental risk assessment is a complex investigation of environmental exposure to active substances in a veterinary medicinal product and the resulting adverse effects. The guideline EMEA/CVMP/ERA/418282/2005 structured in two evaluation phases, Phase I (VICH GL6) and Phase II (VICH GL38) is the basis of an environmental risk assessment. Environmental risk assessment requirements by procedure type are included in the EMEA/CVMP/182112/2006 guideline approved in 2009. These guidance documents have been developed by VICH to harmonize data requirements and the risk assessment process basis for approval, authorization of veterinary medicines in participating countries. Regulation (EU) 2019/6 of January 2022 aims to update the regulatory framework for veterinary medicinal products and replace Directive 2001/82/EC.

Under the new regulation, the Commission has 3 years from the date of entry into force (January 2025) to study the feasibility of transferring environmental risk assessment from a product-based approach to a substance-based approach. Substance-based risk assessment would focus on the development of comprehensive monographs of the active substance that new or generic product applicants can refer to when preparing their product authorization application and that there would be no need to replicate studies that already exist, would lead to harmonized and robust risk assessments, providing a more comprehensive risk characterization.

Risk mitigation is an essential part of product evaluation and can be used to restrict the risk associated with a product to an acceptable level, or even to eliminate such risk altogether. In principle, the applicant must propose measures to reduce the risk, and, if applicable, the effectiveness of such measures must be proven by the data in the file.

The technical guidance document of the European Chemicals Agency (ECHA), "Technical Guidance Document supports legislation on assessment of risks of chemical substances to human health and the environment" (TGD) supports legislation on the assessment of risks of chemical substances to human health and the environment and includes new approaches in the risk assessment of veterinary medicinal products (Directive 93/67/EEC on the risk assessment of new notified substances, Regulation (EC) No 1488/94 on the risk assessment of existing substances, Directive 98/8/EC of the European Parliament and of the Council regarding the introduction of biocidal products on the market).

The national guideline on the prudent use of antimicrobials in veterinary medicine updated in February 2020, drawn up on the basis of the veterinary sanitary legislation in force, provides useful information in the evaluation of veterinary antibiotics [20-24].

1.2. Environmental impact assessment of veterinary medicinal products

Environmental impact assessment is an assessment of the possible environmental hazards presented by a veterinary medicinal product.

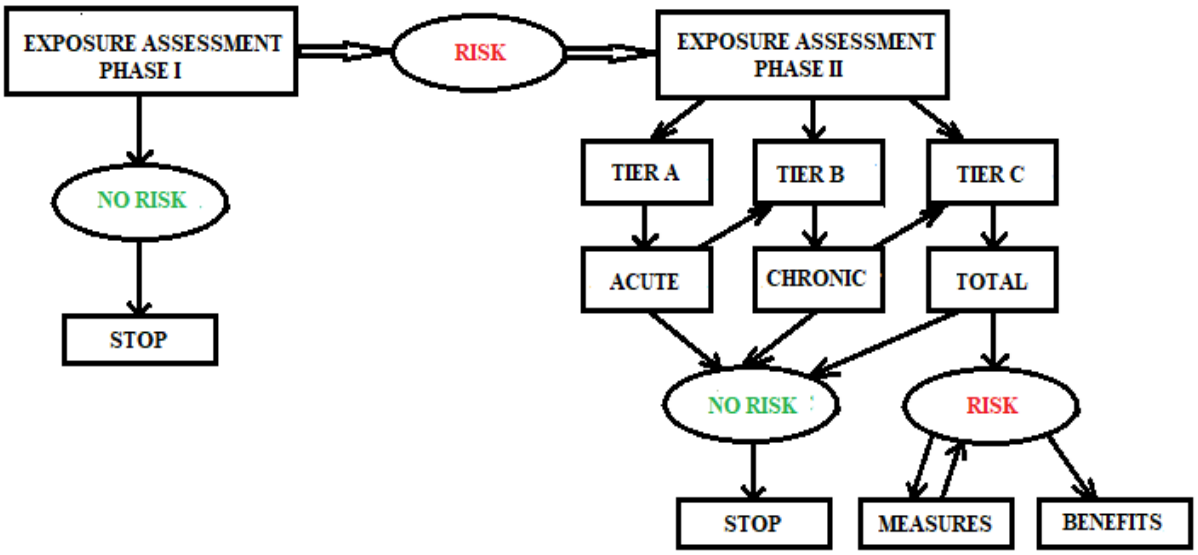


Figure 1.1. Environmental risk assessment for veterinary medicinal products in the EU

The European Medicines Agency (EMEA) considers the environmental risks of a veterinary medicinal product in its benefit/risk assessment and provides guidance to help marketing authorization applicants assess the environmental impact of their medicinal product and implement mitigation measures. risk mitigation (Figure 1.1).

The environmental risk analysis of PMV carried out according to all the provisions of the EMEA guide, ensures the predictability and transparency of the results obtained. The determining factors for the final exposure concentration are the route of distribution and evolution in the environment. The stages of an environmental assessment are: the analysis of environmental exposure (soil, ground water, surface water and sediment) to the active substance in the analyzed veterinary medicinal product, the evaluation of the effects of the products and the characterization of the final risk.

The predictable concentration of the active substance in the environment **(PEC)** is determined according to the calculation formula indicated in the guide knowing the indication for use of the PMV, the physico-chemical properties and data on the release of the active substance into the environment.

The predicted no-effect concentration **(PNEC)** is determined by relating data on available ecotoxicological descriptors to an appropriate assessment factor **(FA)**.

Phase I applies the total residual approach. This means that all the amount of active substance in the applied dose is excreted by the animal and the metabolism data should not be taken into account.

Species of economic interest can be raised indoors (farms) or kept outside (pasture). In the VICH guide, the former are considered to be intensively raised animals and the latter to be pasture-raised animals. Intensively reared animals are those that are reared indoors throughout the production cycle, so the treatment with the veterinary medicinal product is carried out indoors and the residual active substance is eliminated at the rearing site and is incorporated into the manure. This active residue enters the environment when manure is spread on agricultural land and hayfields. In the case of grazing animals, the PMV treatment is carried out on the field and the residue of the active substance from the PVM is eliminated directly in the soil. The calculation of the concentration of active substances (PECsolpasture) for pasture animals is done according to the EMEA guide taking into account the number of animals kept on the land, (stocking density) and the values available in the guide, depending on the species.

The calculation of the predictable concentration of active substance in the soil (PECsol) originating from the PVM, is done taking into account the total residue of the active substance, without taking into account the metabolism. The calculation of PECsol takes into account the proportion of treated animals in the herd that is available in the technical documentation of the product, following the field experiment or

in the scientific literature. When specific information is not available, the values given in the EMEA guidance for different groups of veterinary medicinal products should be used, values that have been stable after discussions with veterinarians in the Member States of the European Union (EU). Data on the number of animals reared, body weight, nitrogen produced and the growth factor entering into the initial soil PEC calculation are available in the EMEA guidance.

The VICH guideline states that veterinary medicinal products administered to intensively raised animals have the potential to affect non-target animal species from the active surface call for indirect transport of the ingredient into water, either into surface water or into groundwater when absorbed into ground Transport to surface water can occur either through discharge or runoff. Veterinary drugs are released into the environment and the transfer to groundwater and surface water is also complex, while real information about their behavior is frequently limited. Environmental exposure pathways for veterinary medicinal products are shown in Figure 1.5.

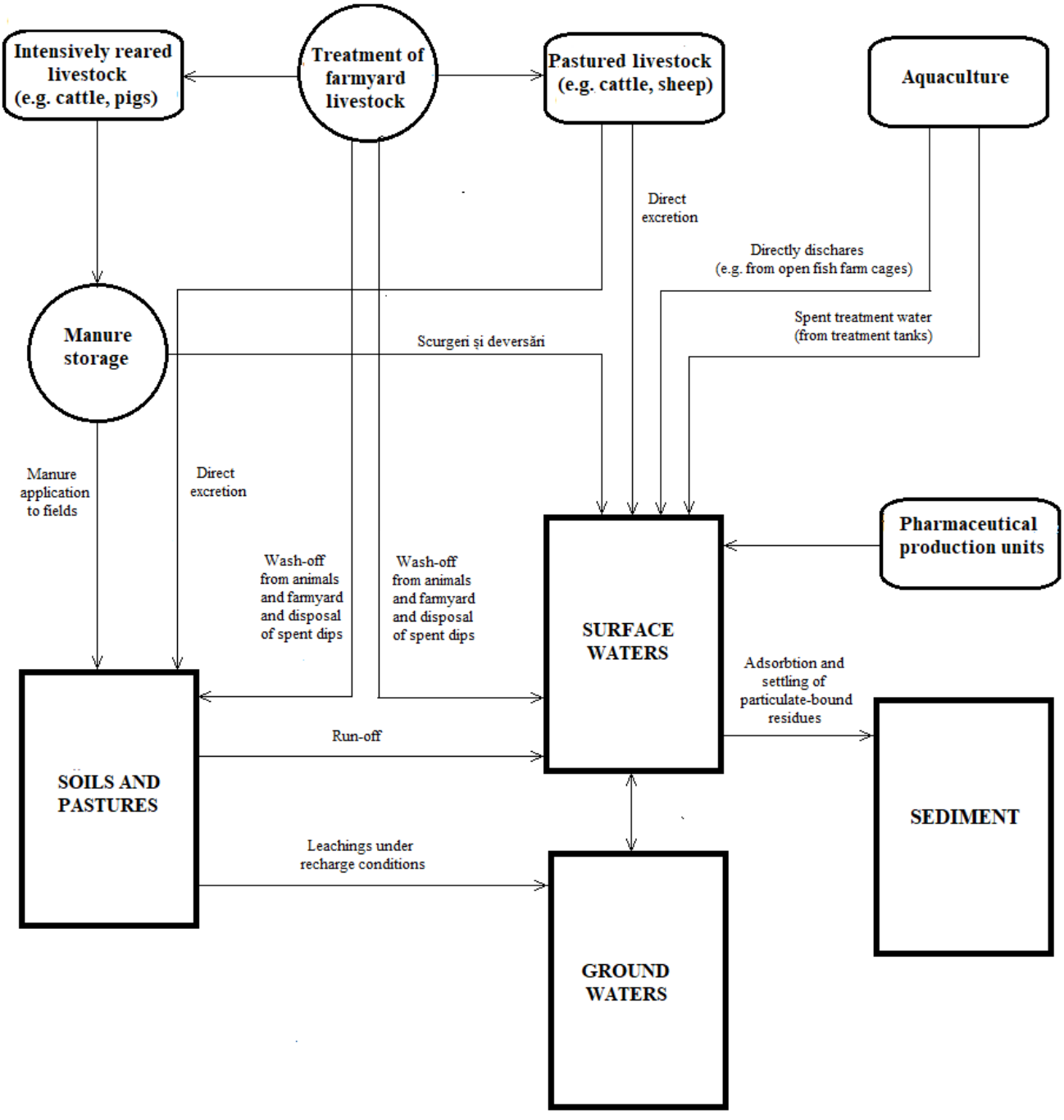


Figure 1.5 Environmental exposure pathways for veterinary medicinal products

It is also possible for the active ingredient to reach ground water with the potential to cause adverse effects on drinking water networks. Therefore, it is necessary to calculate predictable concentration values for both surface water and ground water. It is recommended that when calculating PEC values for groundwater and surface water a stepwise approach be followed, using simple equations for a standard initial analysis and progressing to a more complex approach when refined estimation is required . of exposure.

The calculation of the concentration of residual active substance in groundwater (PEWater table) is made according to the principle of sorption balance in solids, by means of the water-organic carbon distribution coefficient (Koc).

For the determination of the predictable concentration of residual active substance in surface water (PECapa surface), the guideline indicates 1/3 of the concentration in ground water.

The predictable residual active substance concentration in the sediment (PEC sediment) is determined by taking into account the sediment-water partition coefficient (Ksed-water).

The approach (solids sorption balance) for the calculation of PEWater table is described in the Technical Guidance Document on Risk Assessment (TGD) [23]. The calculated PECap value should be compared to the PNEC values for each aquatic species tested. If the risk (R) values are greater than 1, then the PEC can be refined based on metabolism data. If R>1 holds, more sophisticated models can be used to estimate the initial PECap (Projects FOCUS DG SANTE). If still R>1, chronic toxicity data are required to refine the PNEC. This PNEC must then be compared to chronic exposure levels.

Data on the concentration of a substance in any environment under which adverse effects will not occur, (EC50 effective concentration, LC50 mean lethal concentration, NOEC no observed effect concentration) are available depending on the duration of exposure, long-term or short-term .

The PNECs are determined by considering the established ecotoxicity parameters and assessment factors (FA). The evaluation factors applied in the calculation of the PNEC adjust for the differences between the data obtained in the laboratory and in the natural environment and have lower values for the long-term toxicity tests because their uncertainty is small. The more toxicity data we have available from different environmental factor assessment compartments have lower values. To determine the environmental risk, the PNEC determined on the basis of the most sensitive toxic result for a certain species is used.

The determination of the PNEC concentration in soil and sediment is based on the available toxicity data or with the partition balance method having a PNEC calculated on the basis of the toxicity data and the evaluation factor [25].

$$\text{PNEC soil} = (0.1176 + 0.01764 \times \text{Koc}) \times \text{PNEC water} \quad (1.1)$$

$$\text{PNEC sediment} = (0.783 + 0.0217 \times \text{Koc}) \times \text{PNEC water} \quad (1.2)$$

To determine the risk for environmental comparisons (soil, water, sediment) of an active substance, the predicted environmental concentration (PEC) values are related to the predicted no-effect concentration (PNEC) values and in accordance with the provisions of the EMEA guidance, if the PEC / PNEC <1, the risk is acceptable.

In **Phase I**, the decision-making tree diagram containing 17 questions regarding the active substances of the analyzed product, the target specificity, the potential exposure to the environment, taking into account the physico-chemical characteristics of the component ingredients and the method of administration of the veterinary medicinal product, is followed. Calculate the initial predictable concentration of active substances that have entered the soil (PECsol), water (PECapa) and sediment (PECsediment).

For the calculation of PECsol, the parameters available from the technical specification of the product are required.

If the predicted soil concentration values (initial soil PECs) for a target species are greater than 100 µg/kg, the environmental risk assessment must proceed to phase II for all active ingredients, using the worst predicted soil concentration value.

In **Phase II,** the physical/chemical, pharmacological and toxicological properties of the active substances, evolution and stability in soil, water and excreta, effects on non-target organisms will be

analyzed. In phase II, step A, a set of data relating to the evolution and effects of active substances in the environment are presented, very important data, which allow knowledge of the dangers and the possibility to intervene in reducing the risks when using the product.

Phase II, step A of the analysis begins with a more detailed assessment of environmental exposure to the active ingredients of the veterinary medicinal product and the presentation of data on their evolution and effects in the environment. If the calculated risk is greater than 1, as indicated by the EMEA, the environmental risk analysis continues in step B. For an environmental impact analysis all relevant to the assessment of the veterinary medicinal product will be included in the assessment, whether favorable or unfavorable for product (see Annex I of Directive 2001/82/Ec, amended). Relevant literature data should always be included in the documentation. required for Phase I and Phase II analyzes are discussed in the assessment chapters. All are evaluated and summarized to determine their reliability and usefulness. Predictable concentration of the active substance in the soil, initially calculated, greater than 0.1 mg/kg, causes the assessment to move to Phase II, Step A.

Excretion, metabolism, degradation in feces, routes of entry of the active substance into the environment, and agricultural practices influence environmental exposure and the refined risk value (Rraf>1) determines the possibility of moving to a **Phase II, Step B** assessment.

On distribution, the active ingredient may be incorporated into soil or sedimentary material as a latent residue or transformed into metabolites or carbon dioxide. Mineralization or degradation of active substances are considered endpoints of biodegradation studies. In some cases, the organic component metabolites are more hydrophilic than the parent components, so there is a greater possibility of leaching into groundwater. This effect can be taken into account in a special approach to the total residual. Thus, data for metabolites may be requested if such a request can be scientifically justified.

Exposure of birds and mammals through the application of manure with residues of active substances from a veterinary product to agricultural land and hay is possible due to the fact that these non-target species are exposed to products through food and water.

Soil exposure must take into account the spreading of all farm excrement and waste water. Direct soil exposure to grazing animals must also be considered.

Exposure to the aquatic environment must take into account escape and runoff of the active ingredient into surface water and groundwater as well as any other routes of exposure to the aquatic environment. For fish farms there will be direct exposure to the aquatic environment, but there may also be soil exposure due to the spreading of liquid residues from ponds. It is necessary to know the half-life of the active ingredients in the environmental compartments of interest.

On these parts of the evaluation, the physico-chemical properties of the active ingredients, the influence of light, pH, humidity, degradation and other factors must be considered. The kinetics of the removal of the active ingredient from the environmental compartments of interest will provide valuable information about the evolution in the environment .

Chronic toxicity data are less available than acute data and the range of standardized test procedures smaller. Data generated in accordance with Organization for Economic Co-operation and Development (OECD) Test Guideline 210 and 211 are accepted.

Exposure assessment, taking into account all aspects regarding the use of the veterinary medicinal product and regarding the emission of the active substance into the environment leads to the stability of the predictable environmental concentration (PEC) value.

The assessment of effects, taking into account all toxicity data and the assessment factor (FA) increase, leads to the stability of the predicted no effect concentration (PNEC) value.

The risk calculated by relating the PEC to the PNEC may be acceptable (subunit) and the assessment concluded.

If R ≥1, the assessment in phase II step A continues with the refinement of the predictable concentrations in soil, water, sediment, taking into account the metabolism products of the active substances (active metabolism fraction Fa), degradation in manure and degradation in soil (when breakdowns are spread in more than one stage) of the active substances in the analyzed veterinary medicinal product (Figure 1.6).

Refined predicted environmental concentrations are compared to PNECs calculated from ecotoxicity data for each species treated with the veterinary medicinal product, for all environmental compartments (soil, ground water, surface water, sediment), to determine the refined risk (Rraf) .

If Rraf>1, the risk assessment is continued in Phase II Step B and chronic ecotoxicity tests are considered, and a refinement of the PNECs is required.

If the use of the veterinary medicinal product maintains a risk for a compartment, field studies based on more realistic scenarios are presented and the risk assessment is continued in Phase II Step C. After all studies have been performed, following the risk-benefit analysis, a decision is made regarding the introduction to the market of the veterinary medicine [20, 25-36].

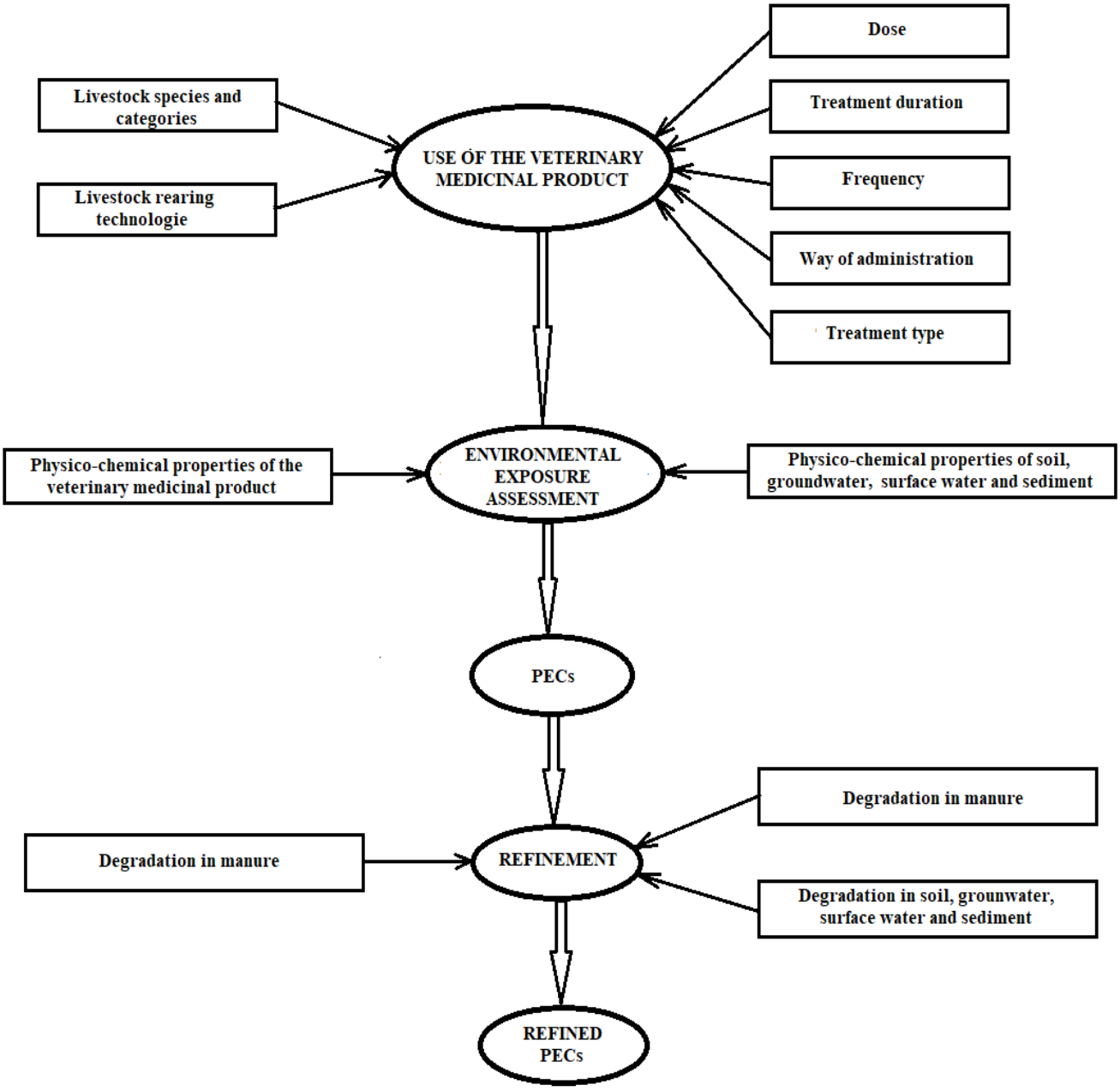


Figure 1.6. Refining PECs

1.3. Current state of research on environmental impact assessment of veterinary medicinal products

The European Union's strategic approach on the impact of pharmaceutical substances on the environment (Brussels, September 2020), effective measures and additional research needed to reduce the impact of pharmaceutical substances on the environment and the need to regulate the level of

pharmaceutical residues in water legislation. The European Council asked to evaluate and define legislative measures to combat the development of antimicrobial resistance, to support the development of pharmaceutical substances less harmful to the environment, to ensure that requests for environmental risk assessment in the search for marketing authorization. has produced so that stability and risk management measures can be published.

Since the monitoring of pharmaceutical substances in the environment, including soil, is still very limited, it is requested to strengthen some surveillance mechanisms, after the introduction to the market, which include data on the impact of the product on the environment.

Competent authorities must create a centralized and secure database that allows all specialists to have access to the results of environmental assessments and to strengthen the European "One Health" plan that links human and animal health and currently also includes the environmental impact assessment of pharmaceutical substances in veterinary products.

Environmental risk assessment, mandatory for obtaining marketing authorization for veterinary medicinal products, is the main means of ensuring environmental safety. However, environmental risks persist. Several states (the Netherlands and Sweden), the European Parliament, third countries (Switzerland), international organizations (the United Nations, HELCOM, the Organization for Economic Co-operation and Development), industry associations and non-governmental organizations have expressed concern and taken measures to reduce pharmaceutical substances in the environment.

At the international level, the 2030 Agenda of the United Nations, especially the sustainable development goal no. 6, and the 2017 ministerial declaration of the United Nations Environment Assembly commitments to action for environmental protection and the World Health Organization (WHO) agreed action on antimicrobial resistance.

As a result of the contamination of soil and water with pharmaceutical residues, the European Commission has published a list of actions, with the aim of reducing drug residues in the environment and to be implemented to reach minimum pollution levels by 2030, stating in at same time the availability of medicines.

Good Manufacturing Practices (GMP) and Best Available Techniques (BAT) are important tools for the prevention and control of environmental pollution emissions that can intervene in support of environmental protection.

The Organization for Economic Co-operation and Development (OECD) made a recommendation in a recent report on pharmaceutical residues in surface water, embodied in a series of measures to limit the release of pharmaceutical residues into the environment.

Sweden, an EU member state, has already proposed that measures to prevent the release of active ingredients from drug manufacturing plants be incorporated into the regulations on veterinary drugs [37, 38].

The current expansion of aquaculture production can only be managed by the use of veterinary medicinal products available for use in aquaculture systems. The environmental risk that can arise from the use of these products has gained increased attention in recent years. Since aquaculture is practiced in very different environmental compartments of risk, it is done according to the real environment (marine or fresh water), based on suitable and easy-to-apply exposure model assessments, to refine predictable concentrations of the veterinary medicinal product.

Current VICH guidelines may not be protective enough to address some adverse effects on non-target organisms (eg, development of antimicrobial resistance).

Environmental risk analyzes are currently based on the most appropriate studies to assess ecotoxicological effects on non-target organisms.

A paradigm shift in current environmental hazard and risk assessments is the recommendation to develop active substance-based assessments to replace product-based risk assessments [39, 40-43].

The Annual Conference on European Environmental Law (2022) meets the requirements of environmental law specialists to keep abreast of the latest developments in legislation, jurisprudence and best practice in this field. The event will facilitate the exchange of experience between legal practitioners, judges and regulators on current challenges and opportunities in EU environmental law.

Antibiotics are used in a wide range of veterinary medicines. A percentage of 20% of veterinary medicines (147 tonnes of active substance per year) sold in France are antibiotics (ANSES 2015). Most are resistant to biodegradation after ingestion and can be persistent in the environment. Antibiotic residues have

been detected as pollutants in various environmental compartments and pose human and environmental threats, especially with regard to the potential emergence and proliferation of antibiotic-resistant bacteria. In this context, the ANTIBIOTOX project (study of antibiotics and associated resistance genes in 210 agroecosystems, ecotoxicological risk for the aquatic environment) is an innovative project funded by ANR france (Agence National de Recherche; contract no. ANR-17-CE34-0003, 2018-2022) which aims to develop a complex approach in the fields of chemistry, biochemistry, environmental microbiology, genetics, molecular biology and ecotoxicology to study the biodegradation pathways and estimate the ecotoxicological impact in the aquatic and terrestrial environment of antibiotics. In this context, the project two proposals to study the fate and environmental impact of sulfonamide antibiotics, sulfamethazine (SMZ) and sulfamethoxazole (SMZ) used in veterinary medicine and to provide new insights into the ecotoxicological impact of veterinary/human antibiotic residues.

Spain was chosen as an example, due to the importance it attaches to biodiversity, and to the use of veterinary drugs (European Union's Horizon 2020 Research and Innovation program, Grant/Award Number: 773830. provided information on the use of antibiotics, animal census and type of rearing (intensively raised or pasture-raised animal) As major antibiotics, tetracyclines and beta-lactams contributed more to soil vulnerability than less commonly used antibiotics. A Shiny Dashboard was developed to visualize environmental risks in detail and to a solid database of veterinary drug use, animal census and husbandry type.

In the European Union, veterinary medicinal products are authorized on the basis of an environmental risk assessment procedure, a centralized applied procedure (at global level) or through a decentralized and mutual recognition procedure (at member state level). Regardless of the level, the risk assessment framework is for any procedure. Following an analysis of the data, only from centrally authorized products, as it was not possible to access the data of nationally authorized products in individual states, it resulted that, in general, veterinary pharmaceutical products (> 95%) they are considered to have a limited release to the environment and their risk assessment is completed after the lowest level assessment (phase I as presented in VICH GL6). Despite the assumption that in most cases contamination with veterinary residues is low and therefore products are exempt from higher level environmental risk assessments that require specific substance safety data, environmental pollution from pharmaceuticals is an emerging problem. Following the entry into force of the revised Water Framework Directive (2008/105/EC) in 2013, and the resulting legislative obligation for the European Commission to develop a strategic approach to reducing water pollution from pharmaceuticals, action has been taken. aimed at reducing the total emissions of veterinary medicinal products and limiting the use of highly hazardous substances in their composition (eg PBT/vPvB substances).

It also calls for or review of the nature of current environmental hazard and risk assessments by recommending the development of active substance-based assessments, moving away from product-based assessments. The potential impact of pharmaceutical residues on humans and wildlife is an issue of increasing concern, as studies of pharmaceutical concentrations in surface waters as well as their effects on wildlife populations are increasingly reported in scientific publications. In conclusion, the EC strategy and the new veterinary regulation indicate that there is a favorable climate for revising the current approach to environmental risk assessment of veterinary medicinal products (eg exploring a substance-based assessment). and, by, to investigate the extension of its scope as well (for example, the assessment of the impact of emissions from production sites) [44].

As part of the animal husbandry systems are now moving towards organic farming and free-range farming, a lower risk of consumption of veterinary drugs is envisaged in general, but animal excretions possibly containing active substances from these products could be released directly into the environment instead of being stored and applied as manure. The first phase of the current environmental risk assessment procedures for veterinary medicinal products in the EU has been critically analyzed from the point of view of changes in animal husbandry. In this respect a large number of default values used in the current environmental risk procedure for veterinary medicinal products were checked for updating. In a three-step approach, first the current trends and changes relevant to animal husbandry in Europe were collected, then the interactions between phase I of the environmental risk assessment procedure for veterinary medicinal products and current animal husbandry systems were assessed. animals and finally the default values used in phase I of the environmental risk assessment procedure for veterinary medicinal products were checked to identify research gaps. From the analysis of the result that several default values used in the current ERA

were identified as outdated. In conclusion, the results of this study indicate that an update of environmental risk assessment procedures for veterinary medicinal products in the EU is necessary to take into account changes in animal husbandry [45].

Regulation (EU) 2019/6 (referred to as the "new NVR veterinary regulation"), applicable from 2022, takes into account the increased interest in environmental protection and adopts certain measures accordingly. For example, among the measures implemented, the NVR requests the EC to investigate the feasibility of a review system based on active substances (the so-called "monograph system") and other potential alternatives suitable for the environmental impact assessment of all veterinary medicinal products in the EU. An analysis of the effectiveness of current and future legislation to protect the environment and reduce the regulatory burden is very useful to identify the weaknesses and strengths of both [46].

Ivermectin (IVM) is an antiparasitic product used throughout the pharmaceutical world for its properties in the treatment of several diseases in humans and animals (a recent study mentions a possible use against COVID-19). Veterinary use of IVM may result in an unacceptable and unsustainable risk to both aquatic and terrestrial ecosystems. IVM showed very high acute and chronic toxicity to crustaceans. In fish, IVM can cause lethargy, dark skin and reduced feeding behavior. Moreover, growth inhibition for algae and high toxicity for terrestrial organisms and dung insects were pointed out. Based on these findings, a reduction (or elimination) of the use of this substance as a veterinary medicine is suggested given the high environmental risk, especially to aquatic ecosystems. If IVM will be authorized, stricter measures should be applied in order to reduce and/or mitigate the impact of IVM on the environment and its organisms [47].

Biosorption has been shown to be an important mechanism in the removal of antibiotic-class veterinary medicinal products by microalgae-based water treatment processes. Adsorption of the veterinary medicinal products: tetracycline (TET), ciprofloxacin (CIP), sulfadiazine (SDZ) and sulfamethoxazole (SMX) on a dry microalgae-bacteria consortium *Scenedesmus almeriensis* was studied at several equilibrium concentrations (20 to 1000 μg/). 000). IT). The study revealed that the *S. almeriensis*-bacteria consortium has a high biosorption power and demonstrated that biosorption is an important mechanism for the removal of ciprofloxacin and tetracycline used in a water treatment process on this microalgal core. However, removals of sulfadiazine and sulfamethoxazole did not exceed 32% [48].

It states that when a product is considered for market authorisation, environmental risks should be considered in the risk-benefit analysis. However, several studies have been published revealing considerable levels of residues in surface water, rivers and lakes across Europe, raising the question of whether the current legislation and system for environmental risk assessment of human and veterinary medicines are sufficient protectors [49].

Environmental risks are not directly comparable to therapeutic benefits, and therefore there is no standardized approach to compare both environmental risks and therapeutic benefits. In this regard, three methods of communicating and comparing therapeutic benefits and environmental risks have been developed for the benefit-risk assessment that supports the EU authorization process. Two of these methods support independent product evaluation (ie, a summative classification and a visual scoring matrix classification); the other supports a comparative evaluation between alternative products, i.e. a comparative ranking [50].

Current EU guidelines for the environmental risk assessment of veterinary medicinal products in groundwater suggest an approach based on the comparison of the calculated concentration in ground water (PEWater table) and a threshold concentration, arbitrary stability, of 0.1 μg/l which reserves . the upper limit of pesticide concentration in groundwater in the EU. If PECapăfreatica calculates does not exceed the threshold, then the risk is considered acceptable. The concentration of 0.1 μg/l is assumed to be safe by default for both humans and exposed organisms in groundwater [51].

Residues of veterinary medicinal products enter the environment through the application of manure on agricultural areas where, in particular, antibiotics can cause phytotoxicity. Tests on terrestrial plants according to OECD guideline 208 are part of the environmental risk assessment of veterinary medicinal products [52].

Based on data from the Dutch FADN system and collected by Wageningen Economic Research, for 17 medicinal products of potential concern in the Netherlands, sources and emissions due to land applications were assessed. It therefore examined the use of veterinary medicinal products in livestock sectors in the Netherlands between 2015 and 2018 and quantified animal excretion rates and dissipation during manure storage. For all veterinary medicinal products, the amounts administered to animals

decreased between 2015 and 2018. The concentrations of active substances in the veterinary drugs under study, over a six-month storage period, decreased between 10 and 98% depending on the compound. The concentrations measured in the suspensions after storage are consistent with the predicted concentrations calculated according to the EMEA guidance [53].

The ecotoxicological risk of pharmaceutical mixtures usually exceeds the individual intrinsic risk, which draws special attention to the fact that monitoring surveys routinely find complex pharmaceutical mixtures in different environmental compartments. However, although the body of data on the ecotoxicology of pharmaceutical mixtures is quite consistent, current guidelines for environmental risk assessment of pharmaceuticals often do not explicitly address the effects of mixtures. Particular attention is paid to the waiver of environmental risk assessments where, based on exposure, the components of a mixture present low, individually non-toxic concentrations, but which in the mixture could combine to produce substantial mixture effects [54].

Based on the One Health concept that human health is closely related to animal and environmental health, some animals such as bees and other pollinators are presented as sentinels for environmental contamination or biological indicators [55].

The latest data reported by the European Food Safety Authority (EFSA) shows that residues of veterinary drugs and other substances found in animals and food of animal origin continue to decrease in the European Union and compliance levels are increasing. In 2021, the percentage of non-compliant samples was 0.17%, the lowest figure recorded in the last 12 years when non-compliance varied between 0.19% and 0.37%. The figure for 2020 was 0.19%. The overall level of non-compliance in targeted samples (ie those taken to detect illegal uses or check non-compliance with maximum permitted levels) also fell to 0.24%, compared to 0.27%-0.35% in the previous four . years. The report (EFSA) includes hormones, antibacterials, environmental contaminants, banned substances and other veterinary drugs [176].

CHAPTER 2
OWN ENVIRONMENTAL RISK ASSESSMENT RESEARCH FOR VETERINARY MEDICINES

2.1. Analyzed veterinary drugs

Veterinary drugs are widely used to treat disease and protect animal health and have the potential to be released into the environment. The behavior of veterinary drugs is investigated more thoroughly to obtain complex information on their impact on the environment. Accordingly, the marketing authorization holder of the veterinary product provides an assessment to the authorities as part of the authorization process. The product is authorized for sale only if the veterinary health authority is satisfied that the environmental risk is sufficiently low. This study is carried out to gain a greater understanding of the environmental risk, to have information on the actual concentrations of veterinary drugs approved to be marketed.

In order to obtain the marketing authorization, we carried out environmental impact assessments, according to the provisions of the European legislation for veterinary drugs, validated by the Institute for the Control of Biological and Veterinary Medicinal Products (ICBMV).

The analytical method according to the EMEA guideline is a complex method that involves many calculations. For a quick and efficient assessment of the environmental impact of veterinary medicinal products, we have created a software method that explicitly goes through all the stages of the guide and supports ERA specialists.

In this thesis, I performed environmental impact assessments using the analytical method for the following veterinary drugs: LIN – SPE – MIX 880 - water-soluble powder for pigs and chickens, Levasol 10% - oral solution for cattle, sheep, goats, pigs and birds, FlorfenicoL FP 10% - oral solution for chickens, broilers, and pigs, Tiasol 10% - oral solution, indicated for pigs, rabbits and birds (chickens, broilers, turkeys).

In this thesis, I performed environmental impact assessments using the two methods, analytical and software, for the following veterinary drugs: Amprolium FP 25% - premix for chickens, broilers, turkeys and Benzylpenicillin potassium 25% - oral powder for piglets, broilers and turkeys.

In the ERA studies carried out, the following were analyzed: the physico-chemical properties of the active substances and the main metabolites, the pharmacological properties, the pharmacokinetic characteristics of the active substance, the metabolism and excretion, the decision tree scheme, the calculation of PECsol for the target animals, the evolution and the behavior in the environment , biotransformation, degradation, hydrolysis, photolysis, soil adsorption, acute and chronic toxicity, bioaccumulation, effect analysis (PNEC calculations), environmental risk characterization, PEC refinements, refined risk characterization, effects determined in long-term exposures, with risk assessment Step B. Following the evaluation of these products, it was found that the levels of residues of active substances in soil, water, sediment, were within appropriate limits and no undesirable effects on the environment were detected.

I have chosen to present in this thesis aspects from the evaluation of some veterinary drugs whose active substances have a high potential to enter the environment but which, following the analysis, proved an acceptable risk for the environment: amprolium, florfenicol, penicillin G, levamisole, tiamulin, lincomycin, spectinomycin.

2.2. Analytical environmental risk assessment for levamisole

The product LEVASOL 10% - oral solution for cattle, sheep, goats, pigs and birds, has as its active substance levamisole hydrochloride, an anthelmintic from the imidazothiazole class. The product contains 100 mg/ml levamisole hydrochloride.

The metabolism of levamisole is very intense, so that the residual substances in tissues, urine and excrement consist mainly of its metabolites. Elimination is very fast, the main routes being through urine and bile.

Administer 10 mg active substance/kg body weight to cattle, sheep, pigs and 20 - 30 mg active substance/kg body weight to birds, once a day. The most important route of environmental exposure is the

excretion of the active substance as such and its metabolites. The resulting excreta are stored and applied to the soil at a later stage.

The resulting concentrations in these environmental compartments will be determined by the physico-chemical properties of the active substance, its partitioning in soil and sediments, the abiotic and biotic degradation, the degradation of the active substance in liquid and solid wastes and the environmental characteristics (including the type soil, climatic conditions).

Calculate the initial PECsol (according to the EMEA guideline) for each target species and technology category. (Table 2.1).

Table 2.1. Initial PEC soil, groundwater, surface water and sediment for levamisol

Parameter / Species and category	PEC_{soil} [mg/kg]	$PEC_{groundwater}$ [mg/l]	$PEC_{surfacewater}$ [mg/l]	$PEC_{sediment}$ [mg/kg]
pigs intensive	0,086	0,0004	0,0004	0,082
turkeys	0,132	0,0007	0,0006	0,126
broilers intensive	0,266	0,001	0,0004	0,082
ducks	0,185	0,001	0,0009	0,176
cattle intensive	0,058	0,0003	0,0004	0,082
cattle pasture	0,020	0,0001	0,0001	0,019
sheep pasture	0,016	0,00008	0,00008	0,015

The most unfavorable PECsol value will be used. As the initial PECsol value for the target species broilers is greater than 0.1 mg/kg, the environmental risk assessment should proceed to Phase II, step A.

The molecular formula of levamisole hydrochloride is C11 H13 Cl N2 S and the structural formula, (S)-6-Phenyl-2,3,5,6tetrahydroimidazo [2,1b] [1,3] thiazole hydrochloride, is shown in Figure 2.1.

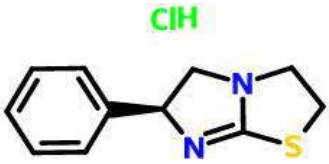


Figure 2.1. Structure of levamisole hydrochloride [177]

In Phase II step A. the potential of the LEVASOL 10% product to affect organisms in the environment (aquatic, terrestrial) is evaluated.

Levamisole is evenly distributed throughout the body of the treated animal and is almost completely metabolized, except for a percentage of 6%, which is excreted unchanged through the urine.

Levamisole hydrochloride is photodegradable (Sweetman). The value of 15.02 mg/l for EC50 (Basnyat, 2010), tested on activated sludge bacteria indicates the toxicity of levamisole to sludge bacteria. The available data on levamisole used in the environmental risk assessment are presented in Table 2.2.

Table 2.2. Levamisole environmental risk assessment parameters

Property	Parameter	Parameter value
Physicochemical	Solubility in water	210000 mg/l (25 ºC)
	Melting point	227-229ºC
	Vapor pressure	0,0148 Pa
Mobility in soil	Koc	3764 l/kg
Persistence in soil	DT 50 (zile)	soil, 20 °C = 720 hours
		water, 20 °C = 360 hours
		air, 20 °C = 7,72 hours
Bioaccumulation	Log Kow	1,84
Degradation in manure	Fraction of active substance still present in manure after 24 days (%)	-

National Library of Medicine PubChem

Available and predictable experimental toxicity values indicated that the toxicity of levamisole to aquatic and terrestrial organisms is low.

The determined water PNEC is presented in Table 2.3.

Table 2.3. PNECwater levamisole

Species	**Parameter / value mg/l**	**FA VICH**	**PNEC (mg/l)**
Fish 96 hours	LC_{50}= 8,038	100	0,080
Algae or other aquatic plants 96 hours	EC_{50}= 17,231	100	0,172
Aquatic invertebrates	EC_{50}=300	100	3
Shellfish (Daphnia magna) 384 hours	EC_{50}= 1,967	100	0,019
Zebra fish	LC_{50}= 32	100	0,32

(OECD 201, OECD 202, OECD 203, OECD 220/222)

The most sensitive toxicity value was considered in the following calculations:

Shellfish 384 hours, EC50 = 1,96, mg/l, FA =100 , ***PNECwater = 0,019mg/l***

The calculation of soil PNEC and sediment PNEC taking into account PNECapa (determined from the result of experimentally determined effects), is carried out using EPM (balanced partitioning method), according to relations 1.1 and 1.2. They resulted in PNECsol = 1.26 mg/kg and PNECsed =1.56 mg/kg.

In order to be able to estimate the adverse effects on soil ecosystems, a risk analysis was carried out. To determine the risk (R), the initially calculated PECs are compared with the PNEC calculated from available ecotoxicity data (R = PEC/PNEC) (Table 2.4).

Table 2.4 The risk of levamisole, R.

Parameter / Species and categoriy	**$R_{inisoil}$**	**$R_{inigroudwater}$**	**$R_{inisurfacewater}$**	**$R_{inisediment}$**
broilers	0,21	0,05	0,02	0,05
turkeys	0,10	0,03	0,03	0,08
ducks	0,14	0.05	0,04	0,11
cattle intensive	0,04	0.01	0,02	0,05
cattle pasture	0,01	0,005	0,005	0,012
sheep pasture	0,01	0,004	0.004	0,009
pigs	0.06	0,02	0.02	0,05

The very low environmental risk values (R < 1) for all species make the evaluation stop here and it follows that the veterinary medicinal product would not present any risk to the ecosystem.

Comparison of the calculated actual daily intakes for levamisole with the acceptable daily intake (ADI) suggests that for the studied compound consumer exposure to veterinary medicinal products in soil via plants is considered to be below the ADI and that the direct risk to human health is therefore low.

The results of the studies indicate that there is no possibility of levamisole contamination of the ground water. However, there is a risk of contamination in case of accidental spills or very poor farm management practices.

Degradation of levamisole in manure during storage (DT50 = 5 -75 days at 25°C), lead to a value for refined PECsol based on degradation in manure very low for all treated species [56 -70].

2.3. Analytical environmental risk assessment for tiamulin

The product TIASOL 10% - oral solution for pigs, rabbits and birds (chickens, broilers, turkeys) has as its active substance tiamulin hydrogen fumarate, a bacteriostatic antibiotic, semi-synthetic derivative

(carboxypenicillin) belonging to the group of pleuromutilins. The product contains 100 mg/ml tiamulin hydrogen fumarate.

Thiasol solution is almost completely metabolized and eliminated through bile and feces. In total, 11 metabolites were identified and quantified in bile. These identified metabolites and the remaining parent compound comprise 60% of the total excreted, with the remaining 40% comprising metabolites present at concentrations too low to allow isolation and identification. The individual concentrations of the excreted metabolites were below 20% of the applied amount of tiamulin and therefore did not have to be included in the risk assessment.

Administer 12 mg tiamulin/kg body weight to pigs, rabbits for 7 days and 20 mg tiamulin/kg body weight to birds (chickens, broilers, turkeys).

The most important route of entry into the environment results from the excretion of the active substance as such and its metabolites. The resulting excreta are stored and applied to the soil at a later stage. The resulting concentrations in these environmental compartments will be determined by the physico-chemical properties of the active substance, its partitioning in soil and sediments, the abiotic and biotic degradation, the degradation of the active substance in liquid and solid wastes and the environmental characteristics (including the type soil, climatic conditions).

Calculate the initial PECsol (according to the EMEA guideline) for each target species and technology category. (Table 2.5).

Table 2.5. *nitial* PECsoil, ground water, surface water and sediment for tiamulin

Parameter / Species and category	PEC_{soil} [mg/kg]	$PEC_{groundwater}$ [mg/l]	$PEC_{surfacewater}$ [mg/l]	$PEC_{sediment}$ [mg/kg]
pigs intensive	0,729	0,028	0,025	0,721
turkeys intensive	0,618	0,023	0,021	0,598
broilers intensive	0,866	0,033	0,030	0,856
rabbits intensive	0,605	0,023	0,021	0,598

The most unfavorable PECsol value will be used. As the initial PECsol value for the target species broilers is greater than 0.1 mg/kg, the environmental risk assessment should proceed to Phase II, step A.

The molecular formula of tiamulin hydrogen fumarate is C32H51NO8S and the structural formula is (3aS,4R,5S,6S,8R,9R,9aR,10R)-6-Ethenyl-5-hydroxy-4,6,9,10-tetramethyl-1-oxodecahydro -3a,9-propano-3acyclopentacycloocten-8-yl[[2(diethylamino)ethyl]sulfanyl]acetate monofumarate, is presented in Figure 2.2.

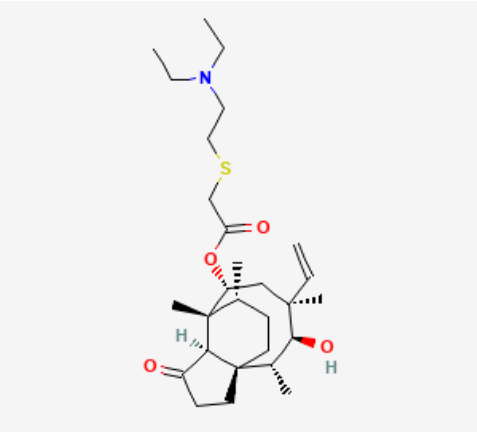


Figure 2.2. Structure of Tiamulin hydrogen fumarate [178]

In Phase II step A. the potential of the TIASOL 10% product to affect aquatic and terrestrial organisms is evaluated.

The absorption spectrum of tiamulin can be used to predict its potential to photodegrade. The data show that tiamulin absorbs light in the spectral range between 200 nm and 280 nm and does not absorb any light at a wavelength greater than 280 nm. Natural sunlight has very little energy in the wavelength range

greater than 280 nm. Thus, it is predicted that, under natural light conditions, tiamulin will not be photodegradable.

The available data on Tiamulin hydrogen fumarate used in the environmental risk assessment are presented in Table 2.6.

Table 2.6. Environmental risk assessment parameters for Tiamulin hydrogen fumarate

Property	**Parameter**	**Parameter value**
Physicochemical	Solubility in water	7580mg/l
	Dissociation constant in water, pKa	7,44
	Vapor pressure	0,06 Pa
Mobility in soil	Koc	536 l/kg
Persistence in soil	DT 50 (zile)	10,2 (soil, 20 °C)
Degradation in manure	DT 50(zile)	49 days (at 25 ° C) 590 de days (at 10 °C)
Bioaccumulation	Log Kow	1,2 (pH 5,5) 3,4 (pH 7) 4,4 (pH 8,5)

(OECD 307, OECD 106, OECD 112, OECD 117, OECD 201, OECD 105)

The determined PNEC for water is presented in Table 2.7.

Tabel 2.7. PNECapă Tiamulin hydrogen fumarat

Species	**Parameter / value (mg/l / mg/kg)**	**FA VICH**	**PNEC (mg/l / mg/kg)**	
Fish 96 hours	LC_{50}= 15,1	1000	0,015	
Algae or other aquatic plants 96 hours	EC_{50}= 11,7	100	0,117	
Shellfish (Daphnia magna) 384 hours	EC_{50}= 17,3	100	0,017	
Earthworms si enchytraeids	NOEC = 1000	10	100	

(OECD 201, OECD 202, OECD 203, OECD 220/222)

The most sensitive toxicity value was considered in the following calculations:

Algae 96 hours, EC50 =11.7, mg/l, FA =100, PNECwater = 0.117mg/l

The calculation of soil PNEC and sediment PNEC taking into account PNECapa (determined from the result of experimentally determined effects), is carried out using EPM (balanced partitioning method), according to relations 1.1 and 1.2. They resulted in PNECsol = 1.119 mg/kg and PNECsed =1.452 mg/kg.

In order to be able to estimate the adverse effects on soil ecosystems, a risk analysis was carried out. To determine risk (R), initially calculated PECs are compared to PNECs determined from available ecotoxicity data (R = PEC/PNEC) (Table 2.8).

Tabel 2.8. The risk of Tiamulin hydrogen fumarat, R.

Parameter / **Species and category**	**$R_{inisoil}$**	**$R_{inigroundwater}$**	**$R_{inisurfacewater}$**	**$R_{inisediment}$**
broilers	0,77	0,28	0,25	0,58
turkeys	0,55	0,19	0,17	0,41
rabbits	0,54	0.19	0,17	0,41
pigs	0,65	0,23	0,21	0,49

The very low environmental risk values (R < 1) for all species make the evaluation stop here and it follows that the veterinary medicinal product would not present any risk to the ecosystem.

The low toxicity values indicate that the environmental impact of tiamulin is acceptable. The rapid degradation of tiamulin during manure storage (DT50 <13 days), the fact that it does not persist in the soil, there is no possibility of accumulation in the environment, indicates that there is no risk of tiamulin on the environment [71-90].

2.4. Analytical environmental risk assessment for florfenicol

The product FLORFENICOL FP 10% - oral solution for chickens (broilers, replacement youth) and pigs, has as its active substance the antibiotic florfenicol, a thiamphenicol derivative. The product contains 100 mg/ml florfenicol.

Florfenicol is rapidly absorbed in the bodies of treated animals and half of the dose of florfenicol administered is eliminated from the body in its initial form. Amine, oxamic acid and alcohol, the main metabolites of florfenicol have a very low antimicrobial activity. Florfenicol is non-volatile, exhibits ultraviolet (UV) absorption (absorption maximum 224 nanometers) and its melting point is 154 °C. Compared to florfenicol, the metabolism products are much more soluble (solubility > 500 g/l) and much less lipophilic.

Florfenicol in the drug under consideration is excreted in urine (97%), and in faeces (3%).

Administer 2 mg florfenicol/kg body weight to pigs, 7 days and 20 mg florfenicol/kg body weight to birds (chickens, broilers), 5 days. The resulting excreta are stored and applied to the soil at a later stage. The resulting concentrations in these environmental compartments will be determined by the physico-chemical properties of the active substance, its partitioning in soil and sediments, the abiotic and biotic degradation, the degradation of the active substance in liquid and solid wastes and the environmental characteristics (including the type soil, climatic conditions).

Calculate the initial PECsol (according to the EMEA guideline) for each target species and technology category. (Table 2.9).

Table 2.9. Initial PECsoil, groundwater, surface water and sediment for florfenicol

Parameter / **Species and category**	**PEC_{soil} [mg/kg]**	**$PEC_{groundwater}$ [mg/l]**	**$PEC_{asurfacewater}$ [mg/l]**	**$PEC_{sediment}$ [mg/kg]**
pigs intensive	0,082	0,068	0,062	0,156
broilers intensive	0,886	0,736	0,667	1,681
hens intensive	0,196	0,163	0,147	0,372

The most unfavorable PECsol value will be used. As the initial PECsol value for the target species broilers is greater than 0.1 mg/kg, the environmental risk assessment should proceed to Phase II, step A.

The molecular formula of florfenicol is C12H14Cl2FNO4S and the structural formula 2,2-dichloro-N-[(1R,2S)-3-fluoro-1-hydroxy-1-(4-methylsulfonylphenyl)propan-2-yl]acetamide is shown in Figure 2.2.

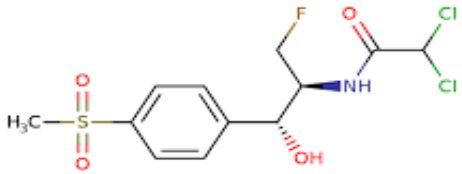


Figure 2.3. Structure of Florfenicol [179]

In Phase II step A. the potential of the considered product to affect aquatic and terrestrial organisms is evaluated.

From the available studies on the photolysis and hydrolysis of florfenicol and its metabolites, it appears that these processes do not significantly influence their degradation in the environment (Connor, 1995; Fackler, 1991a-d).

Florfenicol, with substantial water solubility and an extremely low partition coefficient, log Kow, tends to remain in water.

The degradation of florfenicol (DT50, DT90) in three different sediment-water systems, the solid-water distribution coefficient (Kd) and the organic carbon sorption coefficient (Koc), are presented in Table 2.10.

Table 2.10 Degradation of florfenicol in sediment-water

Sourse	**Sediment type**	**Organic carbon%**	**Sediment/water degradation (days)**		**Kd (l/kg)**	**K_{oc} (l/kg)**
			DT_{50}	**DT_{90}**		
salt water	fertile soil	3.2	13.0	43.1	0.293	9.1
water	fertile soil	2.4	8.4	27.8	0.434	18.1
water	infertile soil	0.76	19.4	64.5	0.250	32.9

The values of the sorption characteristics Kd and Koc for florfenicol are small (Kd were determined in the range 0.07-0.59 and Koc, 10-27, in the range 10-27) (Table 2.11).

Table 2.11 Adsorption of florfenicol in soil

Soil adsorption	**Florfenicol**	**Metabolites of florfenicol**		
		Amine	**Alcohol**	**Oxamic acid**
Sorption (%)	2-10	23.9-39.9	1.3-8.2	7.5-43
Kd (l/kg)	0.07-0.59	1.56-3.35	0.07-0.45	0.41-3.78
Koc (l/kg)	10-27	162-241	7-76.5	36.4-642
Mobility	Very mobile to mobile	Moderate	Very mobile to moderate mobile	Mobile to less mobile
Reference	Fackler (1990)	Fackler (1991a)	Fackler (1991b)	Fackler (1991c)

The available data on Florfenicol used in the environmental risk assessment are presented in Table 2.12.

Table 2.12. Florfenicol environmental risk assessment parameters

Property	**Parameter**	**Parameter value**
Physicochemical	Solubility in water	1,32mg/l
	Dissociation constant in water, pKa	7,5 l/kg Amine metabolit 1,99 l/kg Acid oxamic metabolit
	Vapor pressure	0,06 Pa
Mobility in soil	Koc	18,38 l/kg
Persistence in soil	DT 50 (zile)	13 (sol, 20 °C)
Persistence in manure	DT 50(zile)	3,6
Bioaccumulation	Log Kow	0,37 (pH 7)

(OECD 307, OECD 106, OECD 112, OECD 117, OECD 201, OECD 105)

Acute toxicity tests performed in accordance with OECD 202, in Daphnia magna exposed to florfenicol, at concentrations up to 330 mg/l (LeLievre, 1991a) allowed the determination of EC50 and NOEC values (Table 2.13)

Table 2.13 Acute toxicity of florfenicol and major metabolites to Daphnia magna

Soil adsorption	**Florfenicol**	**Metabolites of florfenicol**		
		Amine	**Alcoolul**	**Acidul oxamic**
EC50 (mg/l)	>330	>18	>14	>24
NOEC (mg/L)	<100	18	8.9	24
Reference	LeLievre (1991a)	LeLievre (1991b)	LeLievre (1991c)	LeLievre (1991d)

The determined PNEC for water is presented in Table 2.14.

Table 2.14. PNECwater for florfenicol

Species	**Evaluation/value parameter EC_{50} or LC_{50} (mg/l)**	**FA VICH**	**PNEC (mg/l)**
Oncorhynchus mykiss	>780	100	7.8
Lepomis macrochirus	>830	100	8.3
Daphnia magna	>330	100	3.3
Navicula pelliculosa	61	10	6.1
Pseudokirchneriella subcapitata	1	10	0.1
Lemna gibba	0.76	10	0.076
Anabaena flos-aquae	0.23	10	0.023

[168.169]

Toxicity values vary, fish (O. mykiss and L. macrochirus) having the highest reported acute values, greater than 780 and 830 mg/l, respectively A. flos-aquae having the lowest value, of 0.23 mg / l. The latter value indicates that freshwater cyanobacteria,

A. flos-aquae, is the most sensitive freshwater species for which Phase II, Tier A assessment data are available.

Studies on land plants have examined both seedling emergence and growth. The latter represented the more sensitive objective, so PNECs are derived based on growth (wet weight) data. The PNEC (ratio between toxicity values and AF) for terrestrial organisms ranges from 0.005 mg/kg for flatworms to 0.156 mg/kg for earthworms. (Table 2.15).

Table 2.15 PNECsoil at invertebrates and terrestrial plants for florfenicol

Specie	**Parametru/valoare (mg/kg)**	**FA VICH**	**PNEC (mg/kg)**
Earthworm (reproduction)	NOEC: 1.56	10	0.156
Nasturtium	EC50: 0.5	100	0.005
Mustard	EC50: 1.7	100	0.017
Wheat (weight)	EC50: 6.7	100	0.067
Nasturtium	EC50 > 1	100	>0.01
Cabbage	EC50: 0.859	100	0.009
Mustard	EC50: 0.705	100	0.007

[168,169]

The most sensitive toxicity value was considered in the following calculations:

Algae 96 h, EC50 = 0.23 mg/l, AF = 10, PNECwater = 0.023mg/l

The calculation of soil PNEC and sediment PNEC taking into account PNECapa (determined from the result of experimentally determined effects), is carried out using EPM (balanced partitioning method), according to relations 1.1 and 1.2. They resulted in PNECsol = 0.01016 mg/kg and PNECsed =0.02718 mg/kg.

In order to be able to estimate the adverse effects on soil ecosystems, a risk analysis was carried out. To determine the risk (R), the initially determined PECs are related to the PNECs determined from the ecotoxicity data (R = PEC/PNEC) (Table 2.16).

Table 2.16. Risk (R) for florfenicol

Parameter / Species and category	$R_{inisoil}$	$R_{inigroundwater}$	$R_{inisurfacewater}$	$R_{inisediment}$
broilers	87,2	32	29	61,84
hens	19,29	7,08	6,39	13,68
pigs	11,9	4,34	3,95	8,46

The environmental risk values, R >1, for all species make the evaluation continue with PEC refinement based on metabolism (dose fraction considered active is 0.5), degradation in droppings and soil, refinement performed according to the guidelines EMEA (Table 2.17).

Table 2.17. Refined risk (Rraf) for florfenicol

Parameter / Species and category	$R_{metabolism}$	$R_{degradationinmanure}$	$R_{degradationinsoil}$ (after 2 spreads)
broilers	43,6	1,37	1.37
hens	9,64	0,004	0,004
pigs	5,90	0,06	0,06

Since the refined environmental risk values remain Rraf >1 even after the refinement of the PECs, for the broiler species, the evaluation continues in Phase II Step B with the refinement of the PNECs.

Tier B risk characterization considers chronic effects on aquatic and terrestrial organisms. The PNECs are calculated based on the available ecotoxicity data and the corresponding evaluation factors.

Aquatic effects, PNEC calculation

Data on Tier B aquatic effects are available for three trophic levels: aquatic plants, invertebrates and fish. Algal and cyanobacterial growth inhibition studies that have been performed [168] can be used to assess chronic effects at level B compared to acute effects at level A. For invertebrates, data are available from a Daphnia life cycle study [169], a rotifer reproduction study and a 28-day, benthic midge study. For fish, an early-life study provides data for Tier B assessment. If more than one toxicity value was available, the lowest value (indicating the highest toxicity) was selected (Table 2.18).

Tabel 2.18. PNECapă rafinat, florfenicol Treapta B [168,169]

Species and reference	Endpoint toxicity	Toxicity value (mg/l)	AF VICH	PNECwater refined (mg/l)
Pseudokirchneriella subcapitata	NOEC, 96-h	0.75	10	0.075
Lemna gibba	NOEC, 7zile	0.39	10	0.039
Navicula pelliculosa	EC10, 72h	18.7	10	1.87
Anabaena flos-aquae	***NOEC, 96-h***	***0.11***	***10***	***0.011***
Daphnia magna	NOEC, 21zile	1.5	10	0.15
Brachionus calyciflorus	NOEC, 2zile	0.76	10	0.076
Pimephales promelas	NOEC, early stage of life	5.5	10	0.55

Terrestrial effects, PNEC calculation

According to the EMEA guide, terrestrial effects studies at level B include nitrogen transformation studies extended to 100 days and terrestrial plant growth tests. Available toxicity data for florfenicol that meet these requirements are presented below. The study on the underpants, by Bealing et al. (1999), found that the most sensitive effect measured was on the longest leaf of the primary and secondary leaf pairs, and the NOEC based on this effect was 0.16 mg/kg. The study by Gray (2007) provided NOEC values for cabbage and mustard based on weight, which was the most sensitive final effect (Table 2.19, Table 2.20).

Table 2.19 PNECsoil (refined for terrestrial organisms) for florfenicol tier B [168,169]

Species and reference	Endpoint toxicity	Toxicity value (mg/l)	AF VICH	PNECsoil refined (mg/l)
Nasturtium	NOEC growth	0.16	10	0.016
Cabbage	NOEC weight	0.123	10	0.0123
Mustard	***NOEC weight***	***0.123***	***10***	***0.0123***

Table 2.20. PNEC sediment (refined for sediment organisms) for florfenicol, tier B [168,169]

Species and reference	Endpoint toxicity	Toxicity value (mg/l)	AF VICH	PNECsediment refined (mg/l)
Chironomus riparius	***NOEC, 28-d***	***25***	***10***	***2.5***

Where more than one toxicity value was available, the lowest value (indicating the highest toxicity) was selected.

Refined risk assessment Tier B (PECrefined / PNECrefined)

The refined PECs, calculated on the basis of degradation in faeces and taking into account the Fa metabolism factor (PEC degradation in faeces x Fa), are compared with the refined Tier B PNECs derived from the toxicity values and assessment factors used in level B to determine the Risk (Raf) Tier B for each treated species (Table 2.21, 2.22, 2.23).

Table 2.21 Rrefined pigs

Compartment	PECrefined mg/l, mg/kg	Toxicity	PNECrefined mg/l, mg/kg	Rrefined
Water	0,0007	*Anabaena flos-aquae* (Gallagher et al., 2008a) NOEC 0,11, AF=10	0,011	0,06
Soil	0,0007	Mustar (Gray, 2007) NOEC 0,123, AF=10	0,0123	0,05
Sediment	0,0007	*Chironomus riparius* (Bradley, 2009) NOEC 25, AF=10	2,5	0,0003

Table 2.22 Rrefined broilers

Compartment	PECrefined mg/l, mg/kg	Toxicity	PNECrefined mg/l, mg/kg	Rrefined
Water	0,0007	*Anabaena flos-aquae* (Gallagher et al., 2008a) NOEC 0,11, AF=10	0,011	0,63
Soil	0,0007	Mustar (Gray, 2007) NOEC 0,123, AF=10	0,0123	0,56
Sediment	0,0007	*Chironomus riparius* (Bradley, 2009) NOEC 25, AF=10	2,5	0,003

Table 2.23 Rrefined hens

Compartment	PECrefined mg/l, mg/kg	Toxicity	PNECrefined mg/l, mg/kg	Rrefined
Water	0,00006	*Anabaena flos-aquae* (Gallagher et al., 2008a) NOEC 0,11, AF=10	0,011	0,005
Soil	0,00006	Mustar (Gray, 2007) NOEC 0,123, AF=10	0,0123	0,005
Sediment	0,00006	*Chironomus riparius* (Bradley, 2009) NOEC 25, AF=10	2,5	0,00002

The refined risk (Raf) Tier B for each environmental compartment is presented in Table 2.24.

Table 2.24 Refined risk (Rre) in tier B for florfenicol

Parameter / Species and category	$R_{refsoil}$	$R_{refwater}$	$R_{refsediment}$
broilers	0,63	0,56	0,003
hens	0,005	0,005	0,00002
pigs	0,06	0,05	0,0003

The calculated step B risk coefficients were ≤ 1 for all indicated species in the three environmental compartments: water, soil, sediment. From the analysis of the environmental impact of the product FLORFENICOL FP 10% oral solution for chickens (broilers, replacement youth) and pigs, it follows that there would be no risk to the ecosystem species.

The activity of florfenicol on prokaryotic microorganisms is not negligible: the minimum inhibitory concentration (MIC) for Pasteurella multocida is 0.25 mg/l, for Trichoderma viride and Aspergillus niger it is over 1 mg/l, for Nitrobacter sp.) it is 65 mg/l and for Nitrosomonas europaea it is 2.5 mg/l [91-99].

2.5. Analytical environmental risk assessment for lincomycin and spectinomycin

The product LIN – SPE – MIX 880 - water-soluble powder for pigs and broilers, has as active substance lincomycin (in the form of lincomycin hydrochloride) and spectinomycin (in the form of spectinomycin dihydrochloride pentahydrate). The product contains 293mg/g lincomycin, 293mg/g spectinomycin. Lincomycin is a lincosamide antibiotic obtained from Streptomyces lincolnensis, active against gram-positive bacteria, gram-negative anaerobic bacteria and Mycoplasma spp. Spectinomycin is an aminocyclitol antibiotic obtained from Streptomyces spectabilis; active against aerobic gram-negative bacteria, gram-positive cocci and Mycoplasma spp.

LIN-SPE-MIX 880 is eliminated through urine and faeces either unchanged or in a microbiologically inactive form, about ¾ of the dose, within 6 hours of administration. Lincomycin undergoes hepatic metabolism and is excreted in bile and urine (Aiello, 1998). Spectinomycin is excreted mostly unchanged in feces and urine (Aiello, 1998). Administer 5.5 mg lincomycin and 5.5 mg spectinomycin/kg body weight to pigs and 8.8 mg lincomycin and 8.8 mg spectinomycin/kg body weight to broilers, over a period of 7 days. The resulting excreta are stored and applied to the soil at a later stage. The resulting concentrations in these environmental compartments will be determined by the physico-chemical properties of the active substance, its partitioning in soil and sediments, the abiotic and biotic degradation, the degradation of the active substance in liquid and solid wastes and the environmental characteristics (including the type soil, climatic conditions).

Calculate the initial PECsol (according to the EMEA guideline) for each target species and each active substance. (Table 2.25, 2.26).

Table 2.25. Initial PECsoil, groundwater, surface water and sediment for lincomycin

Parameter / Species and category	PEC_{soil} [mg/kg]	$PEC_{groundwater}$ [mg/l]	$PEC_{asurfacewater}$ [mg/l]	$PEC_{sediment}$ [mg/kg]
pigs intensive	0,334	0,106	0,096	0,437
broilers intensive	0,063	0,020	0,018	0,083

Table 2.26. Initial PECsoil, groundwater, surface water and sediment for spectinomycin

Parameter / Species and category	PEC_{soil} [mg/kg]	$PEC_{groundwater}$ [mg/l]	$PEC_{asurfacewater}$ [mg/l]	$PEC_{sediment}$ [mg/kg]
pigs intensive	0,334	0,011	0,010	0,329
broilers intensive	0,063	0,002	0,002	0,022

The calculation of the total initial soil PEC for LIN-SPE-MIX 880 was performed by summing the initial soil PEC for the two active substances, taking into account the most unfavorable results, obtained on the pig species.

The calculation of the total PEC (groundwater and surface) for LIN-SPE-MIX 880 is carried out by summing the water PEC (groundwater and surface) for the two active substances (lincomycin and spectinomycin), taking into account the most unfavorable results, obtained on the pig species.

PECtotalgroundwater LIN-SPE-MIX 880 = 0.118 mg/l
PECtotalsurfacewater LIN-SPE-MIX 880 = 0.106 mg/l
PECtotalsediment LIN-SPE-MIX 880 = 0.767mg/kg

As the initial PECsol value for the porcine target species is greater than 0.1 mg/kg, the environmental risk assessment should proceed to Phase II, step A.

The molecular formula of levamisole hydrochloride is C18H35ClN2O6S and the structural formula is (2S,4R)-N-[(1R,2R)-2-hydroxy-1-[(2R,3R,4S,5R,6R)-3,4,5- trihydroxy-6-methylsulfanyloxan-2-yl]propyl]-1-methyl-4-propylpyrrolidine-2-carboxamide;hydrochloride, is shown in Figure 2.4.

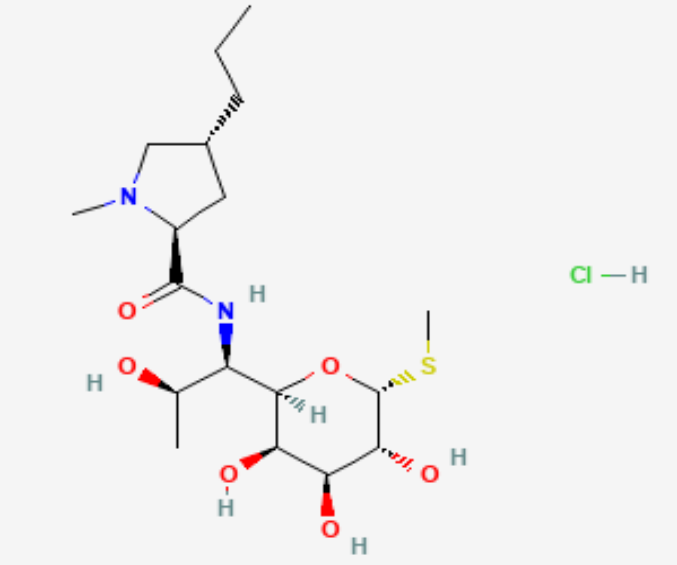


Figure 2.4. Lincomycin Hydrochloride Structure [180]

The molecular formula of spectinomycin dihydrochloride pentahydrate is C14H36Cl2N2O12 and the structural formula is (1R,3S,5R,8R,10R,11S,12S,13R,14S)-8,12,14-trihydroxy-5-methyl-11,13-bis(methylamino)-2,4,9-trioxatricyclo[8.4.0.03,8]tetradecan-7-one;pentahydrate;dihydrochloride (2S,4R)-N-[(1R,2R)-2-hydroxy-1-[(2R, 3R,4S,5R,6R)-3,4,5-trihydroxy-6-methylsulfanyloxan-2-yl]propyl]-1-methyl-4-propylpyrrolidine-2-carboxamide;hydrochloride), is shown in Figure 2.5.

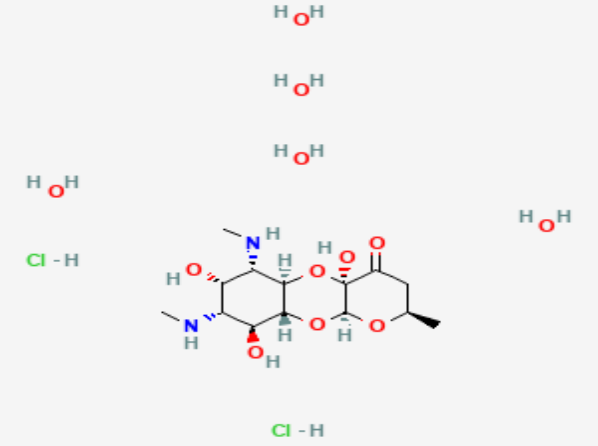


Figure 2.5. Spectinomycin dihydrochloride pentahydrate structure [181]

In Phase II tier A. the potential of the product LIN-SPE-MIX 880 to affect organisms in the aquatic and terrestrial environment is evaluated.

If exposed to daylight, lincomycin and spectomycin may undergo photolysis at the soil-atmosphere interface and on the surface of the litter. The mean time required for 50% lincomycin to dissipate was 31 days. Lincomycin thus belongs to the moderately persistent substances in the soil.

Lincomycin is stable enough during manure storage so that it is present when it is applied to crops or pastures as a plant nutrient source. Monitoring of lincomycin concentrations in manure-amended soils demonstrated that this antimicrobial can persist at distances greater than 5 cm from the soil for several months after application.

Available data on lincomycin and streptomycin used in environmental risk assessment are presented in Table 2.27, 2.28.

Table 2.27. Lincomycin environmental risk assessment parameters

Property	**Parameter**	**Parameter value**
Physicochemical	Solubility in water	900 mg/l
	Melting point	148ºC
	Vapor pressure	0,06 Pa
Mobility in soil	Koc	59 l/kg
Persistence in soil	DT 50 (zile)	31
Bioaccumulation	Log Kow	0,56

National Library of Medicine PubChem

Table 2.28. Spectinomycin environmental risk assessment parameters

Property	**Parameter**	**Parameter value**
Physicochemical	Solubility in water	100 mg/l
	Vapor pressure	0,06 Pa
Mobility in soil	Koc	580 l/kg
Persistence in soil	DT 50 (zile)	-
Bioaccumulation	Log Kow	0,82

National Library of Medicine PubChem

Spectinomycin does not persist in the soil, there is no possibility of accumulation in the environment. For lincomycin and spectinomycin, the bioaccumulative potential is low.

Available and predictable experimental toxicity values indicated that the toxicity of lincomycin and spectinomycin to aquatic and terrestrial organisms is low.

The water PNEC determined from the available ecotoxicity values for lincomycin and spectinomycin and the corresponding assessment factor is presented in Table 2.27, 2.28.

Table 2.27 PNECwater for lincomycin

Species	**Parameter / value (mg/l / mg/kg)**	**FA VICH**	**PNEC (mg/l)**
Fish 96 hours (*Lepomis macrochirus - Bluegill Sunfish*)	LC_{50}= 980	1000	0,98
Algae *(Ceriodaphnia)*	EC_{50}= 721	1000	0,721

(OECD 202, OECD 203)

Table 2.28 PNECwater for spectinomycin

Species	**Parameter / value (mg/l / mg/kg)**	**FA VICH**	**PNEC (mg/l)**
Fish 96 hours *(Oncorhynchus mykiss Rainbow Trout*)	LC_{50}= 118	1000	0,118
Shellfish (*Daphnia magna*) 48 ore	EC_{50}= 1000	1000	1
S. capricornutum (green alga), 72 hours	EC_{50} =1,18	1000	**0,00118**

(OECD 202, OECD 203)

Spectinomycin, which has low stability in stored manure, was not detected in manure applied to crops or grasslands. Consequently, it was not detected in the soil treated with fertilizers.

The most sensitive toxicity value was considered in the following calculations:

Algae PNECwater lincomycin = PNEC Ceriodaphnia = 0.721 mg/l

The calculation of soil PNEC and sediment PNEC taking into account PNECapa (determined from the result of experimentally determined effects), is carried out using EPM (balanced partitioning method), according to relations 1.1 and 1.2. They resulted in PNECsol = 0.834 mg/kg and PNECsed =1.485 mg/kg.

In order to be able to estimate the adverse effects on soil ecosystems, a risk analysis was carried out. To determine the risk (R), the initially determined PECs are compared to the PNEC calculated from the available ecotoxicity data (R = PEC/PNEC) for the porcine species. (Table 2.29).

Table 2.29. The risk (R) of lincomycin

Parameter / Species and category	**$R_{inisoil}$**	**$R_{inigroundwater}$**	**$R_{inisurfacewater}$**	**$R_{inisediment}$**
pigs	0.79	0,16	0.14	0,51

The very low environmental risk values (R < 1) for all species make the evaluation stop here and it follows that the veterinary medicinal product would not present any risk to the ecosystem [100 - 123].

2.6. Dissemination of environmental impact assessment research of veterinary medicines

The software method for evaluating the impact on the environment was carried out based on its own calculation algorithms that were the subject of scientific papers published in the magazine of Romanian veterinary medicine manufacturers, "Medicamentul Veterinar (Veterinary Drug)", listed BDI [148 - 149].

We have carried out research on the analysis of the transfer of levamisole, tiamulin in animal tissue after oral administration, an important factor for ensuring food safety and environmental protection, in order to develop HPLC-MS/MS methods for the determination of tiamulin, levamisole residues.

We carried out research to assess the impact of penicillin G on the environment using the analytical method, the software method, and developed in collaboration with the National Research and Development Institute for Environmental Protection, the SPE-Online-UHPLC-MS/MS method for the determination of penicillin G in water surface.

These researches have been published in articles from ISI-rated specialist journals, level Q1, Q2.

CHAPTER 3
CALCULATION ALGORITHMS IN ENVIRONMENTAL RISK ANALYSIS

3.1. The general calculation algorithm

For the assessment of the environmental risk of the active substances present in the PMV, a proprietary calculation algorithm was drawn up, which was the basis for the development of a special original and interactive software, which would allow the precise determination of the impact of the active substance in a short time . on environmental factors: soil, water, sediment.

The general calculation algorithm integrates all the steps taken according to the requirements of the EMEA guide from the determination of the initial predictable concentrations in soil (initial PECs calculated based on the total residue approach), of the predictable concentrations in water (initial PECs calculated in water, sediment), of predictable concentrations in the environment recalculated based on the type of excretion, metabolism of active substances, based on the degradation of active substances in soil and manure, of predictable no-effect concentrations, PNEC, for each species treated with the veterinary medicinal product, for all environmental compartments, soil , groundwater, surface water, sediment to initial risk stability R and refined, Rraf., Phase II Stage A.

In the EMEA guide the relationships are grouped according to various criteria in different chapters but not in a sequence necessary for the assessment according to the two phases. The original algorithm shows the logical sequence of all the steps you need to go through in order to calculate all the parameters needed to complete the assessment (Figure 3.1).

Figure 3.1. The general calculation algorithm

In structural diagrams of computational algorithms, parallelograms are blocks of given input or given output, rectangles are algorithm steps, and diamonds are logical blocks.

CHAPTER 4
THE SOFTWARE METHOD IN ENVIRONMENTAL RISK ANALYSIS

4.1. Description of the software method

Having completed our own calculation algorithm, presented in the previous chapter, for environmental risk assessment, we developed an original interactive software that:

- it is very easy and convenient to operate, allowing the easy entry of all privileged data on the analyzed veterinary medicinal product and on the animals under treatment;
- follows and concretely explains the steps of the calculation algorithm, having included all the data from the guide necessary for the evaluation, it is no longer necessary to consult it during the analysis;
- contains several options for calculating some parameters, allowing the choice of the most appropriate option;
- allows environmental assessors to carry out particularly detailed environmental risk analyzes in extremely short periods of time;

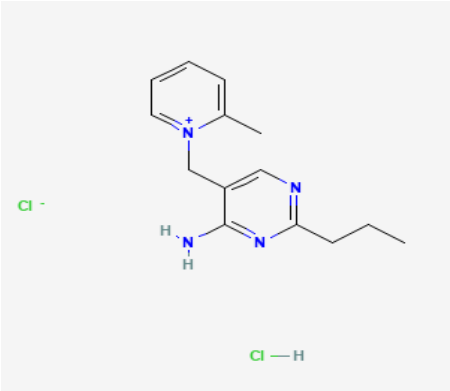
After entering all the product-specific data, according to the administration indications and the information specific to the active substances in the product, the values of all the parameters necessary for the evaluation are displayed.

The software was created in MATHCAD version 16 and updated for current MATHCAD PRIME versions.

I have selected to present as applications of the software method, two environmental impact assessments carried out for the active substances amprolium and penicillin G.

4.2. Environmental risk assessment for amprolium

The environmental risk assessment is an assessment of the exposure and effects of amprolium from a veterinary pharmaceutical product in the form of a premix containing 250 mg/g and administered to chickens, broilers (15.62 mg amprolium /kg body weight/day, in 7 consecutive days) and in turkeys (31.25 mg amprolium /kg body weight/day, in 7 consecutive days).



The structural formula
of the substance amprolium :
(5-[(2-methylpyridin-1-ium-1-yl)methyl]-2-propylpyrimidin-4-amine;chloride;hydrochloride)
is presented in Figure 4.1.
The molecular formula is $C_{14}H_{20}Cl_2N_4$

Figure 4.1. Structure of amprolium [182]

Amprolium is an anticoccidal agent that acts as a competitive inhibitor of thiamine in the parasite's metabolism and interferes with the metabolism of carbohydrate requirements for coccidian multiplication and survival. It is recommended in the control and treatment of eriosis (coccidiosis) produced by *Eimeria spp.*

4.2.1. Analytical environmental risk assessment for amprolium

PEC Calculation (Exposure Analysis)

The exposure assessment was done using the total residue approach. The total amount of the applied dose, excreted by the treated animal, without data on metabolism, was taken into account. The results obtained for each species were:

• PEC initial soil broiler = 969,798 µg/kg
• PEC initial soil laying hens = 113, 297 µg/kg
• PEC initial soil replacement chickens = 214.792 µg/kg
• PEC initial soil turkeys = 966,875 µg/kg
• PEC initial soil cumulative = 2265 µg/kg

Since the initial PECsol values for each target species are higher than 100 µg/kg, the environmental risk assessment continues in phase II.

It will be used in the calculations of the following initial cumulative soil PEC value of all categories of birds.

Calculation of PECwater, PECsediment

The predictable concentrations of penicillin G in groundwater (PEWater table), surface water (PESurface table) and in sediment (PESediment) were calculated, taking into account the molar mass (MW= 315.24), solubility in water (SOL = 540.32 mg/l), vapor pressure (VP = 0.06 Pa) and water-organic carbon distribution coefficient (Koc = 1.006 l/kg) (Table 4.1).

Table 4.1. Calculated predictable concentrations of amprolium in soil, groundwater,surface water and sediment.

Predictable concentration	**Amprolium**
soil	2265 µg/kg
groundwater	4182 µg/l
surface water	5575 µg/l
sediment	20630 µg/kg

Calculation of PNEC (Effect Analysis)

The Predicted No Effect Concentration (PNEC), the concentration of penicillin G that marks the limit below which no adverse effects of exposure in an ecosystem are measured, was calculated with ecotoxicity data available in the literature. Depending on the type of data used, the rating factor was used to account for the reliability of toxicity data that are extrapolated to an entire system (Table 4. 2).

Table 4.2. Estimated No Effect Concentration (PNEC)

Compartment	**Ecotoxicity descriptors**	**FA**	**PNEC**
water	NOECalge: 88,4 mg/l	10	8,84 mg/l
sediment	NOEC: 89,4 mg/kg	10	8,94 mg/kg
soil	NOECworms : 88,4 mg/kg	10	8,84 mg/kg

To determine the risk for the aquatic environment, we selected the lowest toxicity value (PNEC *Anabaena flos-aquae* = 8.84 mg/l).

In the case above, the calculated predicted no-effect concentration in water (PNECwater) is 8.84 mg/l. Using the assessment factor method, the calculated predicted no-effect concentration in soil (PNECsol) is 8.84 mg/kg and the calculated predicted no-effect concentration in sediment (PNECsediment) is 8.94 mg/kg.

Rsol calculation, groundwater, surface water, sediments (Risk Analysis)

The initially calculated PECs are related to the previously calculated PNECs to establish the initial risk. (Table 4.3).

Table 4.3. Cumulative initial risk (Cumulative initial) of amprolium:

Compartment	PECcumulat	PNEC	Cumulative initial risk
soil	2,265 mg/l	8,84 mg/kg	0,256
groundwater/surface water	4,182 /5,575 mg/l	8,84 mg/l	0,473 / 0,631
sediment	20,630 mg/kg	8,94 mg/kg	2,308

The values obtained for PECcumulat sediment and PNEC sediment, lead to a risk R >1, and to a continuation of the risk assessment with the refinement of PECs.

Calculation of Cumulative Refined PECs

The necessary adjustments to process PECs are: refining based on metabolism, on degradation in droppings and soil.
Considering the active dose fraction Fa = 0.68, the refined PECs for each species and the cumulative refined PEC taking into account metabolism (PECraf met cum) were determined.
In the refinement of PECs based on degradation in manure, the time period in which manure is stored and the degradation of amprolium in manure are taken into account, DT50 (days) > 8 (PECraf dej cum). Cumulative refined soil degradation PEC is determined taking into account the number of stages of manure spreading on the agricultural surface (PEC deg sol raf cum).

Calculation of cumulative refined risks
The cumulative refined PECs, calculated based on metabolism, degradation in faeces, degradation in soil, are compared with the PNECs to determine the refined cumulative risk (Table 4.4).

Table 4.4. The refined cumulative risk (Rraf cum) of amprolium:

Compartiment	PECraf met cum	PECraf dej cum	PEC deg soil raf cum	Rraf cum
soil	1,54 mg/kg	0,124 mg/kg	0,120 mg/kg	0,028
ground water/ surface water	-	-	0,45 / 0,6 mg/l	0,05 mg/l / 0,068
sediment	-	-	2,22 mg/kg	0,248

Since Cumulative Rrefined <1, it is not necessary to continue the risk assessment in Step B.

4.2.2. Average risk assessment for amprolium by software method

Matrices of the predictable concentration in the soil (PECsolinitiali), the predictable concentration in ground water (PECapafreaticai), the predictable concentration in surface waters (PECapedesuprafatai) and the predictable concentration in the sediment (PECsedimenti), better content calculated for all categories of intensively raised birds (chickens, broilers, turkeys) and treated with the analyzed product, according to the calculation formulas provided in the guide and presentation in the amprolium software.
The rows of the matrices represent the species of animals treated and the columns the categories within each species. In the case of amprolium, they are found in the common poultry row (third) for the categories broiler, layer hens, replacement hens and turkeys (Figure 4.2).

$$PECsolinitiali = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 969.798 & 113.297 & 214.792 & 0 & 966.875 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

a

$$PECapafreaticai = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 1.791 \times 10^3 & 209.187 & 396.583 & 0 & 1.785 \times 10^3 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

b

$$PECapedesuprafatai = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 2.387 \times 10^3 & 278.916 & 528.778 & 0 & 2.38 \times 10^3 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

c

$$PECsedimenti = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 8.835 \times 10^3 & 1.032 \times 10^3 & 1.957 \times 10^3 & 0 & 8.808 \times 10^3 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

d

Figure 4. 2. PEC matrix calculation - software aspects
a. PECsolinitiali matrix, [µg/kg], b. PEC apafreaticai matrix, [µg/l]
c. PECapedesuprafatai matrix, [µg/l], d. PEC sedimenti matrix, [µg/kg]

The software allows selection of the PEC value for soil, ground water, surface water and sediment (maximum or cumulative) to be used in the following calculations (Figure 4.3).

It will be used in the calculations of the following initial cumulative soil PEC value of all categories of birds.

$$PECsolinitialicum := \sum_{i=0}^{4} \sum_{j=0}^{5} PECsolinitiali_{i,j} \qquad PECsolinitialicum = 2.265 \times 10^3$$

$$PECsolinitialimax := \max(PECsolinitiali) \qquad PECsolinitialimax = 969.798$$

$$PECapedesuprafataicum := \sum_{i=0}^{4} \sum_{j=0}^{5} PECapedesuprafatai_{i,j} \qquad PECapedesuprafataicum = 5.575 \times 10^3$$

$$PECapedesuprafataimax := \max(PECapedesuprafatai) \qquad PECapedesuprafataimax = 2.387 \times 10^3$$

Figure 4.3. PECwater value selection – software aspects

After entering all the significant, available ecotoxicity values of amprolium and the conditions for the evaluation factor method, the results are displayed for PNEC water =8.84 mg/l, PNEC soil=8.84 mg/kg; PNEC sediment = 8.94 mg/l.

The assessment of the initial risks for the soil of amprolium, quantified by the indicators Rsolinii, Rapăfreaticai, Rapedesuprafațai, Rsedimenti, are calculated for all species and categories of intensively raised animals, according to the calculation formulas provided in the guide and present in the amprolium software (Figure 4.4).

$$Rsolinii = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0.11 & 0.013 & 0.024 & 0 & 0.109 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

a

$$Rapafreaticai = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0.203 & 0.024 & 0.045 & 0 & 0.202 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

b

$$Rapedesuprafatai = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0.27 & 0.032 & 0.06 & 0 & 0.269 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

c

$$Rsedimenti = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0.988 & 0.115 & 0.219 & 0 & 0.985 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

d

Figure 4.4 Matrix calculation - software aspects
a. Rsolinii matrix, b. Rapăfreatică matrix
c. Rapedesuprafață matrix, d. Rsedimenti matrix

Since the cumulative risk in the sediment is greater than 1 (Rsedimentcum = 2.308), the assessment continues with refinement of the PECs.
The concentration matrix of PECsolrafmeti [µg/kg] refined in the soil upon administration of the analyzed product, based on the way in which the dose of active ingredient is metabolized by the animal body. (Figure 4.5).

$$Fai = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0.68 & 0.68 & 0.68 & 0 & 0.68 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

a

$$PECsolrafmeti = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 659.463 & 77.042 & 146.059 & 0 & 657.475 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

b

Figure 4.5 Refined PEC based on metabolism
a.matrix of the active ingredient dose fraction b. concentration matrix of PECsolrafmeti

The concentration matrix of refined PECsolrafdejtai [µg/kg] in the soil of arable land and PECsolrafdejfai [µg/kg], in the soil of hayfields when the analyzed product is administered, based on the degradation of the active ingredient in the manure, according to relations 4.1 and 4.2 are named in Figure 4.6.

$$PECsolrafdejtai_{i,j} := \frac{Mti_{i,j} \cdot lrN \cdot 1000}{\rho bsu \cdot 10000 \cdot aps \cdot Nsi_{i,j}} \quad (4.1)$$

$$PECsolrafdejfai_{i,j} := \frac{PECsolrafdejtai_{i,j}}{Na} \quad (4.2)$$

$$PECsolrafdejtai = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 95.097 & 5.814 & 4.36 & 0 & 18.489 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

a

$$PECsolrafdejfai = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 47.548 & 2.907 & 2.18 & 0 & 9.245 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

b

Figure 4.6 Refined PEC in soil based on degradation in manure
a. PECsolrafdejtai in the soil of arab lands b. PECsolrafdejfai in the soil of hayfields

The matrix of PECapafreaticaraftai concentration [μg/l] predictable refined in groundwater, of the active ingredient of the administration of the analyzed product, under the influence of organic fertilization of arab lands is presented in Figure 4.7.

$$PECapafreaticaraftai_{i,j} := \frac{PECsolraftai20cm_{i,j} \cdot RHOsol}{Ksolapa \cdot 1000}$$

$$PECapafreaticaraftai = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 175.582 & 10.734 & 8.05 & 0 & 34.138 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

Figure 4.7 Refined PEC in groundwater - arable land

The matrix of the refined predictable concentration in groundwater, under the influence of organic fertilization of hay (PECapafreaticaraffi) [μg/l] is presented in Figure 4.8.

$$PECapafreaticaraffi_{i,j} := \frac{PECsolraffi20cm_{i,j} \cdot RHOsol}{Ksolapa \cdot 1000}$$

$$PECapafreaticaraffi = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 170.216 & 10.406 & 7.804 & 0 & 33.095 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

Figure 4.8 Refined PEC in ground water - hay

The refined predictable concentration matrix in surface waters under the influence of organic fertilization of arable land (PECapedesuprafataraftai) and hay (PECapedesuprafataraffi) [μg/l) is presented in Figure 4.9 and 4.10.

$$PECapedesuprafataraftai_{i,j} := \frac{\frac{PECsolrafdejtai_{i,j} \cdot RHOsol}{Ksolapa \cdot 1000}}{3}$$

$$PECapedesuprafataraftai = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 234.11 & 14.312 & 10.734 & 0 & 45.517 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

Figure 4.9 Refined PEC in surface water - arable land

$$PECapedesuprafataraffi_{i,j} := \frac{\frac{PECsolrafdejfi_{i,j} \cdot RHOsol}{Ksolapa \cdot 1000}}{3}$$

$$PECapedesuprafataraffi = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 226.954 & 13.875 & 10.406 & 0 & 44.126 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

Figure 4.10 Refined PEC in surface water - hayfields

The matrix of the refined predictable concentration in sediment, under the influence of organic fertilization of arable land (PECsedimentraftai [μg/kg]) and hay (PECsedimentraffi [μg/kg]) according to relation 4.3 and 4.4, is presented in Figure 4.11.

$$PECsedimentraftai_{i,j} := \frac{Ksedimentapa \cdot PECapedesuprafataraftai_{i,j} \cdot 1000 \cdot CONVsediment}{RHOsediment} \quad (4.3)$$

$$PECsedimentraffi_{i,j} := \frac{Ksedimentapa \cdot PECapedesuprafataraffi_{i,j} \cdot 1000 \cdot CONVsediment}{RHOsediment} \quad (4.4)$$

$$PECsedimentraffi = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 839.867 & 51.344 & 38.508 & 0 & 163.293 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix} \quad PECsedimentraftai = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 866.347 & 52.963 & 39.722 & 0 & 168.441 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

Figure 4.11 Matrix of refined predictable concentration in sediment

The assessment of refined risks for the soil under the influence of organic fertilization of arable land (Rsolraftai) and hayfields (Rsolraffi), respectively Rsolrafcum and Rsolrafmax, calculated based on the cumulative and maximum values of the previously refined predictable concentrations is presented in Figure 4.12 and 4.13.

$$Rsolraftai_{i,j} := \frac{PECsolrafdejtai_{i,j}}{1000 \cdot PNECsol} \quad Rsolraftai = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0.011 & 6.576 \times 10^{-4} & 4.932 \times 10^{-4} & 0 & 2.092 \times 10^{-3} & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

Figure 4.12 Refined soil risks - arable land

$$Rsolraffi_{i,j} := \frac{PECsolrafdejfi_{i,j}}{1000 \cdot PNECsol} \quad Rsolraffi = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0.01 & 6.375 \times 10^{-4} & 4.782 \times 10^{-4} & 0 & 2.028 \times 10^{-3} & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

Figure 4.13 Refined soil risks - hayfields

The refined risk assessment for groundwater under the influence of organic fertilization of arable land (Rapafreraftai) and hayfields (Rsolraffi) is presented in Figure 4.14 and 4.15.

$$Rapafreraftai_{i,j} := \frac{PECapafreaticaraftai_{i,j}}{1000 \cdot PNECapa} \quad Rapafreraftai = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0.02 & 1.214 \times 10^{-3} & 9.107 \times 10^{-4} & 0 & 3.862 \times 10^{-3} & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

Figure 4.14 Refined groundwater risks - arable land

$$Rapafreraffi_{i,j} := \frac{PECapafreaticaraffi_{i,j}}{1000 \cdot PNECapa} \quad Rapafreraffi = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0.019 & 1.177 \times 10^{-3} & 8.829 \times 10^{-4} & 0 & 3.744 \times 10^{-3} & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

Figure 4.15 Refined groundwater risks – hayfields

The refined risk assessment for surface water under the influence of organic fertilization of arable land (Rapesupraftai) and hayfields (Rapesupraffi) is presented in Figure 4.16 and 4.17.

$$Rapesupraftai_{i,j} := \frac{PECapedesuprafataraftai_{i,j}}{1000 \cdot PNECapa}$$

$$Rapesupraftai = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0.026 & 1.619 \times 10^{-3} & 1.214 \times 10^{-3} & 0 & 5.149 \times 10^{-3} & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

Figure 4.16 Refined risks for surface water - arable land

$$Rapesupraffi_{i,j} := \frac{PECapedesuprafataraffi_{i,j}}{1000 \cdot PNECapa}$$

$$Rapesupraffi = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0.026 & 1.57 \times 10^{-3} & 1.177 \times 10^{-3} & 0 & 4.992 \times 10^{-3} & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

Figure 4.17 Refined risks for surface water - hayfields

The refined risk assessment for sediment under the influence of organic fertilization of arable land (Rsedraftai) and hayfields (Rapesupraffi) is presented in Figure 4.18 and 4.19.

$$Rsedraftai_{i,j} := \frac{PECsedimentraftai_{i,j}}{1000 \cdot PNECsediment}$$

$$Rsedraftai = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0.097 & 5.924 \times 10^{-3} & 4.443 \times 10^{-3} & 0 & 0.019 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

Figure 4.18 Refined risks for sediment - arable land

$$Rsedraffi_{i,j} := \frac{PECsedimentraffi_{i,j}}{1000 \cdot PNECsediment}$$

$$Rsedraffi = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0.094 & 5.743 \times 10^{-3} & 4.307 \times 10^{-3} & 0 & 0.018 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

Figure 4.19 Refined risks for sediment –hay

Since all cumulative risk values in soil, water, sediment are sub-unit, the evaluation stops here and it can be seen that the product does not pose any risk to the environment.

The full listing of the amprolium environmental impact assessment software is presented in Appendix 1.

The full listing of the penicillin G environmental impact assessment software is presented in Appendix 2.

CHAPTER 5
MONITORING OF PENICILLIN G IN SURFACE WATERS BY SPE-ONLINE-UHPLC-MS/MS

In recent decades, the quality of aquatic ecosystems has been threatened by increasing levels of pollution caused by the release of certain chemicals. Commission Implementing Decision (EU) 2015/495 published in March 2015 draws up a "watch list" of compounds to be monitored at European level including antibiotics.

In this PhD thesis, a methodology based on online solid phase extraction (SPE) ultra-high performance liquid chromatography coupled to a triple quadrupole mass spectrometer (UHPLC-MS/MS) was developed for the simultaneous determination of several compounds, present in veterinary drugs, from which we selected penicillin G. The proposed method offers advantages to many already available methods, such as versatility (more compounds can be analyzed simultaneously), shorter time required for analysis, robustness and sensitivity. The use of online sample preparation minimized sample handling, reduced sample volume required and analysis time, thus making analysis fast and reliable. The method was successfully validated in surface water and influent and effluent wastewater with detection limits of nanogram per liter level. The method developed in this study for the identification and dosage of pharmaceutical product residues in unidentified and degradation-producing surface water matrices that may occur especially in the treatment process, in wastewater treatment plants.

5.1. Sampling and preparation of water samples.

The identification and dosing of Penicillin G was carried out from the Ialomița river, the Buzău-Ialomița hydrographic basin, in the area downstream from the city of Slobozia. Livestock farms and agricultural land are located upstream of the sampling area, as well as the sewage treatment plant of the city of Slobozia. The collection of the 4 samples was carried out weekly, between March 15 and April 5, 2023. The water sample was taken approximately 0.5 m below the water surface and stored in a brown bottle for analysis. After collection, the sample was stored in the ice box and delivered on the day of sample collection to the laboratory, where it was stored at 4 °C in the laboratory refrigerator until analysis. The surface water sample was filtered through 0.2 µm polyethersulfone (PES) filters (Sartorius Stedim Biotech GmbH, Germany) before direct injection into the SPE-online-UHPLC-MS/MS system.

5.2. Equipment, reagents

Equipment

Identification and quantification of Penicillin G was performed using Thermo Fisher Scientific™ SPE-online-UHPLC-MS/MS tandem mass spectrometer, EQuan MAX Plus™UltiMate 3000 System connected to a TSQ triple quadrupole mass spectrometer. equipped with an electro-spray ionization source in positive mode and TraceFinder 3.2 software (USA) for searching and data processing. The technique used is the most sensitive and selective compared to other commonly used analytical techniques, allowing the detection and quantification of emerging pollutants from the pharmaceutical product category at the ng/l level.

Reagenți

The reference standard used for this analysis was Penicillin G (≥97% purity) produced by Dr. Ehrenstorfer, Germany. Ultrapure water was supplied by VWR Chemicals, used in the preparation of all standard solutions. HPLC grade acetonitrile and formic acid for LC/MS were provided by Scharlau Chemie SA and VWR Chemicals.

5.3. Description of the SPE-online-UHPLC-MS/MS method

The method developed in this study for the identification and quantification of Penicillin G by the SPE-online-UHPLC-MS/MS method meets the European Union (EU) requirements regarding the limit of

detection. Identification and quantification were performed in selected reaction monitoring (SRM) mode, recording the transitions between the precursor ion and the two most abundant product ions for each target analyte, thus achieving three identification points for the compound according to the Commission Decision of 2002/657/CE regarding the performance of analytical methods and the interpretation of results.
Online preconcentration column switching was applied as a means to minimize sample pretreatment and shorten analysis time. Chromatographic analysis was performed using a Thermo Scientific™ EQuan MAX™ online sample concentration UHPLC-MS/MS system equipped with a Thermo Scientific™ Hypersil GOLD aQ™ preconcentration column (20 x 2.1 mm, particle size 12 μm) and a Thermo Scientific™ Hypersil GOLD™ analytical column (50 x 2.1 mm, 1.9 μm particle size). Chromatographic separation of the analyzes was performed at a constant flow rate of 300 μL/min. The injection volume was 1 ml and the column temperature was maintained at 20°C. The mobile phase consisted of (A) ultrapure water with 0.1% (v/v) formic acid and (B) acetonitrile with 0.1% (v/v) formic acid; The gradient elution program worked as follows: the gradient started with 2% mobile phase B and 98% mobile phase A until time 1.2 min; in the range 1.2–1.6 min, mobile phase B increased from 2% to 100% and remained at 100% in the range 1.6–9.0 min; in the interval 9–11 min, mobile phase B decreased from 100% to 2% and mobile phase A increased from 0 to 98%. All target compounds were eluted from the column within 14 min. The stream from the LC column was transferred to a triple-quadrupole mass spectrometer equipped with an ESI source. Mass spectrometer analyzes were performed in a triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source operated in positive ionization mode.
Ionization mode: Heated Electrospray (H-ESI) Scan type: SRM Polarity: positive ion mode; Spray voltage [V]: 3400 positive ion; sweep gas pressure [arb]: 0; Vaporizer temperature [°C]: 300; Mantle gas pressure [arb]: 30; Auxiliary gas pressure [arb]: 10; Capillary temperature [°C]: 350; Collision gas pressure [mTorr]: 1.5; Cycle time [s]: 1; Peak width: Q1/Q3 full width of a peak at half maximum height (FWHM) of 0.70 Da.
The optimized ionization mode, fragmentation voltages, collision energies and chromatographic retention times for each analyte are summarized in table 5.1

Table 5.1. Selected reaction monitoring transitions

Compound	Retention Time (min)	Polarity	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy (V)
Penicillin G	5.94	Positive	335.2	176 160 114	14.1 13 31.5

5.4. Results and discussion

The TraceFinder 3.2 software processes the data both for the creation of calibration curves, method validation, and the processing of good method data for the identification of pharmaceutical compounds from different samples and complies with the requirements of the European guidelines for their validation.

The analytical method used in this work was evaluated in terms of linearity, repeatability, accuracy and sensitivity. The manufacturer's widely used 100 μg/mL penicillin G standard solution was used to prepare a 1 μg/mL stock solution in 1:1 (v:v) water - acetonitrile. A 0.01 μg/ml (10 ng/ml) solution was prepared from the stock solution, which was further diluted to 5 levels taking into account 4 pg/ml, 20 pg/ml, 100 pg/ml, 500 pg/ml and 5000 pg/ml (4 ng/l, 20 ng/l, 100 ng/l, 500 ng/l and 5000 ng/l). The standard calibration curve was plotted based on the results obtained with the standard calibration solutions. The linearity of the response was verified. Penicillin G concentration was calculated using the standard calibration curve. The calibration curve obtained for Penicillin G was linear, with a correlation coefficient R2> 0.999 (Figure 5.1). The relative standard deviation (RSD) was 2.5% RSD for repeatability and 3.8% RSD for reproducibility. The precision limit <10% RSD was met, indicating good precision of the method (Figure 5.1).

Method validation was performed by analyzing 10 replicates at 5 different concentrations (4 ng, 20 ng, 100 ng, 500 ng and 5000 ng). Mixed standard working solution with antibiotic concentrations ranging from 4 to 5000 ng/L was set up for detection and analysis. The limit of detection and limit of quantification were calculated by the method based on the calibration curve (Miller and Miller, 1993). The residual standard deviation of the areas, as standard deviation (σ), was calculated. The limit of detection was calculated with the formula LOD = 3.3 σ/p, where is the slope of the curve and the limit of quantification LOQ = 10 σ/p (σ = 0.43; p = 46.5). The limit of detection (LOD) for Penicillin G tested in the water sample was 0.03 ng/l and the limit of quantification (LOQ) 0.09 ng/l. The on-line SPE system eliminates human SPE errors and provides very good reproducibility of the method (Figure 5.2) [185].

Analysis report data: injected concentration: 32.452, retention time: 5.92, area: 1748, height: 2032.

Calibration Summary								
				A0	**A1**	**A2**	**R^2**	
		Manually	**Curve**	**y-Intercept**	**Slope**		**R^2**	
Compound		**Integrated**	**Type**	**Mean RF**			**% RSD**	
penicillin G			L	2,39E+03	4,65E+01		0,999	

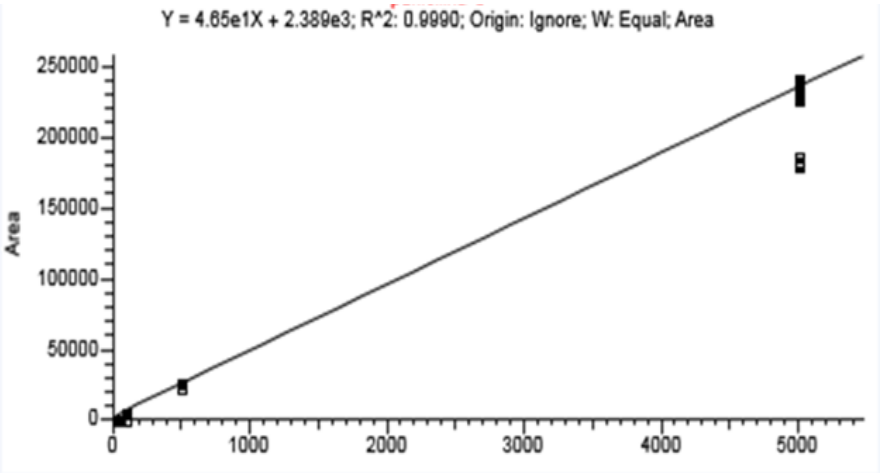


Figure 5.1. Penicillin G calibration curve

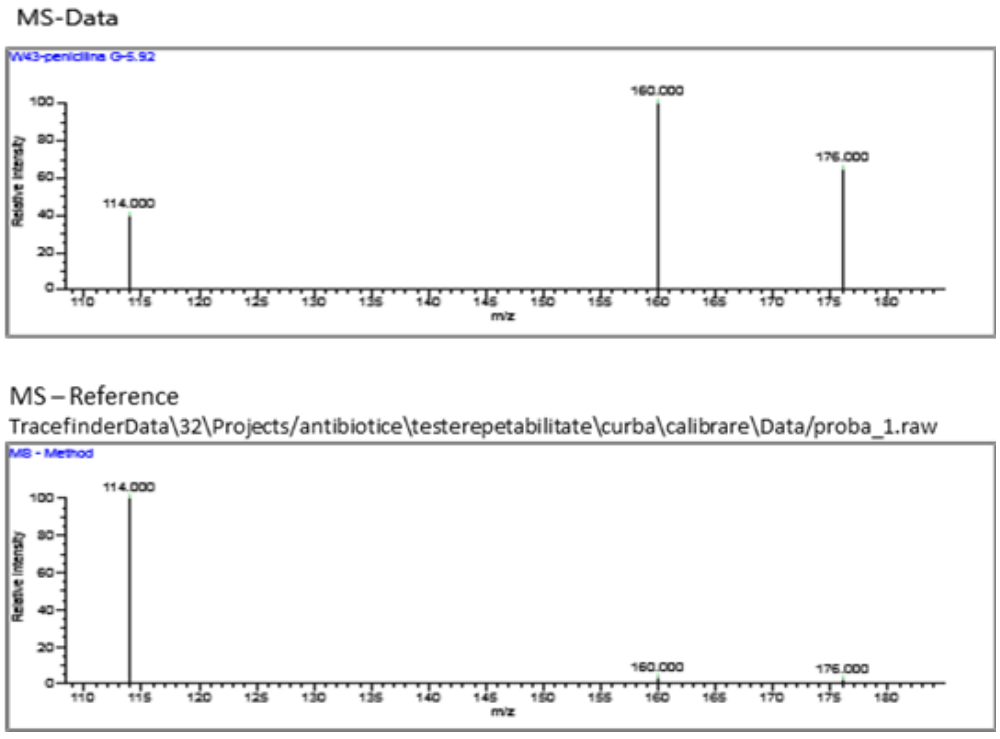


Figure 5.2. Repeatability tests - calibration curve

The results of the optimized ionization mode, fragmentation voltages, collision energies, and chromatographic retention time for Penicillin G are given in the Thermo Scientific Instrument Analysis Report (Figure 5.3).

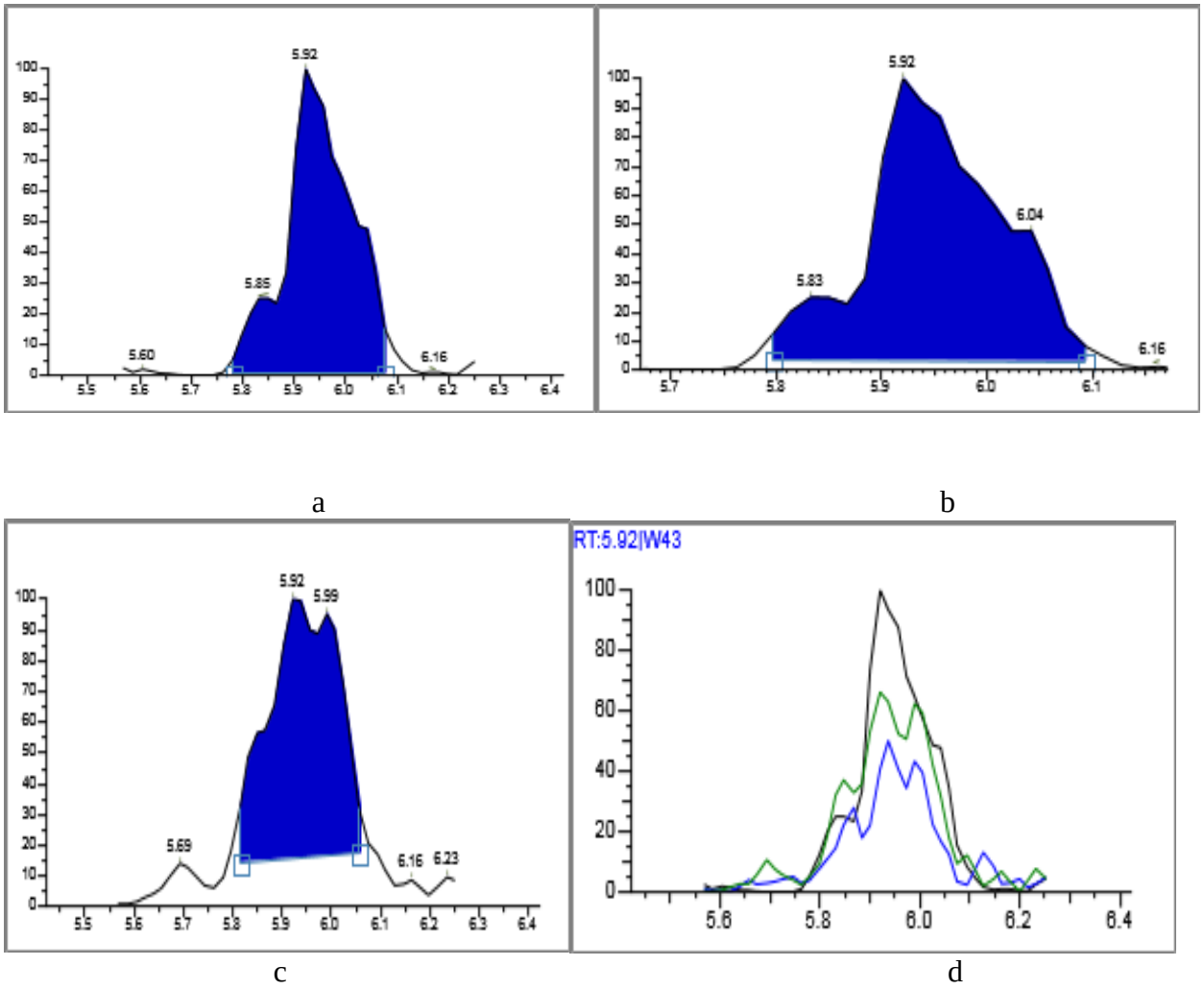


Figure 5.3. Thermo Scientific Instrument Analysis Report
Confirmatory ion chromatograms for penicillin G:
A. Quan Peak: 335,200-> 114,000 mz, b. Ion 1 Quality: 335,200->160,000 mz,
c. Cal Ion 2: 335,200-> 176,000 mz, d. Superimposed chromatograms of the 3 confirmatory ions for penicillin G

Surface water samples were taken at weekly intervals:

- sample 1 on 15.03.2023
- sample 2 on 22.03.2023
- sample 3 on 29.03.2023
- sample 4 on 04.05.2023

The results of Penicillin G monitoring from the analyzed surface water samples are presented in Figures 5.4, 5.5, 5.6, 5.7.

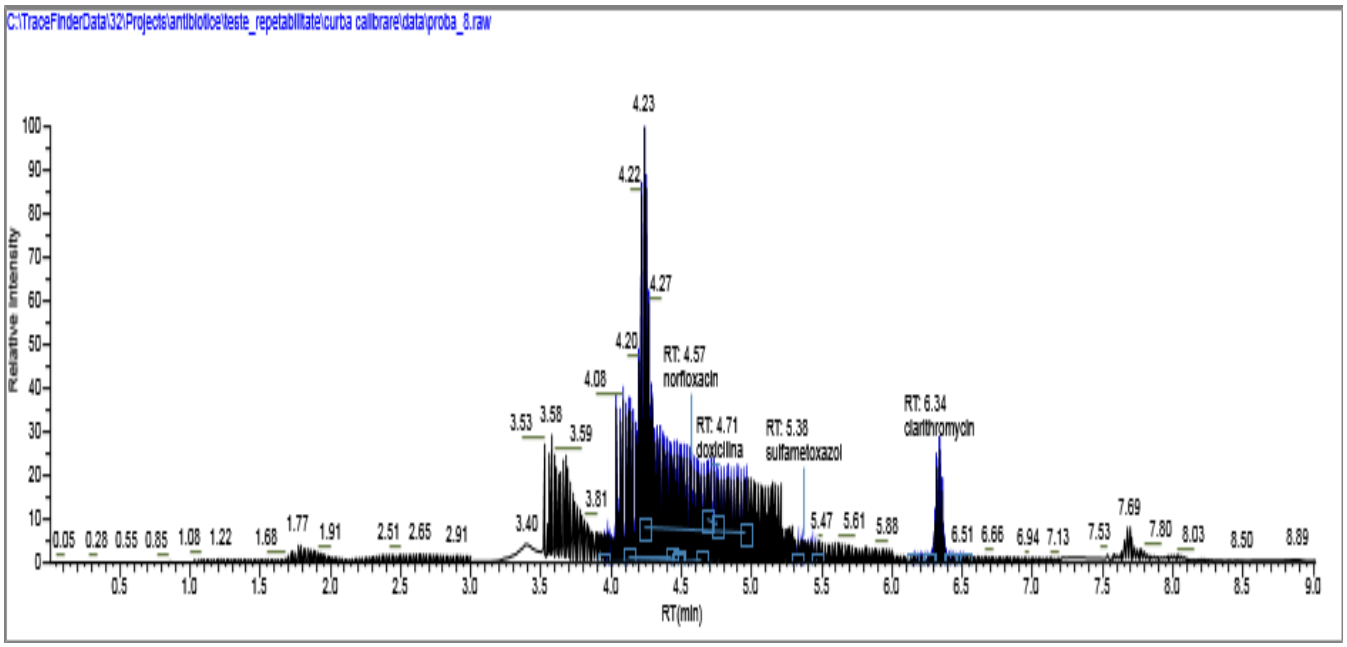


Figure 5.4. Identification, dosage of pharmaceutical pollutants from the Ialomița river – sample 1

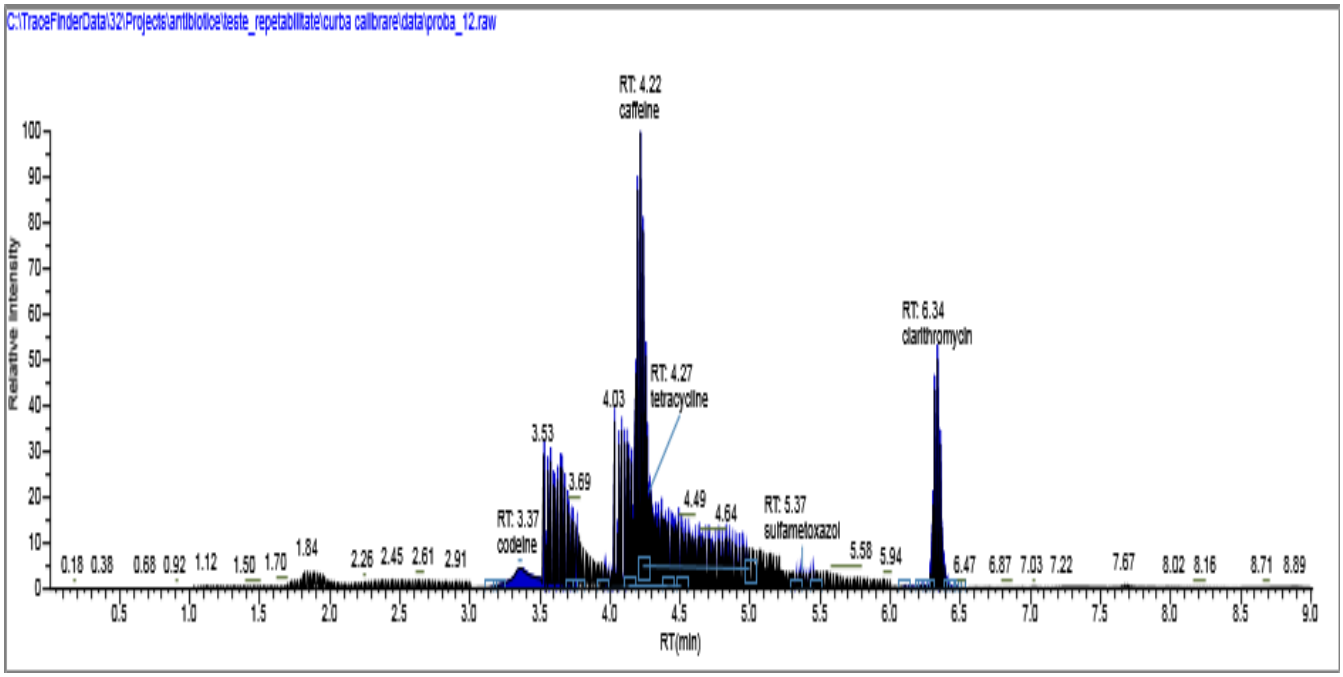


Figure 5.5. Identification, dosage of pharmaceutical pollutants from the Ialomița river - sample 2

Penicillin G was not detected in the analyzed surface water samples 1 and 2 taken on March 15 and 22, 2023 (Figure 5.4, Figure 5.5).

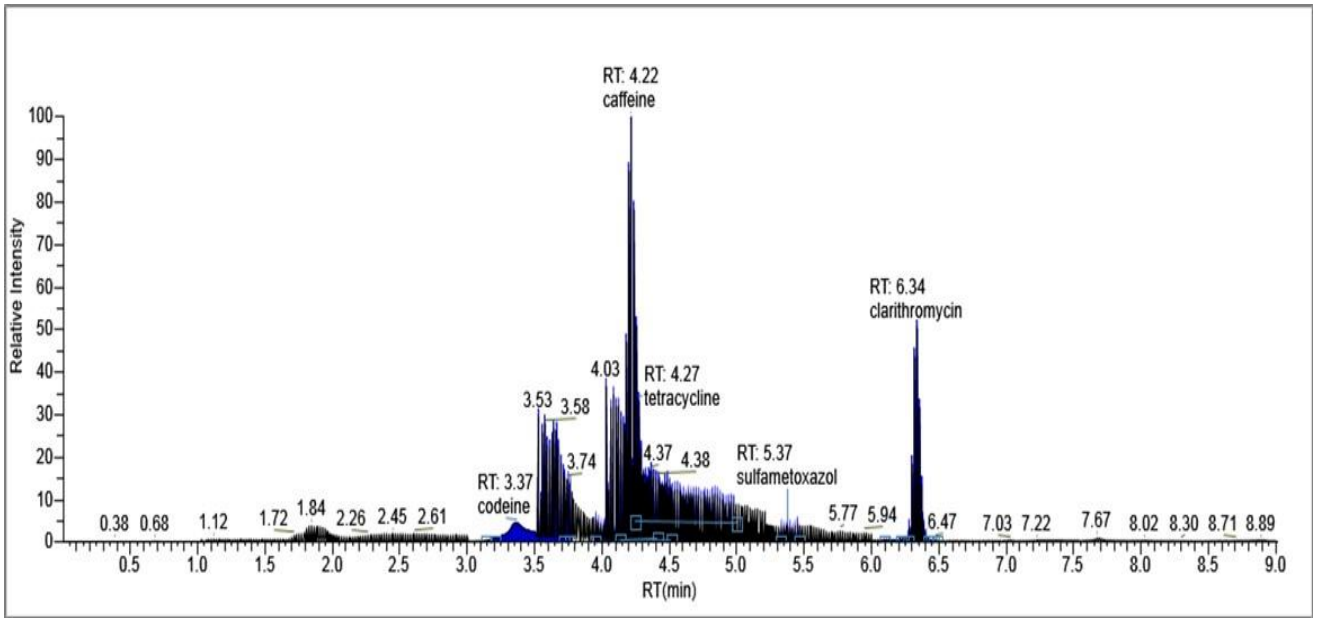


Figure 5.6. Identification, dosage of pharmaceutical pollutants from the Ialomița river – sample 3

Penicillin G was identified and dosed in a concentration of 0.032 µg/l, only in sample 3, taken on March 29, 2023 (Figure 5.6).

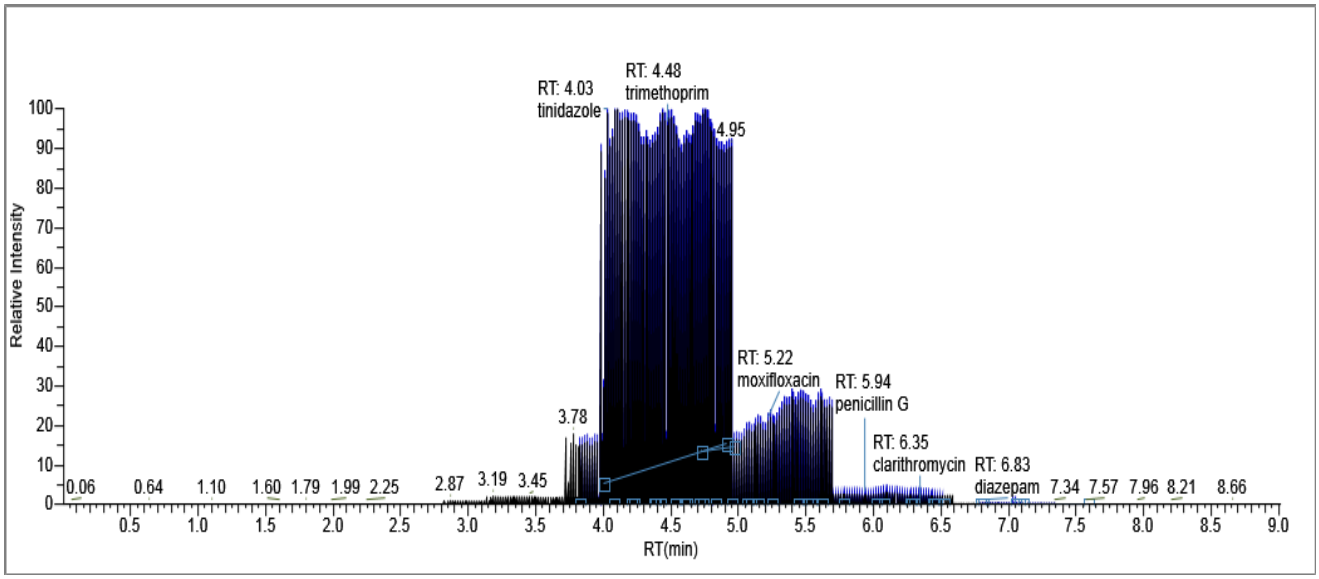


Figure 5.7. Identification, dosage of pharmaceutical pollutants from the Ialomița river - sample 4

Figures 5.4 - 5.7 show, according to identification, the specific dosage analyzed, including penicillin G, depending on the retention time (RT), the confirmation according to MS, for the precursor ion and the product ion (confirmation report for the analyzed sample).

Penicillin G was not detected in sample 4 of surface water taken on April 5, 2023 (Figure 5.7).

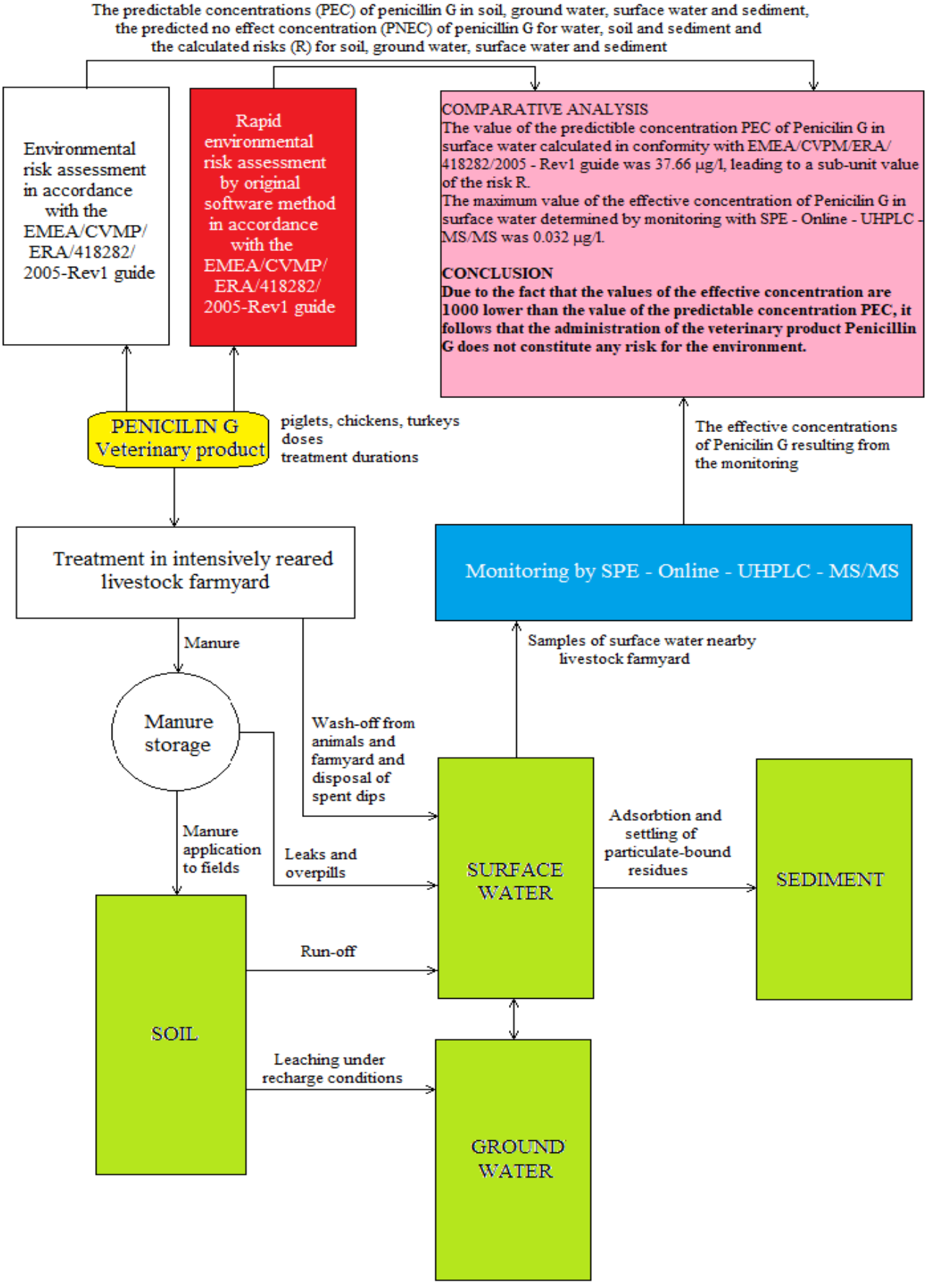


Figure 5.8. Scheme of the environmental impact assessment of penicillin G, described previously

Figure 5.8 shows schematically the evaluation of the environmental impact of penicillin G from a veterinary product according to the EMEA guide, by the analytical method and by the software method. The results obtained for the surface water environmental compartment by the analytical method / software, according to the EMEA guide, are compared with the results obtained by the SPE on line UHPLC-MS/MS analysis method.

Such studies are very important to detect Penicillin G residues and the potential indirect effects of environmental exposure on ecological and human health.

Penicillin G was not detected in the investigated surface water samples.

Only in one sample were traces of Penicillin G detected.

Through this study, SPE-online-UHPLC-MS/MS proved to be a powerful analytical tool that allows highly sensitive detection of antibiotics in surface waters, even when they are present in trace amounts (ng/l). This type of analysis will provide important insights into the occurrence and distribution of antibiotics (and other pharmaceuticals) in drinking water supplies and could help to impose appropriate limits on the amounts of these chemicals that are allowed to enter the environment.

In the environmental risk assessment of penicillin G according to the EMEA guideline, the predicted concentration of penicillin G in surface water ***(37.66 µg/l***) is higher than the experimentally determined one (0.032 µg/l), but the calculated environmental risk (R) is sub-unitary and indicates that it is carried out with the veterinary medicinal product considered to present no risk to the environment.

The method developed for the quantification of Penicillin G was also used for the detection of other pharmaceutical pollutants.

In sample 1, the following active substances were detected: norfloxacin 39 ng/l, sulfamethoxazole 35 ng/l, clarithromycin 53 ng/l and doxycycline 62 ng/l (Figure 5.4).

In sample 2, the following active substances were detected: caffeine 20 ng/l, sulfamethoxazole 47 ng/l, tetracycline 94 ng/l and clarithromycin 85 ng/l (Figure 5.5).

In sample 3, the following active substances were detected: caffeine 41 ng/l, sulfamethoxazole 62 ng/l, tetracycline 45 ng/l and clarithromycin 58 ng/l (Figure 5.6).

In sample 4, the following active substances were detected: trimethoprim 93 ng/l, tinidazole 41 ng/l, moxifloxacin 56 ng/l and clarithromycin 65 ng/l (Figure 5.7).

The monitoring of these active substances was carried out for 3 months. Samples were taken monthly and low concentrations were detected at the ng/l level.

CHAPTER 6
MONITORING OF TRIMETHOPRIM IN SURFACE WATERS BY SPE-ONLINE-UHPLC-MS/MS

The veterinary medicine with trimethoprim was administered to horses, in injectable form, at a dose of 30 mg/kg body weight 2 times a day.

Structural formula: 5-[(3,4,5-rimethoxyphenyl)methyl]pyrimidine-2,4-diamine (Figure 6.1)

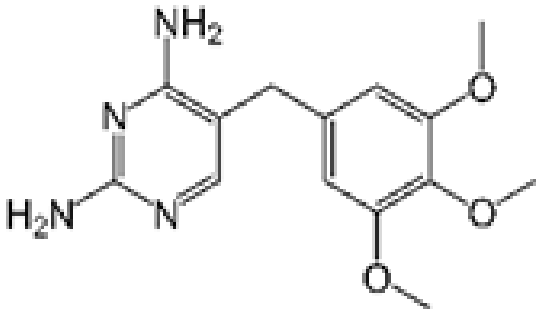


Figure 6.1. Structure of trimethoprim (PubChem) [184]

6.1. Sampling and preparation of water samples

The identification and dosing of trimethoprim was carried out from the Ialomița river, the Buzău-Ialomița river basin, in the area downstream from the city of Slobozia. Livestock farms and agricultural land are located upstream of the sampling area, as well as the sewage treatment plant of the city of Slobozia. The collection of the 4 samples was carried out weekly, between April 5 and April 25, 2023. The water sample was taken approximately 0.5 m below the water surface and stored in a brown bottle for analysis. After collection, the sample was stored in the ice box and delivered on the day of sample collection to the laboratory, where it was stored at 4 °C in the laboratory refrigerator until analysis. The surface water sample was filtered through 0.2 µm polyethersulfone (PES) filters (Sartorius Stedim Biotech GmbH, Germany) before direct injection into the SPE-online-UHPLC-MS/MS system.

6.2. Equipment, reagents

Equipment

Identification and quantification of trimethoprim was performed using Thermo Fisher Scientific™ SPE-online-UHPLC-MS/MS tandem mass spectrometer, EQuan MAX Plus™UltiMate 3000 System connected to a TS triple quadrupole mass spectrometer. an electrospray ionization source in positive mode and TraceFinder 3.2 software (USA) for application and data processing. The technique used is the most sensitive and selective compared to other commonly used analytical techniques, allowing the detection and quantification of emerging pollutants from the pharmaceutical product category at the ng/l level.

Reagents

The reference standard used for this analysis was trimethoprim (≥97% purity) produced by Dr. Ehrenstorfer, Germany. Ultrapure water was supplied by VWR Chemicals, used in the preparation of all standard solutions. HPLC grade acetonitrile and formic acid for LC/MS were provided by Scharlau Chemie SA and VWR Chemicals.

6.3. Description of the SPE-online-UHPLC-MS/MS method

The optimized ionization mode, fragmentation voltages, collision energies, and chromatographic retention times for each analyte are summarized in Table 6.1**.**

Table 6.1. Selected reaction monitoring transitions

Compound	Retention Time (min)	Polarity	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy (V)
				261,1	25.1
Trimetoprim	4.48	Positive	291,2	230	23.5
				123,1	26,4

The results of the optimized ionization mode, fragmentation voltages, collision energies, and chromatographic retention times for trimethoprim are given in the Thermo Scientific Instrument Analysis Report (Figure 6.2).

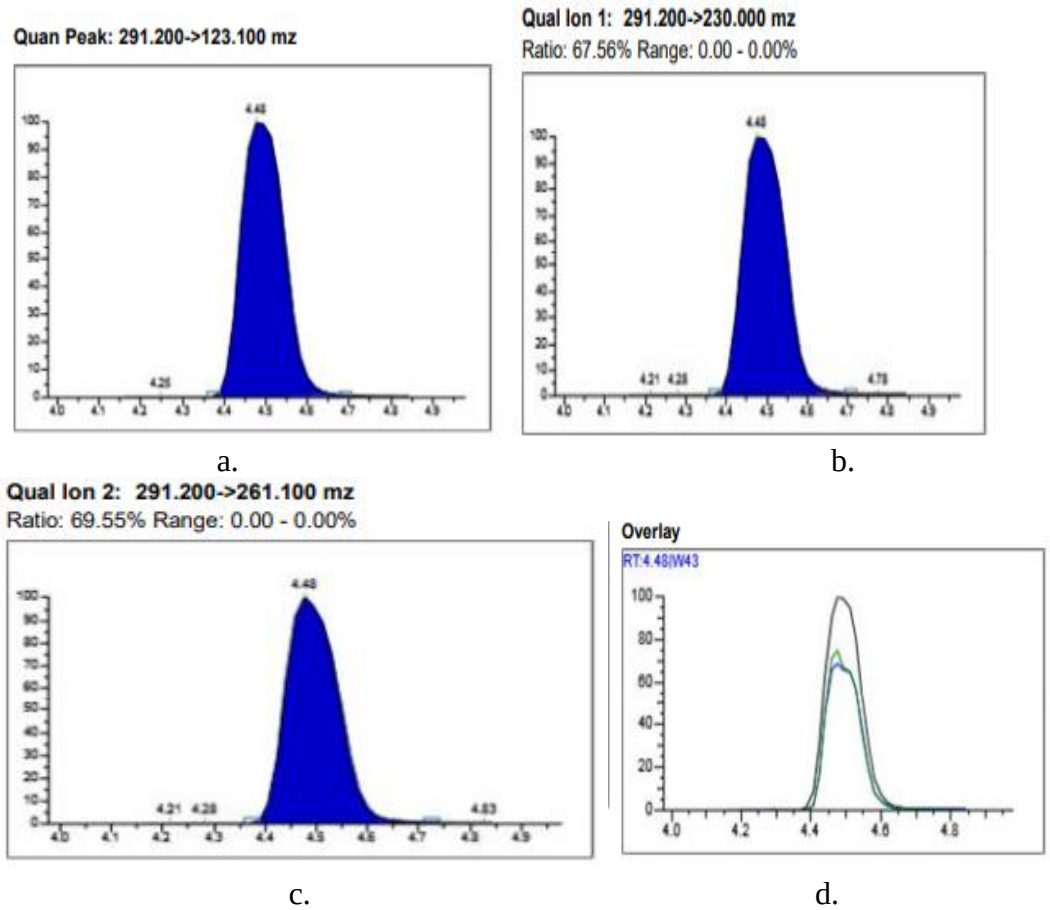


Figure 6.2. Thermo Scientific Instrument Analysis Report
Confirmatory ion chromatograms for trimethoprim:
A. Quan Peak: 291,200-> 123,100 mz, b. Ion 1 Quality: 291,200->230,000 mz,
c. Cal Ion 2: 291,200-> 261,000 mz, d. superimposed chromatograms of the 3 confirmatory ions for trimethoprim

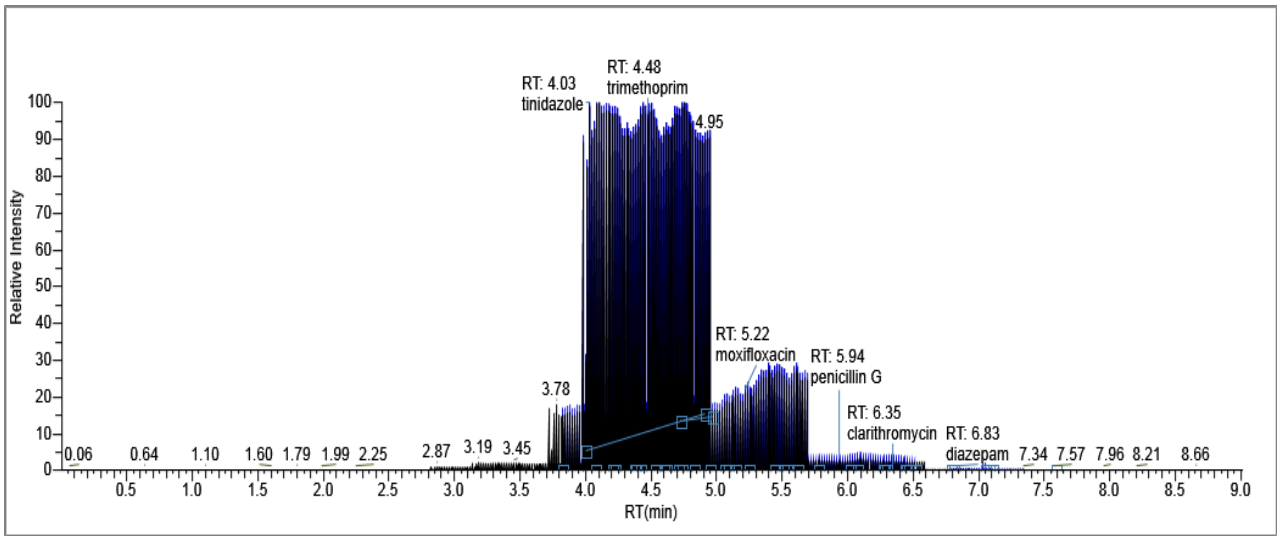


Figure 6.3. Identification, dosage of pharmaceutical pollutants from the Ialomița river – sample 1

Trimethoprim was detected in a single surface water sample, sample 1 (taken on April 5, at a concentration of 93 ng/l.

Figure 6.3 shows corresponding identification results, the main analyzed dosage, including trimethoprim, depending on the retention time (RT), confirmation according to MS, for the precursor ion and the product ion (confirmation ratio for the analyzed sample).

Based on studies [162-174], it is found that 1 µg/l would be a reasonable protective exposure limit for trimethoprim in aquatic environments β-lactam antibiotics (penicillin β-lactam antibiotic discovered) are not usually detected in the aquatic environments analyzed in Europe extremely due to their high latencies to heat, light, pH, metal ions, oxidizing and reducing agents, nucleophiles and solvents, such as. would be water, which lead to their hydrolysis under ambient temperature and pH conditions. However, based on several studies carried out in the EU, the ranges of β-lactam concentrations in water were determined, namely: 18–6196 ng/l in the influent of wastewater treatment plants, 47–1205 ng/l , in wastewater treatment plant eluent and 3.57–552 ng/l, in surface water [175].

CHAPTER 7
GENERAL CONCLUSIONS, ORIGINAL CONTRIBUTIONS AND PERSPECTIVES

7.1 General conclusions

1. In the European Union, veterinary medicinal products are authorized on the basis of an environmental risk assessment procedure, a centralized applied procedure (at European level) or through a decentralized and mutual recognition procedure (at member state level) and regardless of level, the risk assessment framework is the same for any procedure followed.

2. The environmental risk assessment contains the analysis of potential adverse effects resulting from environmental exposure to a veterinary medicinal product and is carried out in accordance with the EMEA guidelines (VICH GL6) and VICH GL38).

3. Environmental risk assessment requirements by procedure type are included in the guideline EMEA/CVMP/182112/2006, developed by VICH and approved in 2009, to harmonize the data requirements and the basic risk assessment process for approval, authorization of veterinary medicinal products in participating countries.

4. Regulation (EU) 2019/6 of January 2022, aims to update the regulatory framework for veterinary medicinal products and replaces Directive 2001/82/EC, but the knowledge of the environmental risks of all authorized veterinary medicinal products and the consistency of assessments remains quite similar between both legislations.

5. The process of assessing the environmental risks of veterinary medicinal products is progressive, in two phases, and can be completed after phase I, of lower level, for veterinary medicinal products that do not pose a risk to the environment, or after completion of phase II, of level superior, for veterinary medicinal products with a risk for the environment; depending on the degree of risk to the environment in phase II, steps A, B and C can be completed by using procedures for refining the risk parameters determined in phase I based on specific data for the safety profile of the analyzed substances or on some analyzes risks-benefits.

6. The issue of environmental risk assessment of veterinary drugs is of great importance worldwide, being intensively debated in a multitude of specialized works covering a wide area, main current concerns mainly referring to the problems related to the practical implementation of the new legislation in the field of veterinary medicinal products from the perspective of the environmental risk assessment process, to analyzes of the environmental risk data of centrally authorized veterinary medicinal products, to critical analyzes of the data contained in the current evaluation guidelines with proposals for updating them according to the current trends in the evolution of animal breeding systems, to data related to risk parameters in soil, water, sediment, for different veterinary drugs (especially antibiotics, but also other classes), to toxicity studies related to different animal species specific to different environmental compartments, to non-conventional techniques for assessing the environmental risks of veterinary medicinal products.

7. Environmental impact assessments were carried out using the analytical method for the following veterinary medicines: LIN – SPE – MIX 880 - water-soluble powder for pigs and chickens, Levasol 10% - oral solution for cattle, sheep, goats, pigs and birds, FlorfenicoL FP 10% - oral solution for chickens, broilers, and pigs, Tiasol 10% - oral solution, indicated for pigs, rabbits and birds (chickens, broilers, turkeys).

Environmental impact assessments were carried out by the two methods, analytical and software, for the following veterinary drugs: Amprolium FP 25% - premix for chickens, broilers, turkeys and Benzylpenicillin potassium 25% - oral powder for piglets, broilers and turkeys.

8. Following the evaluation of these products, it was found that the levels of residues of active substances in soil, water, sediment, were within appropriate limits and no undesirable effects on the environment were detected.

9. The environmental risk assessment study for levamisole was completed in phase I, requiring only calculations to determine PECs, PNECs and risks, which had sub-unit values, concluding that the product poses no risks to environment.

10. The environmental risk assessment study for tiamulin was completed in phase IIA, requiring, in addition to the phase I calculations, also PEC refinement calculations, based on degradation in manure and

soil, after which the risk values have became subunit, drawing the conclusion that the environmental impact of the product is acceptable

11. The environmental risk assessment study for florfenicol was completed in phase IIB, requiring in addition to phase I calculations, PEC refinement calculations, based on degradation in manure and soil, and PNEC refinement calculations -s, based on in-depth toxicological studies, after which the risk values became sub-unit, drawing the conclusion that it is acceptable from the point of view of environmental impact, especially taking into account its effectiveness as a product against a wide spectrum pathogenic microorganisms.

12. The environmental risk assessment study for the product with two active ingredients, lincomycin and spectinomycin, has been completed at the lower level, phase I, with the conclusion that the product does not pose a risk to the environment.

13. Based on the prescriptions of the EMEA guidelines, an original environmental risk assessment algorithm for veterinary medicinal products corresponding to phase I and phase IIA was developed.

14. The algorithm is composed of several successive sections for determining: initial PECs, PNECs, initial risks (corresponding to Phase I), refined PECs, refined risks for soil, ground water, water surface and sediment (corresponding to Phase II step A).

15. Based on the evaluation algorithm, an original interactive software was developed in the MATHCAD environment which:

- it is very easy and convenient to operate, allowing the easy entry of all data regarding the analyzed veterinary medicinal product and the animals under treatment;
-it follows and concretely explains the steps of the calculation algorithm, having incorporated all the data from the guide specific to them, it is no longer necessary to consult it during the analysis;
- carries out matrix calculations to take into account environmental risk assessment parameters simultaneously for all species and categories of animals in treatment;
- contains several options for calculating some parameters, allowing you to choose the most appropriate option;
- allows the performance of particularly detailed environmental risk assessment analyzes in extremely short periods of time.

16. The software contains all the specific data and relationships from the guides, which are no longer needed during use.

17. Using the software, an original rapid method of environmental risk assessment of medicinal products was developed, which is a particularly useful and effective tool for specialists in the field.

18. The paper presents two applications of the rapid method of environmental risk assessment of medicinal products, for AMPROLIUM and PENICILINA G products, which contain the software listings applied to the two veterinary medicinal products, in addition to the specialty specifications .

19. In this PhD thesis, a methodology based on online solid-phase extraction (SPE) ultra-high performance liquid chromatography coupled to a triple quadrupole mass spectrometer (UHPLC-MS/MS) was developed for the simultaneous determination of several compounds, present in veterinary medicines, from which we selected penicillin G and trimethoprim. The proposed method offers advantages over already available methods, such as versatility (several compounds can be analyzed simultaneously), shorter time required for analysis, robustness and sensitivity. The method has been successfully validated in surface water and influent and effluent wastewater, with detection limits at the nanogram per liter level.

7.2 Original contributions

1. The documentary study regarding the current state in the field of environmental risk assessment of veterinary medicinal products.
2. Environmental risk assessment study of levamisole product.
3. Environmental risk assessment study of tiamulin product.
4. Environmental risk assessment study of the florfenicol product.
5. Environmental risk assessment study of the combined product lincomycin and spectinomycin.
6. The general environmental risk assessment algorithm for veterinary medicinal products.
7. The algorithm for determining PECs.

8. Algorithm for determining PNECs.
9. The risk determination algorithm (according to phase I).
10. The algorithm for determining refined PECs.
11. Refined risk determination algorithm (according to phase IIA).
12. Interactive environmental risk assessment software for veterinary medicinal products.
13. Application of software to AMPROLIUM.
14. Application of software to PENICILLIN G.
15. Rapid environmental risk assessment study for AMPROLIUM.
16. Rapid environmental risk assessment study for PENICILLIN G.
17. Penicillin G monitoring study in surface waters by the SPE-online-UHPLC-MS/MS method.
18. Monitoring study of trimethoprim in surface waters by SPE-online-UHPLC-MS/MS method.
19. Rapid environmental risk assessment of Penicillin G in a veterinary product using an original software method and monitoring by SPE-Online-UHPLC-MS/MS.
20. Analysis of transfer of tiamulin to animal tissue after oral administration: an important factor for ensuring food safety and environmental protection (HPLC-MS/MS).
21. Research on the determination of levamisole residues in bovine, ovine, caprine, porcine and poultry tissue (HPLC-MS/MS).

7.3 Future research perspectives

1. Continuation of research regarding the environmental risk assessment of veterinary medicinal products, especially for phase II Tier B and phase II Tier C.
2. Designing rapid methods for assessing environmental risks applicable in phase II Tier B
3. Development of experimental research to determine the content of active substances from veterinary drugs in surface water and wastewater samples.
4. The development of databases that contain references about the physicochemical properties of the active ingredients of veterinary medicinal products, respectively about their toxicological characteristics in the environmental compartments, which allow quick access to the data necessary to run the software.
5. The environmental impact assessment methods presented in this paper can form the basis of future assessments for other classes of active substances in veterinary pharmaceutical products.
6. Development of FOCUS simulation models for use in calculating predictable concentrations of veterinary products in groundwater and surface water.
7. Development of experimental research on the influence of active substances in veterinary medicines on algae.

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ABBREVIATIONS

EMEA - European Medicines Agency

PMV - veterinary medicinal product

SPE-online-UHPLC-MS/MS - online solid phase extraction (SPE) ultra-high performance liquid chromatography coupled to a triple quadrupole mass spectrometer (UHPLC-MS/MS)

ERA - environmental risk assessment

CVMP - Committee for medicinal products for veterinary use

EPAR - European Public Assessment Report

ERAWP - Environmental Risk Assessment Working Group

VICH - International Veterinary Conference on Harmonization

ECHA - European Chemicals Agency

TGD - Technical guidance document

EU – European Union

PEC - Estimated concentration in the environment

PNEC - Predicted No Effect Concentration

FA - evaluation factor

PEC groundwater - Estimated concentration in groundwater

Koc - water-organic carbon distribution coefficient

PESurface layer - Estimated concentration in surface water

PEC Sediment - Estimated concentration in sediment

Ksed-water - sediment-water partition coefficient

EC50 - average effective concentration

LC50 - mean lethargic concentration

NOEC - no observed effect concentration

EC10 - effective concentration

OECD - Organization for Economic Cooperation and Development

R – Environmental risk

Rraf - refined environmental risk

UNU - United Nations Organization

WHO - World Health Organization

EFSA - European Food Safety Authority

GMP - Good Manufacturing Practice

BAT - Best Available Techniques

NVR - the new veterinary regulation

IVM – ivermectin

TET – tetracycline

CIP – ciprofloxacin

SDZ – sulfadiazine

SMX – sulfamethoxazole

SEM - scanning electron microscopy

FTIR - Fourier transform infrared spectroscopy

UHPLC-MS/MS - ultra performance liquid chromatography

ICBMV - Institute for the Control of Biological Products and Veterinary Medicines

DT 50 - The time required for the chemical concentration to decrease to 50% of the application amount

Kow - octanol-water partition coefficient

DT90 – persistence in soil

Kd - solid-water distribution coefficient

SCIENTIFIC ACTIVITY

Works published during the doctoral internship

ISI indexed journal papers

Viviana Ciuca, Safta Victor Viorel, Rusanescu Carmen Otilia, Paraschiv Gigel, Deak Gyorgy, Ilie Mihaela, Cananau Sorin, Rapid Environmental Impact Assessment of Penicillin G in an Veterinary Product Using an Original Software Method and Monitoring by SPE-Online-UHPLC-MS/MS.Molecules, Volume 28, Issue 17, , DOI 10.3390/molecules 28176227, Accession Number MEDLINE:37687057, eISSN 1420-3049, **F.I. 4.6**

- Chapter in a specialized book published by the international publishing house:

Liquid Chromatography in Analysis of Bioactive Compounds for Pharmaceuticals, Cosmetics, and Functional Food Interest, Editors Jan Oszmianski Sabina Lachowicz-Wisniewska, Published: November 2023, Pages: 320, ISBN 978-3-0365-9229-9 (hardback); ISBN 978-3-0365-9228-2 (PDF).

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Lucrarea: **“RESEARCH ON WASTEWATER TREATMENT USING ACTIVATED SLUDGE TECHNOLOGY IN THE ANAEROBIC-ANOXIC-AEROBIC”**

Autori : Eugen MARIN, Carmen Otilia RUSĂNESCU, Gigel PARASCHIV, Carmen Viviana CIUC

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