Attachment 2a

SELF PRESENTATION

Development and application of new analytical tools in the assessment of the presence, mobility, stability and ecotoxicity of selected pharmaceuticals in the environment

Anna Białk-Bielińska

Faculty of Chemistry, University of Gdansk

Gdansk, 2018

1. Name and surname: Anna Białk-Bielińska

2. Scientific degree and titles:

- **PhD in Chemistry**

  University of Gdansk, Faculty of Chemistry, 15.02.2012

  Title of PhD dissertation: *Analytics and evaluation of ecotoxicological risk of the residues of sulfonamides in the environment*

  Supervisor: prof. dr hab. Piotr Stepnowski

- **Engineer**

  Gdansk University of Technology, Faculty of Chemistry, Field of studies: Environmental Protection Technologies, Specialization: Analytics and Environmental Monitoring, 25.02.2008

  Title of engineering thesis: *Determination of antibiotic residues in the environmental samples*

  Supervisor: prof. dr hab. Marek Biziuk

- **M. Sc. in Environmental Protection**

  University of Gdansk, Faculty of Chemistry, Field of studies: Environmental Protection, 11.06.2007

  Title of M. Sc. thesis: *Analysis of steroid hormones in the woman urine using gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC-MS)*

  Supervisor: dr hab. Edmund Maliński, prof. UG

3. Informacje o dotychczasowym zatrudnieniu w jednostkach naukowych

2016 -	Head of the Laboratory of Chemical Environmental Risks
2013 -	Director of the Postgraduate Studies *"Risk assessment of chemicals in the REACH system",* University of Gdansk
2012 -	Assistant professor, Faculty of Chemistry, University of Gdansk
2010 – 2012	Assistant, Faculty of Chemistry, University of Gdansk
2009 – 2012	Specialist for monitoring and reporting in the project „*University of Gdansk promoter of the modern economy – increasing the number of graduates in natural science and science (PRO-GOS)",* Faculty of Chemistry, University of Gdansk

4. Scientific achievement based on art. 16 act. 2 of the treaty from March 14th 2003 about scientific degrees and titles as well as degrees and titles in art (Dz. U. 2006 pos. 882 with changes in Dz. U. 2016 pos. 1311.):

A) Title of scientific achievement:

A series of publications on the topic:

"Development and application of new analytical tools in the assessment of the presence, mobility, stability and ecotoxicity of selected drugs in environment"

B) Author / authors, title / titles of the publication, year of publication, name of the publisher

[H1] M. Borecka M., **A. Białk-Bielińska***, G. Siedlewicz, K. Kornowska, J. Kumirska, P. Stepnowski, K. Pazdro, 2013. A new approach for the estimation of expanded uncertainty of results of an analytical method developed for determining antibiotics in seawater using solid-phase extraction disks and liquid chromatography coupled with tandem mass spectrometry technique. Journal of Chromatography A, 1304, 138-146. **IF_{2013} = 4,258**

[H2] M. Borecka, G. Siedlewicz, Ł.P. Haliński, K. Sikora, K. Pazdro, P. Stepnowski, **A. Białk-Bielińska***, 2015. Contamination of the southern Baltic Sea waters by the residues of selected pharmaceuticals: method development and field studies. Marine Pollution Bulletin, 94, 62-71. **IF_{2015} = 3,099**

[H3] **A. Białk-Bielińska***, J. Kumirska, M. Borecka, M. Caban, M. Paszkiewicz, K. Pazdro, P. Stepnowski, 2016. Selected analytical challenges in the determination of pharmaceuticals in drinking/marine waters and soil/sediment samples. Journal of Pharmaceutical and Biomedical Analysis, 121, 271-296. **IF_{2016} = 3,255**

[H4] J. Maszkowska, S. Stolte, J. Kumirska, P. Łukaszewicz, K. Mioduszewska, A. Puckowski, M. Caban, M. Wagil, P. Stepnowski, **A. Białk-Bielińska**, 2014. Beta-blockers in the environment: Part I. Mobility and hydrolysis study. Science of the Total Environment, 493, 1112-1121. **IF_{2014} = 4,099**

[H5] K. Mioduszewska, J. Maszkowska, **A. Białk-Bielińska**, O. Krüger, U. Kalbe, B. Liberek, P. Łukaszewicz, P. Stepnowski, 2016. The leaching behavior of cyclophosphamide and

ifosfamide from soil in the presence of co-contaminant - mixture sorption approach. Science of the Total Environment, 542, 915–922. **IF_{2016} = 4,900**

[H6] J. Maszkowska, S. Stolte, J. Kumirska, P. Łukaszewicz, K. Mioduszewska, A. Puckowski, M. Caban, M. Wagil, P. Stepnowski, **A. Białk-Bielińska**, 2014. Beta-blockers in the environment: Part II. Ecotoxicity study. Science of the Total Environment 493, 1122-1126. **IF_{2014} = 4,099**

[H7] M. Wagil, **A. Białk-Bielińska***, A. Puckowski, K. Wychodnik, J. Maszkowska, E. Mulkiewicz, J. Kumirska, P. Stepnowski, S. Stolte, 2015. Toxicity of anthelminitic drugs (fenbendazole and flubendazole) to aquatc organisms. Environmental Science and Pollution Research 22, 2566-2573. **IF_{2015} = 2,760**

[H8] **A. Białk-Bielińska***, E. Mulkiewicz, M. Stokowski, S. Stolte, P. Stepnowski, 2017. Acute aquatic toxicity assessment of six anticancer drugs and one metabolite using biotest battery - Biological effects and stability under test conditions. Chemosphere, 189, 689-698. **IF_{2016}=4,208**

[H9] M. Borecka, **A. Białk-Bielińska***, Ł. P. Haliński, K. Pazdro, P. Stepnowski, S. Stolte, 2016. The influence of salinity on the toxicity of selected sulfonamides and trimethoprim towards green algae *Chlorella vulgaris*. Journal of Hazardous Materials, 308, 179-186. **IF_{2016} = 6,065**

[H10] **A. Białk-Bielińska***, M. Caban, A. Pieczyńska, P. Stepnowski, S. Stolte, 2017. Mixture toxicity of six sulfonamides and their two transformation products to green algae *Scenedesmus vacuolatus* and duckweed *Lemna minor*. Chemosphere, 173, 542-550. **IF_{2016} = 4,208**

[H11] A. Puckowski, S. Stolte, M. Wagil, M. Markiewicz, P. Łukaszewicz, P. Stepnowski, **Białk-Bielińska A.,** 2017. Mixture toxicity of flubendazole and fenbendazole to *Daphnia magna*. Journal of Hygiene and Environmental Health, 220, 575-582. **IF_{2016} = 4,643**

[H12] **A. Białk-Bielińska***, M. Matzke, M. Caban, S. Stolte, J. Kumirska, P. Stepnowski, 2017. Effects of five sulphonamides on duckweed (*Lemna minor*) after prolonged exposure time and their dependency on photoradiation. Science of the Total Environment, 618, 952-960. **IF_{2016}=4,900**

(* corresponding author)

C) Description of the scientific goals and achieved results that constitute the scientific achievement together with the description of their potential use

I. Introduction

Pharmaceuticals are classified as so-called "new emerging" pollutants. This is mainly due to the fact that they are widely used in medicine and veterinary practice. Therefore they are introduced into the environment *via* different routes, where they can cause certain effects even at very small doses. This occurs because pharmaceutical substances have – by nature – been designed to pose a specific biological activity responsible for the proper therapeutic effect. After reaching the environment, they may, however, cause unusual toxicological effects in the so-called "non-target" organisms. An often-cited example of such an effect is the extinction of over 95% of the Bengal vulture population in India as a result of the consumption of carrion of cows treated with diclofenac. However, the death of vultures was not the result of analgesic or anti-inflammatory activity of this drug, but resulted from the unexpected acute renal failure of these birds [1]. Despite the fact that pharmaceutical concentrations in the environment are very small (in the range of ng/L – μg/L), their continuous introduction into the environment in combination with their physicochemical properties and biological activity may be sufficient to cause negative toxicological effects.

Research on the presence of drug residues in the environment has been conducted for 20 years, therefore the state of knowledge on this subject has already increased significantly. A recently published report by the German Federal Environment Agency [2] summarizes over 1,000 original articles and reviews in this area published in the past. The results of this review indicate the presence of 631 active pharmaceuticals (or their metabolites and degradation products) in the different environmental samples and 17 have been detected in all five regions of the United Nations. Nevertheless, if the number of commercially available active ingredients on the market is taken into account (reaching around 4,000), this picture is still incomplete, also due to the fact that there is a lack of reliable data for the full environmental risk assessment (ERA) of these substances.

Taking into account the current ERA guidelines for new pharmaceutical products, as for other chemical substances, it is necessary to provide reliable information on the level of exposure (assessment of environmental pollution and fate) and the actual hazard (ecotoxicological risk assessment). Presently, the proposed ERA procedures differ not only depending on the country,

but also on usage of specific product: in veterinary medicine (in European Union countries: CVMP (2008) [3], VICH GL 6 (2000) [4], VICH GL 38 (2005) [5]) or in medicine (in European Union countries: CHMP (2006) [6]). Only for veterinary medicines the same guidelines are applied in the European Union countries, United States and Japan. Regardless of differences in detailed solutions of recommended procedures, the assessment of environmental risk of pharmaceuticals can take place in two phases (**Figure 1**).

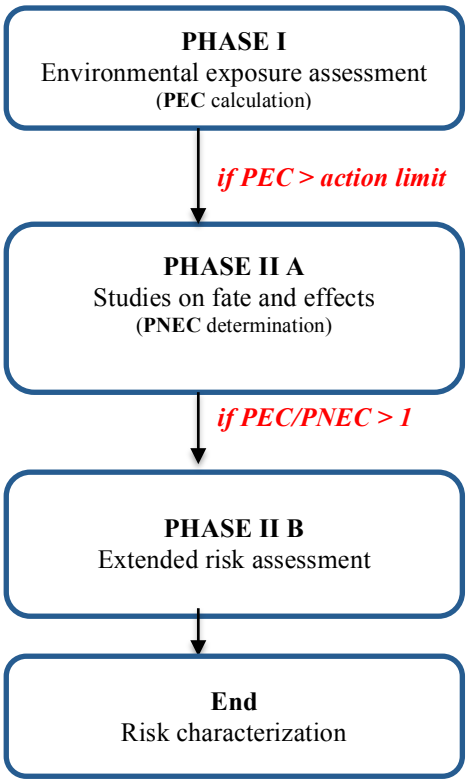


Figure 1. The general scheme of the procedure of ERA of pharmaceuticals,
where: **PEC** – *Predicted Environmental Concentration*, **PNEC** – *Predicted No-Effect Concentration*

The ERA process of pharmaceutical products begins with a preliminary exposure assessment (Phase I). At this stage – depending on the usage of the drug – the PEC parameter is calculated. This refers to the expected concentration of this substance: in soil (PEC_{soil}) for pharmaceuticals used in veterinary medicine; in surface waters (PEC_{sw}) for those agents that are administered in human medicine; with the exception of drugs used in fish farms (aquacultures) for which an analogous parameter is calculated – the Environmental Introduction Concentration $EIC_{aquatic}$. The guidelines present also proper mathematical formulas for calculating the values of these parameters.

However, with some exceptions, the assessment of environmental fate and ecotoxicological effects (Phase II) is only required if the calculated PEC is greater than the set thresholds (action limits) above which it is estimated that the substance can have the significant impact on the environment. Considering the EU guidelines, such tests are performed when: $PEC_{soil} \geq 100$ µg/kg, $PEC_{sw} \geq 0.01$ µg/L or $EIC_{aquatic} \geq 1$ µg/L. In contrast, in the United States such activities are taken into account when the $PEC_{sw} \geq 1$ µg/L, which clearly indicates how significant differences in the approach to ERA drugs in different countries are.

In the second phase, the environmental fate as well as the ecotoxicological risk are assessed for pharmaceuticals used in veterinary and human medicine in accordance with the procedures, i.e. OECD, ISO. Thanks to the ecotoxicological tests carried out in Phase II A it is possible to determine the PNEC parameter. It is necessary to calculate the Risk Quotient (RQ) - as the ratio of the predicted environmental concentration (PEC) and the predicted no effect concentration (PNEC). Sometimes, instead of the PEC parameter (if such data is available), the concentration of a given drug measured in the environment (Measured Environmental Concentration, MEC) is taken into account. Ultimately if RQ < 1, the drug is not expected to have a negative impact on the environment.

It should be emphasized that the ecotoxicological tests proposed in the discussed documents (included Phase II A) refer only to three types of organisms (fish, algae and invertebrates). Experiments using other species are made only if a negative effect of the test substance on the previously mentioned organisms is found. In addition, only the toxicity of single substances is considered. Neither the mixture toxicity assessment nor chronic toxicity are considered, even though these substances are normally present in the environment in mixtures, and also continuously entering the ecosystem, which may lead to negative long-term effects. This is reflected in the data presented in the generally available WikiPharma database (www.mistrpharma.se) presenting the results of the ecotoxicological tests carried out so far. The most available information refers to invertebrates *Daphnia magna* (29.1% of all tests carried out), *Ceriodaphnia dubia* (6.9%), *Thamnocephalus platyurus* (5.5%), then for bacteria (*Vibrio fischeri*, 12%), algae (*Raphidocelis subcapitata* earlier *Pseudokirchneriella subcapitata*, 9.5%) and fish (8.7%). In addition, the information presented there indicates that acute toxicity tests based on microorganisms (exposure time ≤ 30 min), algae (exposure time ≤ 72 h), invertebrates (exposure time ≤ 48 h) and vertebrates (exposure time ≤ 96 h) constitute 55% of all collected data [7]. This was also confirmed by Santos et al. [8], who have shown that acute toxicity tests account for more than 60% of all ecotoxicity tests performed for pharmaceuticals.

For these reasons, there is an ongoing discussion in the literature on the correctness of the established threshold values of pharmaceutical concentrations in the environment, above which such assessment should be performed. This also concerns the interpretation and reliability of the results of ecotoxicological tests.

Therefore, the main goal of the performed research, the results of which were included in this scientific achievement, was to provide as much reliable data as possible, which would allow in the future performing complete and reliable assessment of the environmental risk of selected groups of pharmaceutcals in the environment. Conducted in this respect research can be divided into three main areas: (*i*) first, and at the same time - the superior one, was related to the development of different analytical methods and procedures for the determination of selected substances using liquid chromatography with spectrophotometric and/or mass spectrometry detection. The developed analytical tools were used, among others to (*ii*) assess the contamination of coastal waters of the southern Baltic Sea and to quantify the processes related to the assessment of mobility and stability of selected groups of pharmaceuticals, and (*iii*) quantitative and qualitative description of observed biological effects in ecotoxicological tests.
The objectives of this research were 26 drugs commonly used in human and/or veterinary medicine, belonging to various therapeutic groups and their selected transformation products.

II. Description of scientific achievements

In the literature on the subject, a large number of publications presenting the achievements of many authors in the field of developing new analytical methodologies for determining residues of selected drugs in environmental samples can be found. Nevertheless, these publications generally refer mainly to the analytics of these substances in wastewater (influents and effluents) samples as well as in surface waters. However, appropriate methodological solutions for the determination of these analytes in soils, sediments, and especially in marine and/or estuarine waters are much less available. In terms of solid matrices, this is most likely associated with a much greater difficulty in selecting appropriate conditions for isolation and enrichment of analytes, thus ensuring adequately effective extraction conditions for their reliable determination. On the other hand, in the case of marine water samples, there was a long-standing belief that due to the high dilution, which occurs after such substances enter the marine

ecosystem, the risk they might pose is low and therefore has not been taken into account. However, if the hydrological conditions are considered of the seas such as the Baltic Sea, characterized with very limited water exchange with the North Sea, by analogy to the typical organic and inorganic contaminants, it can be assumed that also in the case of the so-called "new-emerging" or "pseudo-persistent" pollutants may accumulate, especially in the coastal zone. It is also necessary to take into account the fact that all these substances, after introducing into the marine environment, will be able to undergo various biotic and abiotic transformation processes (like biodegradation, hydrolysis or photolysis) or sorption to sediments and suspensions. Nevertheless, the fact that they are introduced continuously, the risks they may pose should not be underestimated.

Taking this into consideration as well as the very limited state of knowledge on the marine pollution by pharmaceuticals (not only the Baltic Sea, but also other marine waters around the world), there was a need to develop proper analytical tools to determine residues of selected drugs in this type of environmental samples with relatively high salinity on adequately low concentration levels. In this context, it has become extremely important to propose methodological solutions that would lead to obtain reliable analytical results. Assurance of the reliability and quality of the obtained data is still the "Achilles' heel" of many Authors of different literature reports. Often, the validation procedure is described in a very laconic manner, and the qualitative analysis is based on insufficient criteria. Authors do not define also how they have determined the recovery values. All this makes it impossible to reliably compare the analytical solutions presented in the literature. Moreover, apart from two scientific articles [9-10], the authors of the available publications do not determine the expanded uncertainty of the obtained results of pharmaceutical residues determination in environmental samples, despite the fact that it is very important in the case of trace analysis.

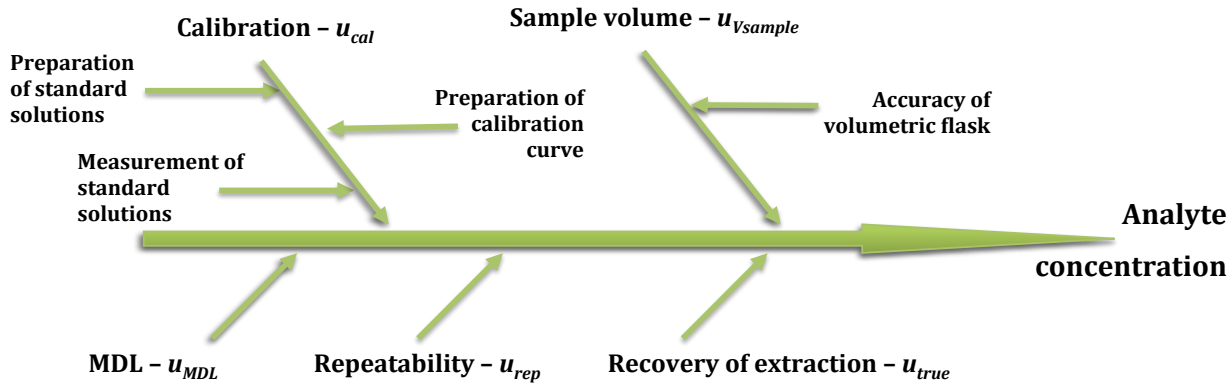
For these reasons, the research tasks in the analytical area were primarily aimed at developing an original methodology for the determination of selected drugs in marine water samples using the high-performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) technique [H1-H2]. In these papers, a new methodological approach to the determination of drug residues in environmental samples was proposed. It was suspected that these substances along with the run-off of surface water, as well as waters discharged from fish farms situated along the streams of the Pomeranian rivers, or along with discharged effluents, could be introduced into the marine and estuarine ecosystem.

Firstly, the objectives of research were selected antibiotics commonly used mainly in veterinary medicine (trimethoprim, sulfathiazole, sulfapyridine, enrofloxacin, sulfamerazine, sulfamethazine, sulfamethiazole, sulfachloropyridazine, sulfamethoxazole, sulfisoxazole and sulfdimethoxine) [H1]. In addition to selecting the appropriate conditions for chromatographic separation (**Figure 2**, [H1]), which in the future enabled the addition of subsequent analytes to one run ([H2]), the most important challenge was to develop the method appropriate for trace amounts of analytes. It was based on the Multiple Reaction Monitoring (MRM) mode, taking into account the characteristic transitions of the pseudomolecular ion and fragmentation ion for each analyte (**Table 1**, [H1]). However, considering the potential impact of various components of individual samples both on the extraction efficiency (EE) and matrix effects (ME) observed in the ion source (electrospray ionization (ESI) applied) and thus on absolute recovery (AR), in order to assure proper quality and reliability of the obtained results these parameters were determined (ME, AR and EE) not only at the stage of selecting the extraction conditions (**Table 2**, [H1]), but also during the analysis of each sample.

In addition to the extensive validation methodology, the factor that distinguishes the presented analytical approach [H1] from similar solutions was a different approach to the selective extraction of these compounds from seawater, through the use of SPE speed disks. Their geometry and smaller particle diameter (8 – 12 μm) than in classic SPE cartridges (40 – 80 μm), cause that they have a larger sorption surface, and allow the use of faster flow rates, leading to the possibility of extracting larger sample volume in shorter period of time. This results in lowering the limits of quantification and detection, which is of particular importance in the analysis of marine water samples. Several methods have been tested to ensure the proper conditions for this extraction (**Table 2**, [H1]). They were selected based on available literature data for other analytes as well as previous experience. Method 4 was selected as the best method (**Table 2** and **3**, [H1]), which was characterized not only with satisfactory EE values (> 50%), but also ME (-50% <ME <50%) and thus AR (> 30%). The key aspect in lowering the observed high value of matrix effects (mainly ionization suppression) due to the presence of a number of interferences was the introduction of an additional stage of washing with hexane, which has already been used in the extraction of beta-blockers and beta-antagonists from environmental samples [11].

The developed methodology was subjected to the validation process. However, as mentioned before, in order to ensure the highest quality of this type of results, in this work [H1] the entire process of calculating expanded uncertainty is presented for the first time (**Chapter 2.7**, **Table**

S2 [H1]), which was based on the so-called Ishikawa diagram (**Figure 2**). This presents the effect of uncertainty of individual parameters of the analytical process on the value of total uncertainty of the final result of determination of selected pharmaceuticals in environmental samples.



where:

$u_{Vsample}$ – uncertainty of sample volume;

u_{cal} – relative uncertainty of calibration step;

u_{true} – uncertainty of recovery of extraction;

u_{rep} – uncertainty of precision;

u_{MDL} – uncertainty of method detection limit.

Rysunek 2. Ishikawa diagram for the developed analytical procedure

Finally, the determined metrological parameters as well as the value of the expanded uncertainty (**Table 4**, [H1]) indicated the high applicability of the proposed analytical methodology. Therefore, it has been used to carry out pioneering studies on the assessment of the degree of pollution of marine waters in the southern Baltic coastal zone, proving simultaneously the presence of residues of various medicinal substances in a wide range of concentrations.

At a later stage, the developed methodology was modified and eventually found its application for the following drugs: trimethoprim, sulfapyridine, sulfathiazole, enrofloxacin, sulfamazine, sulfamethazine (sulfadimidine), sulfamethoxazole, oxolinic acid, sulfadimethoxine, ketoprofen, naproxen, ibuprofen and diclofenac. The final determinations were based on the LC-MS/MS technique in the MRM mode, based on the monitoring of three characteristic transitions for a given analyte (**Table S1**, [H2]). Also here, satisfying ME, AR, and EE parameters as well as expanded uncertainty of the analytical results were obtained (**Table 3,** [H2]), and the method was applied to the determination of the selected pharmaceutical residues in seawaters along the entire coast of northern Poland (**Figure 1, Table 2,** [H2]) in 17 points taking into account the

determination of their concentrations not only in horizontal but also vertical profiles. On the basis of the statistical analysis of the obtained results (**Chapter 2.6** and **3.1.1**, **Table 3S** and **Figures S1-S3**, [H2]), it was found that there were no clear relationship between the determined ME, AR or EE parameters of the tested samples and their salinity or suspended matter content, which proves the influence of other physicochemical parameters of water on the value of these parameters.

Obtained results (**Table 4a** and **4b**, [H2]) indicated also the widespread presence of various pharmaceuticals in marine waters, in concentrations ranging from a few to over one hundred ng/L. Trimetroprim was detected in 95% of the analyzed samples, however sulfamethoxazole and enrofloxacin in 68% and 59%, respectively. The frequency of detecting other drugs ranged from 4 to 40%, which is most likely due to their different behavior (stability) under environmental conditions. In addition, it was confirmed that the highest concentrations of the tested substances were located enclosed or semi-closed bodies of water such as bays and river months and also close to the output of wastewater treatment plants. No significant differences in the drug concentrations were observed in the studied vertical profile along with depth. However for some substances a steep gradient was observed along with the distance from the estuaries to the sea. This proves the importance of the river input as a key of pharmaceutical residues in the marine environment.

In the course of further work, a number of other analytical methodologies were also developed that were applied to determine the residues of pharmaceuticals in various environmental components, with particular emphasis on soils, sediments, marine sediments, surface waters and fish tissues. The published results in this respect have been described in the **Section 5** – Description of other scientific and research achievements – described later.

All developed analytical methods and their subsequent modifications were used in the further research to describe quantitatively sorption processes and stability of selected pharmaceuticals in the conducted model experiments; as well as to try to explain the observed biological effects in the performed ecotoxicological experiments [H4-H12]. Each of the applied analytical method was once again subjected to the validation process, including determination of the linearity, working range, precision, accuracy, detection and quantification limits in a given analytical matrix. A detailed description of these methods and their validation parameters have been included in publications [H4-H12] and are summarized in **Table 1**.

The experience and knowledge gained during the conducted analytical research became the basis for writing the review paper [H3], presenting the most important challenges in the analysis

of pharmaceutical residues in the selected environmental components, i.e. marine and drinking waters, soils and sediments.

Table 1. A list of methods for final determination of selected drugs and their transformation products applied during the conducted research

Analyte	Technique	Publication	Method characteristic	Validation parameters
propranolol, metoprolol, nadolol	HPLC-UV	[H4]	Section 2.4 (sorption and hydrolysis studies)	Table 1S (sorption studies) Table 2S (hydrolysis studies)
cyclophosphamide, ifosfamide, metoprolol	HPLC-UV	[H5]	Section 2.5 (for metoprolol like in [H4])	Section 2.5 (for metoprolol like in [H4])
propranolol, metoprolol, nadolol	HPLC-UV	[H6]	Section 2.3 (conditions as in [H4] for sorption studies)	Table 1S (ecotoxicological studies)
fenbendazole, flubendazole	HPLC-DAD	[H7]	Section „Materials and Methods - Instrumental analysis”	Section „Results and Discussion- Instrumental Analysis”
5-fluorouracyl, cyclophosphamide, ifosfamide, imatinib, methothrexate, 7- hydroxymethothrexate, tamoxifen	HPLC-UV/ LC-MS/MS	[H8]	Table 2A	Table 2A
sulfamethozazole, sulfadimethoxine, sulfapyridine, trimethoprim	HPLC-UV	[H9]	Tabela 2	Tabela 2
sulfamethoxazole, sulfadimethoxine, sulfapyridine, sulfadimidine, sulfathiazole, sulfanilamide, sulfanilic acid	HPLC-DAD	[H10]	Table A2 (modification from [H9])	Table A2
mixture of fenbendazole and flubendazole	HPLC-DAD	[H11]	Section 2.7 (as in [H7])	As in [H7]
sulfamethoxazole, sulfadimethoxine, sulfapyridine, sulfadimidine, sulfathiazole, sulfanilamide, sulfanilic acid	HPLC-UV/ LC-MS/MS	[H12]	Table 2 (modification from [H10])	Table 2

Water bodies, both surface and subsurface waters, are almost always the final reservoir for pharmaceuticals. However, it should be remembered that soils are an important environmental component for their transport as well as final deposit. This environment is particularly exposed to these pollutants when it is fertilized with sewage sludge or manure contaminated with the residues of medicines. It has been proven that the concentration of drugs in arable land treated with manure can range from several to even several thousands μg/kg [12]. Transport of chemicals in the soil environment depends on the intensity of their sorption to soil particles. However, the sorption process directly affects the bioavailability of these chemicals, and

therefore plays a crucial role in assessing the exposure of living organisms to their presence in the environment.

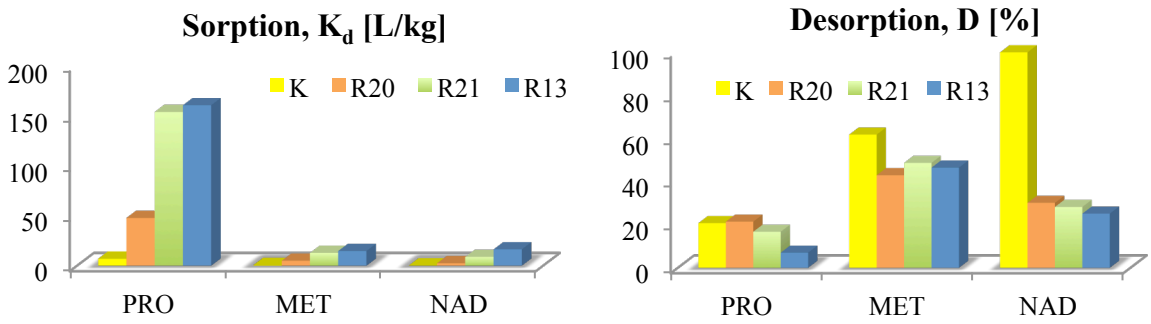
For these reasons, a detailed assessment of the mobility of selected drugs belonging to two common therapeutic groups (beta-blockers [H4] and cytostatic drugs [H5]) was performed for the first time. Research on the assessment of the sorption potential of beta-blockers has been additionally extended by the evaluation of their hydrolytic stability [H4], which has not been the subject of detailed studies so far.

Beta-blockers (BB) belong to the group of beta-blockers commonly used in the treatment of cardiac diseases, especially in the hypertension and ischemic heart disease. They also find their application in veterinary medicine and illegally as doping agents in sport. Their consumption in the world is very high, e.g. in Germany, it reaches 100 tones per year [13]. It is known that some of them are slightly absorbed in the body and only partially metabolized. Moreover, their removal efficiency in the wastewater treatment is small. All this means that in many regions of the world they are detected in various components of the environment at the levels of μg/L. Despite this, knowledge about their ability to accumulate in porous media, with particular emphasis onto natural soils, is small. Due to the widespread use and detection in environmental samples (**Table 1**, [H4]), three drugs belonging to this group were selected in this study: metoprolol (MET), propranolol (PRO) and nadolol (NAD).

Studies on the sorption potential of selected BB representatives were carried out in accordance with the OECD procedure 106 [14] (**Figure 1S_ part A**, [H4]) under the conditions of a static test (batch test) using three types of soil characterized with different physicochemical parameters (R20, R13, R21) (**Table 2**, [H4]). This was complemented with kaolinite - a clay mineral which, thanks to the lack of organic matter, is an excellent reference material reflecting only the interactions with the mineral fraction. The static test a valuable tool to determine the equilibrium adsorption coefficient K_d, which is characterized by the partition of the chemical compound between the aqueous phase and solid, and therefore its determination is based on a fixed ratio of soil to aqueous solution. It is very useful for assessing the impact of changing environmental conditions on the sorption process of a given substance, i.e. pH, ionic strength of the soil solution or the temperature in the system.

Obtained results (**Figure 1** and **Table 3**, [H4]), schematically presented also in the **Figure 3**, allowed to clearly indicate that the mobility of these drugs in soil environment decreases with their hydrophobicity ($logP_{PRO}$ = 3.48, $logP_{MET}$ = 1.95, $logP_{NAD}$ = 0.81) and increase of the content of organic matter (OC) and cation exchange capacity of soil (CEC) (**Table 3**, [H4]). The

highest values of K_d and the lowest desorption value among tested soils were observed for soil R13, characterized with the highest values of the mentioned parameters. However, for kaolinite - a sorbent that does not contain organic matter - the K_d values were the lowest, and the desorption percentage the highest.



	K	R20	R21	R13
pH_{KCl}	6,00	5,62	6,22	6,65
OC [%]	0	2,1	14,9	19,4
CEC [cmol(+)/kg]	3,0	6,2	53,1	85,6
Clay fraction [%]	100	11,3	7,2	16,7

Figure 3. Determined values of the equilibrium adsorption coefficients and the degree of BB desorption under the experiment conditions carried out in accordance with OECD 106 guideline (drawing prepared based on the data in **Table 2** and **3** in [H4])

It was also shown that BB sorption to soils is only partially reversible (the maximum desorption was 48.6 ± 1.4%), while their desorption is inversely proportional to the increasing content of organic matter in the soil. The determined K_d values of BB allow concluding that these pharmaceuticals might be characterized with lower mobility and lower bioavailability in comparison to other pharmaceuticals such as sulfonamides [15-16], which may limit their penetration into water compartments.

In order to describe the obtained experimental results and to explain the sorption mechanism of selected BBs onto soils, it was shown that out of the four investigated isotherms (Freundlich, Langmuir, Dubinin-Radushkevich, Temkin) (**Figure 3S-5S**, [H4]) the best fit was for the Freundlich isotherm (**Table 4**, [H4]). Although the Dubinin-Radushkevich and the Temkin isotherms showed a worse fit, they also indicated a low probability of the existence of chemisorption or the interaction resulting from the transfer of charge (Temkin's isotherm). Based on acid-base properties, these compounds are organic bases and under the experimental conditions (**Figure 2S**, [H4]) they occurred in the cation form, however the Dubinin-Radushkevich isotherm did not show a good fit to the experimental data. This is most likely

associated with a far more complex chemical structure (various possibilities of molecular interactions) than for simple inorganic cations, for which the Dubinin-Radushkevich model is usually well-fitted (the calculated average free energy E_D in the system would confirm the ion exachnge). In addition, taking into account the K_d values of other strongly-absorbed substances for which the described ion-exchange mechanism has been confirmed by the determined E_D free energy values, and the determined values of K_d for BB, it may be assumed that they are too small for unambiguous confirmation of ion exchange with a mathematical model. Therefore, it may be suspected that BB may be attracted by soil particles *via* many additional forces, such as donor-acceptor interactions, π - π interaction or hydrogen bonding. A further interpretation of the isotherms indicated that sorption of BB onto a heterogeneous surface could also be multilayered.

Significant decrease of BB sorption potential at the pH > pKa of these drugs (and thus with decreasing percentage of cationic form in favor of the neutral form) (**Figure 2**, [H4]) as well as with the increase of the ionic strength (**Figure 3**, [H4]) allowed to clearly indicate that the ion exchange mechanism is highly probable. These drugs are basic compounds, thus, at pH below their pKa, they occur in the cationic form - strongly interacting with the negatively charged soil surface (**Figure 2S**, [H4]). This is also explained by the observed decrease in sorption potential along with the increase of ionic strength, hence with the increase of Ca^{2+} ions in the aqueous solution competing with the BB cations for active sites on the soil surface.

The study on BB sorption potential was extended by evaluating their hydrolytic stability in accordance to OECD 111 guideline [17] (**Figure 1S_ part B**, [H4]). Based on the obtained results (**Figure 4**, [H4]), it has been proved that these substances hydrolytically stable under environmental conditions ($t_{1/2}$ > 1 year at 25 ^{o}C). Thus reliable data on their behavior in the ecosystem was provided for the first time. Having in mind these findings as well as limited immobilization in soil structures (comparing the determined K_d coefficients for BB (K_d < 160.8 L/kg) to other typical environmental pollutants (K_d > 1000 L/kg)), it can be assumed that these substances will accumulate in the aquatic environment. For these reasons, the studied drugs were taken into account in conducted ecotoxicological studies, aimed at characterizing the threats they might pose to selected organisms [H6], which will be discussed further in this Self-Presentation.

A successful attempt to assess the mobility of two selected drugs commonly used in anticancer therapies was also performed not only in static test, but also in a dynamic test (column test) [H5] according to the procedures proposed by the German Institute for Standardization E DIN 19527 [18] and DIN 19528 [19], respectively. These are the first studies of this type in the world. These experiments were conducted using two types of soils characterized with different

physicochemical parameters (**Table A.1**, [H5]): loamy sand (LS) and sand with medium grain size (MS). The research object covered the two most commonly used drugs in anticancer therapies: cyclophosphamide (CK) and ifosfamide (IF), which have been used for many years. Cytostatic drugs, to which they belong, due to their high activity (they lead to the inhibition of DNA synthesis in the cell), cytotoxicity and genotoxicity [20], are believed to pose a very serious threat not only to organisms living in the environment, but also indirectly also for human health. Nevertheless, the state of knowledge about the presence, fate and threats that they may pose is still very limited.

Based on the obtained results, it was found that CK and IF show a much greater ability to penetrate to groundwater than the previously discussed beta-blockers. They have been leached from the soil columns already in the first fraction with a liquid/soil ratio L/S of 0.3 L/kg for MS soil (90% decrease in concentration) and in the second fraction of L/S 0.75 L/kg for soil LS (90% decrease in concentration) (**Figure A.1** and **A.2**, [H5]). Based on this, it was observed that leaching was slower in the case of LS soil, which suggests that CK and IF sorption is preferred in soils with higher organic matter content as well as a higher content of clay fraction.

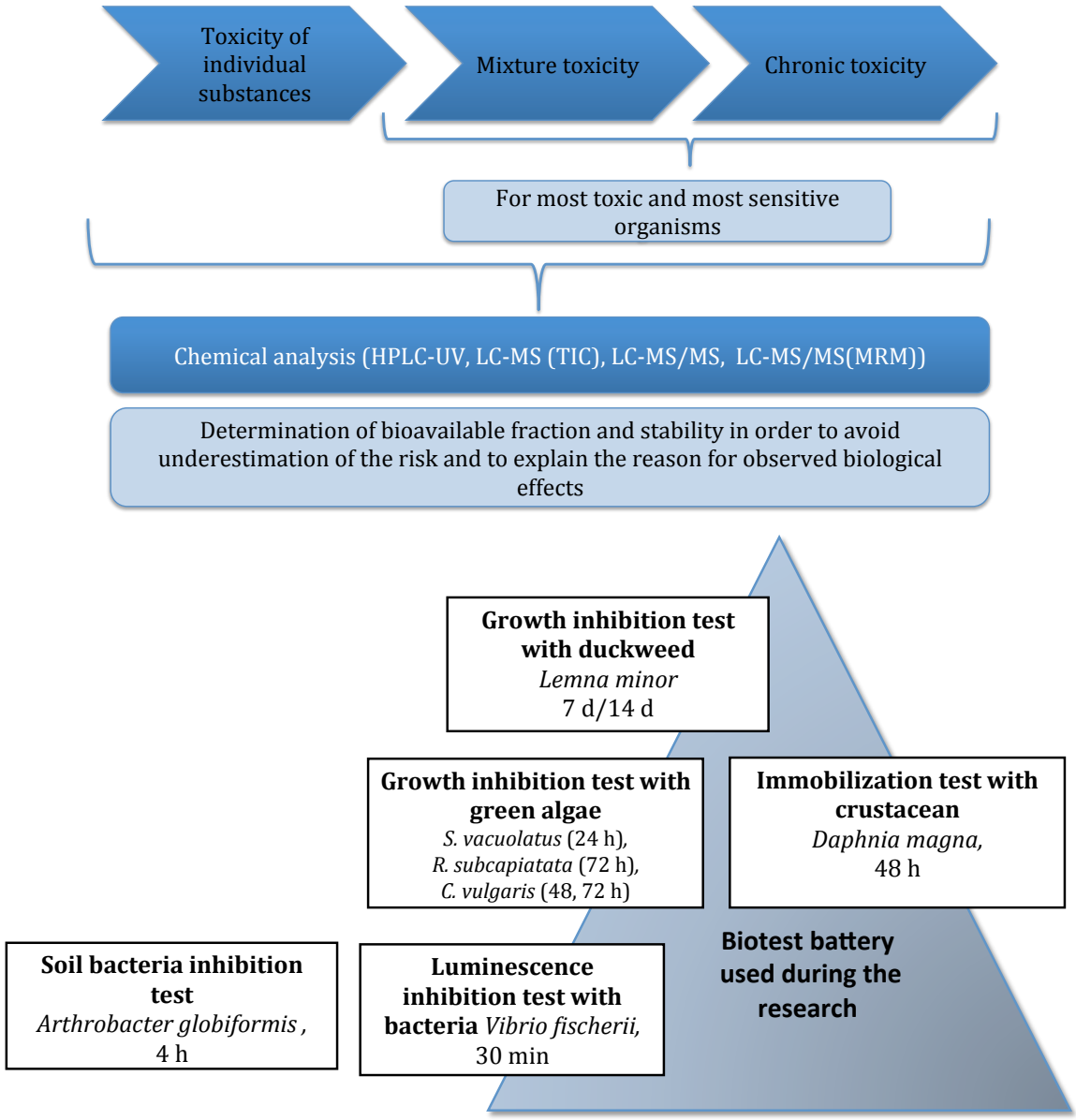
In these studies, the evaluation of the impact of the presence of other pollutant (co-contaminant) on the behavior of these compounds in soil structures was also taken into account. In routine sorption studies of any kind of contamination, this type of experiment is usually not carried out, and as it results from the obtained data, it has a significant impact on the intensity of and reversibility of sorption. As a model substance - considering the previously discussed results - metoprolol was selected. It was expected that this compound will show much less mobility under the conditions of the dynamic test than the discussed anti-cancer drugs. In addition, the cationic form, in which it will occur under the experimental conditions, may favor additional interactions with anticancer drugs, leading to their different behavior in the soil environment. Taking into account the determined cumulative release (**Figure A.1** and **A.2**, [H5]) in the individual fractions, it was confirmed that metoprolol is not only much less mobile than two selected anticancer drugs, but primarily significantly reduces IF mobility (by 29% for MS soil and 26% for LS soil) and CK (by 21% for LS soil, but insignificantly for MS soil) in the soil environment.

Considering the determined additional physicochemical parameters of the collected fractions (like: pH, conductivity, turbidity, total organic carbon content) (**Table B.1**, [H5]) during the dynamic test, it can be presumed that the selected anticancer agents under the test conditions are present in the anionic form due to the loss of the proton from the phosphoamide group in the

structure of CK and IF, which have a greater affinity for positively charged metoprolol molecules adsorbed on the negatively charged surface of the soil. The possibility of multilayer sorption is consistent with previous observations during the study of BB sorption potential in a static test [H4]. In addition, taking into account the observed negative relation between turbidity and the column effluent conductivity, the particle-facilitated transport of the anticancer drugs, can not be excluded, despite the observed reduced mobility in the presence of metoprolol. This is in accordance with the results of the static test that has been carried out for these compounds. (**Figure B.1**, [H5]). In the case of tests without the presence of co-contaminant, the concentrations of the investigated anticancer drugs were lower in the static test than in the dynamic test. This may be due to the additional portion of these compounds eluted from the column with the suspended fraction; whereas in the static test, this fraction is completely centrifuged.

Obtained results on the assessment of mobility of structurally different BB and representatives of anticancer drugs indicate that both groups of pharmaceuticals after entering the environment might be transported to both surface and subsurface waters.

Taking into consideration discussed above results and the previously mentioned problems related to current guidelines for the ERA of pharmaceuticals, detailed ecotoxicological studies were performed. Conceptual approach of these studies is presented in **Figure 4**. They included, in the first place, the evaluation of toxicity of many drugs (as individual substances) and their selected transformation products using biotest battery based on organisms representing different levels of biological organization [H6-H10]. Afterwards, for the most toxic substances and the most sensitive organisms, mixture toxicity [H10-H11] and chronic toxicity [H12] was assessed. All ecotoxicological tests were accompanied by chemical analyses using liquid chromatography technique with various detection systems (**Figure 4**), which enabled both determining the bioavailable fraction for individual organisms of test substances, as well as determining analyte stability under biotest conditions to avoid underestimating the risk and to explain potential causes of observed biological effects. **Such holistic approach (taking into account the use of analytical techniques in ecotoxicological tests) provides a much more reliable biological observation. However, it differs significantly from those routinely carried out in this area around the world. Therefore, hopefully it will become the methodological standard of the ecotoxicology of "new-emerging" environmental pollutants, taking into account the obtained results presented here.**



Rysunek 4. Conceptual approach of the performed ecotoxicological studies

All ecotoxicological data obtained during the research is presented in the publications [H6-H12] and was summarized in **Table 2a** and **2b**. All tests were carried out in accordance with the OECD or DIN guidelines, which was described in detail in [H6-H11]. Based on the determined (if it was possible in the tested concentration range) EC_{50} values for individual tested substances as well as selected transformation products, these chemicals were classified according to EU Directive 93/67/EEC [21]. It should be added that the highest tested concentrations of analytes in the individual ecotoxicological tests were selected based on the solubility of a given substance in water as well as the appropriate biological medium. For well soluble in water and biological medium compounds it was set for 100 mg/L (except for trimethoprim and the evaluation of the

influence of the salinity on its toxicity [H9]), because in accordance to the above-mentioned Directive, substances with the EC_{50} higher than 100 mg/L are considered as non-toxic to aquatic organisms. Among the tested compounds selected transformation products, 7-hydroxymetotrexate (metabolite and biodegradation product for methothrexate) [H8] as well as sulfanilamide and sulfanilic acid (the most commonly detected transformation products of sulfonamide - drugs with antibacterial properties commonly used in veterinary medicine, but also medicine) [H10] were investigated. However, as test organisms, those which are commonly found in the environment and already routinely used in ecotoxicological studies have been selected, but at the same time they were representing different levels of biological organization. This was aimed at assessing the potential impact of these substances on the functioning of the ecosystem.

Table 2a. Ecotoxicological results obtained during performed research

Organism/ Substance	***MARINE BACTERIA*** *V. fischeri* (30 min)	***GREEN ALGAE*** *S. vacuolatus* (24 h)* *R. subcapiatata* (72 h)** *C. vulgaris* (48, 72 h)***	***PLANTS*** *L. minor* (7 d)	***CRUSTACEANS*** *D. magna* (48 h)	***SOIL BACTERIA*** *A. globiformis* (4 h)
propranolol[H6]	> 100	24*	> 100	n. d.	> 100 / 218 (with/ without soil)
metoprolol[H6]	> 100	75*	> 100	n. d.	> 100
nadolol[H6]	> 100	100*	> 100	n. d.	> 100
fenbendazole[H7]	> 0,3	> 1*	> 1	0.019	n. d.
flubendazole[H7]	> 0,3	> 1*	> 1	0.045	n. d.
5-fluorouracyl[H8]	> 100	0.075**	2.45	> 100	n. d.
cyclophosphamide[H8]	> 100	> 100**	> 100	> 100	n. d.
ifosfamide[H8]	> 100	> 100**	> 100	> 100	n. d.
imatinib[H8]	23.06	5.08**	61.05	72.43	n. d.
tamoxifen[H8]	> 0.2	> 0.2**	0.18 – 0.23 (depending on the test conditions)	> 0.2	n. d.
methothrexate[H8]	> 100	9.51**	0.08 – 0.16 (depending on the test conditions)	> 100	n. d.
7-hydroxy-methothrexate[H8]	> 10	> 10**	8.3	n. d.	n. d.

n. d. – not determined

Toxicty to aquatic organisms classification according to the Directive EU 93/67/EEC [21]:

< 0,1 mg/L Highly toxic	**10 – 100 mg/L** Harmful
0,1 – 1 mg/L Very toxic	**> 100 mg/L** Non-toxic
1 – 10 mg/L Toxic	

Based on the obtained results, it was observed that the most sensitive organism is duckweed (*L. minor*), and then the green algae. Some of the tested drugs have been classified as highly toxic to aquatic organisms such as 5-fluorouracil, fenbendazole, flubendazole and methothrexate, as well as very toxic like tamoxifen. It has also been proven that the toxicity of the methotrexate metabolite is less than the native form of this drug. Among the tested organisms, luminescent bacteria (*V. fischeri)* should be considered the least sensitive, which may be due to very short time (30 min) of this test. In the case of the test with the use of duckweed (*L. minor*), which lasts for 7 days, the exposure time seems to be long enough to observe specific biological effects of the selected drugs. If, for example, selected cytostatic drugs (except CK and IF), which lead to inhibition of DNA synthesis in the cell by various mechanisms, are considered, and during this test intensive reproduction of genetic material occurs, their mechanism of toxic action towards the selected plant species may result from their pharmacological mode of action (MoA) [H8]. On the other hand, both CK and IF were found to be non-toxic to any of the tested organisms, which may primarily result from the fact that these drugs are activated only in the human body and their metabolites exhibit biological activity, not native forms [H8]. Taking into account the previously demonstrated high potential of penetration of CK and IF into water reservoirs [H5], as well as the demonstrated low toxicity [H8], it can be assumed that the environmental risk for these substances is small.

Similarly, also residues of beta-blockers should be characterized with a relatively low risk for aquatic organisms (**Table 2**, [H6]). It was only observed that with the increase of their hydrophobicity their toxicity to green algae (*S. vacuolatus*) increases. Interestingly, the toxicity of propranolol towards soil bacteria *A. globiformis* was higher in the test without soil than in the presence of soil (**Figure 3S**, [H6]), which confirms the fact that sorption of these compounds to soil structures leads to a reduction of the bioavailable fraction for a organism.

Moreover, it is also important to highlight that among investigated pharmaceuticals only imatinib with anticancer activity [H8] showed a different toxicity towards all tested organisms. This may result from its specific MoA, which is based on the inhibition of tyrosine kinases. These, in turn, are a key element of the information transfer system in all cells and constitute a necessary link responsible for contact and communication with the external environment.

It has been also proved that fenbedazole (FEN) and flubendazole (FLU) are highly toxic towards *D. magna* (**Table 3**, [H7]). Taking into account the determined EC_{50} values: $EC_{50,\ FLU}$ = 0.045 mg/L, $EC_{50,\ FEN}$ = 0.019 mg/L respectively, and their values of $logP_{FLU}$ = 2.91 and $logP_{FEN}$ = 3.93, it was suspected that their toxicity may result from their hydrophobic character. Additional

calculations were performed using available models to predict the mechanism of toxic action of substances called 'baseline toxicants'. However, on this basis, it was shown that the biological effect observed towards *D. magna* results from their specific mechanism of action. Considering the fact that their MoA relies on the binding of these drugs to beta-tubulin and inhibition of the formation of microtubules in cells, which are present in both animal, plant and bacterial cells – it is impossible at the moment to clearly indicate the reasons for the strong toxicity of these two drugs.

In the course of further work (obtained results are summarized in **Table 2b**) it was decided to assess the toxicity of the two most commonly identified transformation products of sulfonamides (SAs), indicating that only one of them (sulfanilamide, SN) poses some biological activity, however lower than the native forms of these drugs [H10]. The main reason for the performed research on the toxicity evaluation of sulfonamides degradation products [H10], the assessment of their mixtures [H10] and long-term effects [H12], were previously obtained results for their individual substances towards various organisms [22]. It was proved then that these drugs have strong phytotoxic effects towards *L. minor* and toxic towards green algae (*S. vacuolatus*), which is also shown in **Table 2b**. For these reasons, these studies were continued and significantly expanded during my eight-month postdoctoral internship in the Center for Environmental Research and Sustainable Technologies (UFT) at the University of Bremen in Germany.

Table 2b. Ecotoxicological results obtained during performed research – continued (color classification as in **Table 2a**)

Organism/ Substance	***MARINE BACTERIA*** *V. fischeri* (30 min)	***GREEN ALGAE*** *S. vacuolatus* (24 h)* *R. subcapiatata* (72 h)** *C. vulgaris* (48, 72 h)***	***PLANTS*** *L. minor* (7 d)	***CRUSTACEAN*** *D. magna* (48 h)	***SOIL BACTERIA*** *A. globiformis* (4 h)
12 sulfonamides[22]	> 20	**2.22 – 32.25***	**0.02 – 4.89**	n. d.	> 80
sulfanilamide[H10]	n. d.	25.83*	5.09	n. d.	n. d.
sulfanilic acid[H10]	n. d.	> 100*	> 100	n. d.	n. d.
Influence of the salinity[H8]					
sulfamethoxazole		[48 h]: 0.98 – 9.31*** [72 h]: 0.95 – 1.53*** (depending on the salinity)			
sulfadimethoxine		[48 h]: 4.94 – 26.65*** [72 h]: 1.02 – 7.65*** (depending on the salinity)			
sulfapyridine		[48 h]: 1.79 – 3.20*** [72 h]: 0.28 – 1.81*** (depending on the salinity)			
trimethoprim		[48 h]: 123 – n. d.*** [72 h]: 90 – n. d.*** (depending on the salinity)			
Mixture toxicity[H10, H11]		Mixture toxicity of 6SAs and 6SAs+SN towards *S. vacuolatus* i *L. minor*[H10]		Mixture toxicity of FEN + FLU towards *D. magna*[H11]	
Chronic toxicity[H12]			Toxicity of 5SAs towards *L. minor* in the prolonged test, 14 d		

n. d. – not determined

As part of this study, the impact of salinity on the toxicity of the three most commonly detected in marine waters, collected from the coastal zone of the Southern Baltic (taking into account the results described in [H1-H2]), sulfonamides (sulfamethoxazole, sulfapyridine, sulfadimethoxine) and trimethoprim – which is very often used with these drugs [H9], were investigated for the first time. As the representatives of organisms living in the marine environment, including Baltic Sea, green algae - *Chlorella vulgaris* (a species of cosmopolitan algae, occurring not only in sea waters but also freshwater and estuarine) were selected. Simultaneously a significant toxic effect of SAs on these organisms was expected. Literature data for this species of algae was limited and available only for sulfadimethoxine and sulfamethoxazole. Toxicity of these drugs after 48 h and 72 h was evaluated with salinity in the range of 0 to 9 PSU (0, 3, 6 and 9 PSU), reflecting the salinity range of the Baltic Sea [H9]. Based on the determined EC_{50} values after 48 h and 72 h time of exposure as well as in the

dependence on the salinity (**Table 4**, [H9]) and statistical analysis of the obtained data, it was proved that: *i*) SAs show toxic effects on *C. vulgaris* regardless of the tested salinity; *ii*) trimethoprim is a non-toxic compound towards *C. vulgaris*, and its toxicity decreases with increasing salinity; *iii*) in most cases, the lowest SAs toxicity was observed at the highest salinity (9 PSU), with the exception of sulfadimethoxine (72 h), which was the least toxic in unsalted water (0 PSU); *iv*) an increase in the exposure time of these organisms to the presence of the tested drugs has led, in most cases, to increased toxicity, especially in the most saline environments. Considering the results from chemical analysis, obtained in the course of these studies, it was shown that tested pharmaceuticals are stable under the conditions of toxicological tests and very soluble in the used biological media. Observed biological effects resulted, therefore, from the action of the native form of the studied drugs, not from the decreasing concentration of the bioavailable fraction of drug to organisms with the increase of salinity or the formation of more toxic degradation products. Taking into account their acid-base properties, and thus the confirmed presence of these drugs under the test conditions in neutral form (trimethoprim approx. 90%, sulfapyridine approx. 85%) or anion (sulfamethoxazole approx. 95%, sulfadimethoxin approx. 84 %) the potential interaction of analytes present in the form of a cation with chloride anions, present in the largest amounts in marine waters, leading to their reduced bioavailability - is also impossible. In addition, as for FEN and FLU, using appropriate prediction models of the mechanism of toxic action of chemicals towards *C. vulgaris* (as described in **Chapter 2.6** and **3.4** [H9]), it was proved that the mechanism of toxicity of these drugs is specific and therefore does not result from their hydrophobicity and the ability to overcome biological barriers.

As already mentioned, chemical analysis of samples (solutions of analytes in a given test medium) collected before and at the end of the ecotoxicological test were carried out. This was done in order to determine the bioavailable fraction of analytes and assess the analytes' stability under the test conditions. The obtained test results are presented in the publications [H6-H12] and summarized in **Table 3**. Based on the presented data it might be concluded nominal concentrations of each analyte used in ecotoxicological tests were close to real concentrations (the difference according to the OECD recommendations was in the range of 80 - 100%, which was marked in green color), and thus the bioavailable fraction for organisms. The determined toxicity of the tested substances was therefore correctly estimated, with the exception of tamoxifen, for which due to its low solubility in water, some underestimation of the risk should be considered.

Table 3. Determined content of bioavailable fractions for the highest concentration of a given analyte under the conditions of ecotoxicological tests

Organism/ Substance	***BACTERIA*** *V. fischeri* (30 min)	**GREEN ALGAE** *S. vacuolatus* (24 h)*/ *R. subcapiatata* (72 h)**/ *C. vulgaris* (48, 72 h)***	**PLANTS** *L. minor* (7 d)	**CRUSTACEANS** *D. magna* (48 h)
propranolol[H6]	102.6	100.8*	101.9	
metoprolol[H6]	100.5	102.9*	102.8	
nadolol[H6]	102.4	100.2*	99.5	
fenbendazole[H7]	83.0	74.4*	75.0	95.6
flubendazole[H7]	96.1	78.2*	75.7	98.4
5-fluorouracyl[H8]	105.5	103.8**	98.5	100.3
cyclophosphamide[H8]	101.2	98.2**	99.7	97.2
ifosfamide[H8]	99.8	100.3**	104.5	98.7
imatinib[H8]	97.0	104.3**	96.9	88.4
tamoxifen[H8]	47.9	53.5**	38.6	56.8
methothrexate[H8]	89.2	98.9**	112.9	90.2
7-hydroxymethothrexate[H8]	93.9	97.6**	94.9	86.4
sulfanilamide[H10]		> 80*	> 80	
sulfanilic acid[H10]		> 80*	> 80	
Influence of the salinity[H9]				
sulfametoxazole		99.4 – 101.8*** (depending on the salinity)		
sulfadimethoxine		97.2 – 99.2*** (depending on the salinity)		
sulfapyridine		98.2 – 100.4*** (depending on the salinity)		
trimethoprim		98.9 – 99.5*** (depending on the salinity)		
Mixture toxicity[H10,H11]				
6SAs		> 80*	> 80	
6SAs + SN		> 80*	> 80	
FEN + FLU				> 95
Chronic toxicity, 14 d [H12] 5SAs			87.7 – 107.9	

> 80 %	50 – 80 %	< 50 %	not applicable

Similarly, the results of research on the stability assessment (**Table 4**) of the tested substances under the test conditions proved that in most cases (for which stability was > 80%, marked with green color) the observed biological effect resulted mainly from the presence of the native form of these substances. The exceptions were methothrexate (MET), 7-hydroxymethothrexate (7-OH-MET) and tamoxifen (TAM), for which native form was not observed at the end of different tests. Therefore their toxicity could have resulted from the degradation products potentially produced under the experimental conditions of the tests.

Table 4. Determined stability of a n investigated analytes under ecotoxicological tests conditions

Organism/ Substance	***BACTERIA*** *V. fischeri* (30 min)	**GREEN ALGAE** *S. vacuolatus* (24 h)*/ *R. subcapiatata* (72 h)**/ *C. vulgaris* (48, 72 h)***	**PLANTS** *L. minor* (7 d)	**CRUSTACEANS** *D. magna* (48 h)
propranolol[H6]	n. d.	n. d.	n. d.	
metoprolol[H6]	n. d.	n. d.	n. d.	
nadolol[H6]	n. d.	n. d.	n. d.	
fenbendazole[H7]	n. d.	n. d.	n. d.	> 80
flubendazole[H7]	n. d.	n. d.	n. d.	> 80
5-fluorouracyl[H8]	n. d.	75.9**	83.2	100.2
cyclophosphamide[H8]	n. d.	96.6**	85.0	99.2
ifosfamide[H8]	n. d.	97.2**	98.0	98.1
imatinib[H8]	n. d.	80.9**	107.3	90.0
tamoxifen[H8]	n. d.	n. d. **	0	0
methothrexate[H8]	n. d.	0**	0	n. d.
7-hydroxymethothrexate[H8]	n. d.	0**	0	n. d.
sulfanilamide[H10]		> 80*	> 80	
sulfanilic acid[H10]		> 80*	> 80	
Influence of the salinity[H9]				
sulfametoxazole		98.0 – 100.7*** (depending on the salinity)		
sulfadimethoxine		100.9 – 102.3*** (depending on the salinity)		
sulfapyridine		99.1 – 101.8*** (depending on the salinity)		
trimethoprim		100.2 – 101.2*** (depending on the salinity)		
Mixture toxicity[H10,H11]				
6SAs		> 80	> 80	
6SAs + SN		> 80	> 80	
FEN + FLU				77 – 92
Chronic toxicity, 14 d [H12] 5SAs			0 – 94.9	

> 80 %	50 – 80 %	< 50 %	not applicable

Having in mind also the fact that some of the selected for the study drugs were characterized with relatively high logP values (especially tamoxifen (TAM), as well as FEN and FLU), it was suspected that under the ecotoxicological test conditions using polypropylene test vessels, the observed loss of the drug could also result from their adsorption on their surface. Therefore, additional chemical analysis were carried out using both glass and polypropylene vessels. It was found that in the case of FEN and FLU and the test using *D. magna* [H7] the loss of these drugs in the polypropylene vessels did not exceed 20% (**Figure 1**, [H7]), and thus it could be regarded as insignificant. Similar observations have been made for MET, its metabolite 7-OH-MET and TAM under the test conditions using duckweed (*L. minor*) (**Figure 2A**, [H8]).

The possibility of biosorption or bioaccumulation of these substances on the surface/to the studied organisms was also taken into account. However, tests carried out in this field for FEN and FLU in the test with *D. magna* (under the test conditions in the presence of or without organisms) showed no statistically significant differences between these tests (**Figure 2**, [H11]). In the case of 7-OH-MET and TAM (**Figure 2A**, [H8]), the obtained results indicated that their biosorption/bioaccumulation into plant material (*L. minor*) can not be completely excluded.
Furthermore, the possibility of photodegradation of the tested compounds under the experiment with duckweed conditions was also taken into account, because this test is conducted with continuous irradiation (6 klx) for 7 days. The obtained results (**Figure 2**, [H8]) confirmed that for all three analytes (MET, 7-OH-MET, TAM) gradual photodegradation of these compounds was observed. For these reasons, extended ecotoxicological studies were carried out for TAM and MET (**Figure 1A**, [H8]) in so-called semi-static conditions, i.e. with the exchange of the analyte solution in medium during the experiment. Based on these results (**Table 4**, [H8]) it was found that MET toxicity increased with the increased frequency of medium exchange, i.e. with the delivery of a fresh portion of native form: $EC_{50,\ daily\ exchange}$ = 0.08 mg/L, $EC_{50,\ exchange\ at\ day\ 3\ and\ 5}$ = 0.11 mg/L, $EC_{50,\ without\ exchange}$ = 0.16 mg/L. In contrast, toxicity of TAM remained at a similar level: $EC_{50,\ daily\ exchange}$ = 0.23 mg/L, $EC_{50,\ exchange\ at\ day\ 3\ and\ 5}$ = 0.22 mg/L, $EC_{50,\ without\ exchange}$ = 0.18 mg/L. It has been proved that the produced degradation products of MET are less toxic than the native form of this drug, in contrast to the degradation products of TAM, which most likely have similar toxicity as the native form. Using the LC-MS/MS technique, an attempt was made to identify the resulting degradation products, confirming for example the presence of the two most commonly identified TAM photodegradation products with *m/z* = 370 [H8].

Taking into consideration obtained results for the most toxic substances as well as the most sensitive organisms, the toxicity of mixtures was assessed. Since the number of mixture combinations is infinitely great, mixtures of FEN and FLU towards *D. magna* [H11] and six sulphonamides (6SAs) with their transformation product - sulfanilamide (6SAs + SN) towards not only the *L. minor*, but also green algae *S. vacuolatus* [H10], were selected as model systems. The selected sulfonamides were: sulfathiazole, sulfamerazine, sulfadimidine, sulfamethoxazole, sulfadimethoxine and sulfapyridine, representing substances with a wide spectrum of toxicity to the tested organisms [22] and often detected in environmental samples. These studies included the use of two recommended in the literature models to predict the toxicity of mixtures of various substances: Concentration Addition (CA) and Independent Action (IA). According to the

basic assumptions of the CA model: substances in the mixture have a similar mechanism of action, differ only in strength, and each substance affects the toxicity of the whole mixture in proportion to its dose. Moreover, what is more important, substances in the mixture in the concentrations lower or equal to their NOECs (No-Observed Effect Concentrations) can exert toxic effect. Model IA assumes, however, that the substances in the mixture act independently and substances in the mixture have a different mechanism of action, and when they appear together in concentrations lower or equal to their NOECs they will not exert toxic effect. In the mixture toxicity studies, it is suggested to use the CA model as the default and worst-case estimation to predict the toxicity of given mixtures, which was also included in this work [H10-H11].

The scheme of performed mixture toxicity tests for FEN and FLU is presented in **Figure S1** in [H11], while for 6SAs and 6SAs + SN in **Figure A1** in [H10]. As a result, based on a comparison of the predicted value of the EC_{50} parameter (or the expected biological effect [%]), calculated in accordance with the discussed models (described, among others, in **Chapter 2.5**, [H11]) and experimentally determined values of this parameter (**Table 3**, [H11] and **Table 4**, [H10]), the additivity model was proved to be a sufficiently safe model to predict the toxicity of pharmaceutical mixtures with the same mechanism of action for the mixture of two benzimidazoles [H11] and sulfonamides with their transformation product [H10]. For example, to confirm this statement, obtained during these studies data in the **Figure 5** is presented. It clearly indicates that whenever individual sulfonamides were tested in the concentration not causing toxic effect, while they were mixed together such toxic effect was observed (although smaller than predicted with the CA model).

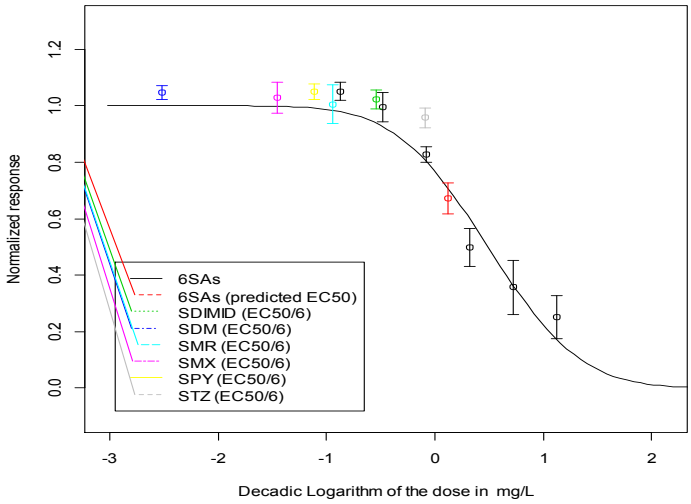


Figure 5. Determined toxicity of given sulfonamides as single substances at a concentration equal to 1/6 of the EC_{50} value (without a significant biological effect – response close to 1) and their mixtures of exactly the same doses (causing toxic effect – marked in red)

These studies constitute a significant contribution to the state of knowledge about the risks that these drugs might pose in the environment. At the same time, they prove that the toxicity of pharmaceutical mixtures in the environment should not be ignored, because even when these compounds are present in the environment at the concentrations that do not exert toxic effects, the biological effect can be observed for their mixtures. This justifies the concern of many researchers about the adequacy of the currently applied ERA guidelines of pharmaceuticals, which do not take into account the toxicity assessment of mixtures.

Finally, for the first time, chronic toxicity assessment of selected representatives of sulfonamides was carried out under the conditions of a prolonged test using duckweed as the most sensitive organism for this group of drugs [H12]. Five substances belonging to this group were selected in this study: sulfadimethoxine (SDM), sulfamethoxazole (SMX), sulfamerazine (SMR), sulfadimidine (SDIMID) and sulfathiazole (STZ) [H12]. The experiments consisted of: *i*) toxicity assessment of five selected SAs in the prolonged test (14 days test) with the use of duckweed *L. minor*, which was compared with that determined under standard conditions (7 days test); *ii*) assessment of toxicity of sulfonamide solutions (at 3 selected concentrations, **Table 1** [H12]) after irradiation (ageing them in the environmental chamber); *iii*) performing a series of chemical analysis using the HPLC-UV technique (for quantitative analyzes) and LC-MS/MS in the MRM mode (for qualitative analysis) to determine the degree of elimination of the native form during the experiment and to confirm the presence of the native form of the

drug and the two most commonly detected photodegradation products of sulfonamides – sulfanilic acid with sulfanilamide in the tested samples.

On this basis, it was found that the order of acute toxicity differs from chronic toxicity (**Figure 3**, [H12]). For two of the tested sulfonamides – the most toxic in the standard test (SDM and SMX), a decrease in toxicity was observed (for SDM: $EC_{50,\ 7\ days}$ = 0.02 mg/L *vs.* $EC_{50,\ 14\ days}$ = 0.15 mg/L, for SMX $EC_{50,\ 7\ days}$ = 0.21 mg/L *vs.* $EC_{50,\ 14\ days}$ = 1.90 mg/L), and for others the increase in the toxicity in the extended test was assessed (for SMR: $EC_{50,\ 7\ days}$ = 0.68 mg/L *vs.* $EC_{50,\ 14\ days}$ = 0.28 mg/L, for STZ: $EC_{50,\ 7\ days}$ = 4.89 mg/L *vs.* $EC_{50,\ 14\ days}$ = 2.76 mg/L, for SDIMID: $EC_{50,\ 7\ days}$ = 1.74 mg/L *vs.* $EC_{50,\ 14\ days}$ = 0.85 mg/L).

Taking into account the obtained results from chemical analysis (**Table 3**, [H12]) it was shown that it is impossible to explain this phenomenon simply by the loss of the native form under the test conditions, because all of the tested sulfonamides photodegradated to some extent during this test. While this loss for standard solutions (stored in the environmental chamber at exactly the same time and conditions at which the prolonged test was conducted) was smaller, it was much larger for samples taken during and at the end of the extended test, which confirms the possibility of biosorption/bioaccumulation of onto/in the duckweed. Therefore, it was expected that produced photodegradation products and their toxicity may be the reason for the observed differences in the toxicity in the prolonged test. Toxicity of such degradation mixtures was determined under the conditions of the 7-day standard test (results are summarized in **Figure 4**, [H12]). Also during this experiment, appropriate chemical analyses were carried out (**Table 3**, [H12]). The obtained results confirmed that SAs undergo photodegradation under conditions of such "irradiation". However, only for SDM and SMX a significant decrease in toxicity was observed, in contrast to the other three drugs, where the toxicity remained almost the same. Perhaps the observed biological effect results from the reduced concentration of the bioavailable drug fraction to organisms or from the toxicity of the resulting degradation products. Considering the previously determined relatively low toxicity of the two most frequently identified photodegradation products SAs [H10], which could have been the reason for the observed decrease in toxicity, it was decided to check their presence in the collected samples. However, they were not identified in these samples. For these reasons, while the observed decrease in SDM and SMX toxicity under the chronic test could be explained by the loss of their bioavailable fraction to organisms and the production of potential degradation products with lower toxicity, this hypothesis is inappropriate for the other three sulfonamides (STZ, SDIMID and SMR). This highlights the fact that the threat posed by the residues of these three substances,

despite the loss of their native form, can not be reduced, and even, as shown by the data obtained in the prolonged test - it can increase. It can therefore be assumed that the reason for the observed differences in chronic toxicity SAs towards *L. minor* was another mechanism of their toxic action and transportation through biological membranes, which perhaps was related to the form of their occurrence, because only for these three drugs (SDIMID, STZ, SMR) the percentage of neutral form significantly exceeded 90%, in comparison to the other two (SMX, SDM) (**Table 4**, [H12]).

III. Summary

The obtained results of the studies presented in the scientific achievement allow to formulate the following conclusions:

- It has been proven how important and valuable it is to poses and use appropriate analytical tools not only to assess the level of exposure (degree of contamination or environmental fate), but also to characterize the risks that residues of pharmaceuticals may pose in the environment (in the ecotoxicological studies - which allows to understand the reason for the observed biological effects);
- For the first time in the studies on the occurrence of drug residues in the environmental samples the problem of the need to determine the expanded uncertainty of the obtained analytical results was highlighted. This is particularly significant in the analysis of trace environmental pollutants, which include pharmaceutical residues and should be routinely taken into account;
- Thanks to the developed methodology for the determination of selected drugs in marine waters and conducted monitoring studies in the coastal zone of the southern Baltic, the state of knowledge on the Baltic Sea pollution by these substances has been increased, which has already been taken into account in the recently published HELCOM Committee report (2017) entitled: "*Pharmaceuticals in the aquatic environment of Baltic Sea region – A status report*";
- Mobility of the selected drugs (from the group of beta-blockers and cytostatic drugs) in the soil environment was determined, which pose a significant contribution to the state of knowledge about their environmental fate;
- New toxicological data was presented for many compounds not yet fully taken into account in the environmental risk assessment;

- For the first time, the effect of salinity on the toxicity of drugs (sulfonamides and trimethoprim) on aquatic organisms (green algae) was examined;
- Pioneering studies on determination of mixture toxicity of sulfonamides and one of their transformation products towards green algae and duckweed, as well as benzimidazoles towards *Daphnia* were conducted, proving their significance in the interpretation of ecotoxicological results;
- A significant dependency was found between exposure time of organisms to sulfonamides in the aquatic environment and their toxicity;

A set of new, original analytical and toxicological data was provided that could be used in the future to perform a full environmental risk assessment of selected group of pharmaceuticals.

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5. Description of other scientific and research achievements

My other scientific and research interests result from the desire to constantly broaden the knowledge and look holistically at the problem of the presence of „new-emerging" pollutants in the environment. Among my scientific interests, apart from pharmaceuticals, there are also other substances such as ionic liquids. Each time, if possible, I intend to obtain as much data as possible for complete environmental risk assessment of these chemicals. These interests have become the main reason for my involvement in the realization of several different projects as the Project Leader (MNiSW 0012/IP3/2015/73), the Main Investigator (NCN DEC-2011/03/B/NZ8/03009), as well as Investigator (NCN DEC-2011/03/B/NZ8/03010, MNiSW N306 300536, MNISW N204 260237). The list with short description of the scientific and research achievements (after obtaining the PhD degree – **Chapter 5.1**; before obtaining this degree – **Chapter 5.2**) is presented below.

5.1. Scientific and research achievement after obtaining the PhD degree

My other scientific and research achievement might be dived into the presented thematic areas:

A) Development of a number of additional analytical methodologies and assessment of the occurrence of other groups of pharmaceuticals in different environmental components (continuation or extension of research connected with the scientific achievement)

As the part of this research task, a number of analytical methodologies have been developed and subjected to the validation process for the determination of, among others, veterinary medicines, including those used as feed additives and/or in fish farming (aquaculture) in surface waters, bottom sediments and fish tissues [U1-U5]; antibiotics in marine sediments and bottom waters collected from the southern Baltic coastal zone [U6-U8]; and antibiotics used in veterinary medicine in soils collected from the Northern Poland region [U9] using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) technique. Furthermore, the influence of the matrix composition on the of matrix effects observed during the determination of selected

sulfonamides in various environmental samples using LC-MS [U10] was also evaluated. Moreover, the possibility of using chemometric methods to assess the efficiency of the derivatization process of analytes during their determination using gas chromatography (GC-MS) [U11] was investigated. The application of the developed analytical methodologies allowed to assess the degree of pollution of various environmental components of selected regions of our country with the residues of these drugs.

B) Study on the sorption potential and mechanism of selected sulfonamides (continuation of studies started as the part of the doctoral dissertation) and beta-blockers in soils (continuation and extension of research included in the scientific achievement)

These tests were carried out both in the static [U12-U14] and dynamic [U14] test and included the assessment of the influence of not only factors such as pH and ionic strength [U12], but also the temperature on the sorption of these compounds to soils [U13].

C) Stability assessment of other drugs in biotic and abiotic degradation processes (extension of research included in the scientific achievement)

As part of this research task, the susceptibility to biodegradation of antidiabetic drugs was assessed and the basic degradation products of the selected drugs from this group were identified along with the proposition of their degradation pathways and ecotoxicological evaluation of the resulting degradation mixtures [U15-U16]. The hydrolytic stability of other veterinary drugs with antimicrobial activity in the aqueous environment was also evaluated [U17].

D) Evaluation of the (eco) toxicological risk of other chemical substances, including pharmaceuticals, using biotest battery (continuation or extension of research included in the scientific achievement)

Comprehensive risk characterization resulting from the presence in the environment of various chemical substances was performed, which included: *i*) the three most commonly used in veterinary fluoroquinolones (enrofloxacin, norfloxacin and ciprofloxacin) towards aquatic organisms, like luminescent batteries *Vibrio fischeri*, green algae *Scenedesmus vacuolatus*, duckweed *Lemna minor* and crustacean *Daphnia magna* [U4]; *ii*) veterinary medicines commonly used as feed additives and/or in fish farms (metronidazole, doramectin, florfenicol and oxytetracycline) towards the above-mentioned aquatic organisms [U18]; *iii*) methyltrioxorhenium and derivatives [U19] and ionic liquids [U20].

In these studies [U4, U18-19], analytical techniques were also used to obtain complementary results to ecotoxicological data.

E) Searching for alternative methods of removing polar pollutants from waters together with determining the ecotoxicity of potential degradation products

My research interests were also focused on searching for alternative to currently applied methods/technologies of wastewater treatment, which would be characterized with better efficiency of removing not only drug residues [U21], but also ionic liquids [U20, U22]. This research [U20-U21] was carried in the cooperation with the Department of Environmental Technology of the University of Gdansk and the Center for Environmental Research and Sustainable Technologies of the University of Bremen. In addition to the standard evaluation of the proposed electrolysis processes for the removal of selected sulfonamides [U21] and ionic liquids [U20] from wastewater, the ecotoxicity of the degradation mixtures was assessed. This allowed to obtain the better view on the efficiency/effectiveness of the proposed method. In addition, the assessment of the use of carbon nanotubes as potential adsorbents for removing ionic liquids from water matrices [U22] was also performed.

F) Publication of review papers

The scientific experience and knowledge gained during the implementation of the above-mentioned research tasks allowed to prepare and publish 12 reviews on the state of knowledge, problems and challenges related to analysis of the residues of pharmaceuticals in various environmental components [U23-U35], as well as on the assessment of exposure and risk posed by the presence of selected pharmaceuticals in the environment [U32-U34], including this resulting from the presence of sulfonamides in the soil ecosystem [U32] and the summary of available data on the mixture and chronic toxicity of this group of medicines in the environment [U33].

Additionally, just recently, the paper of an experimental and review nature was published in Trends in Analytical Chemistry. It concerns the determination of pKa values of selected drugs used in chemotherapy [U35]. The necessity of such research was dictated by the fact that the information presented in the literature on the acid-base properties of these compounds was inconsistent, hence the interpretation the obtained test results was impossible.

List of supplementary publications published after obtaining the PhD degree:

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[U10] M. Borecka, **A. Białk-Bielińska**, G. Siedlewicz, P. Stepnowski, K. Pazdro, 2014. The influence of matrix effects on trace analysis of pharmaceutical residues in aqueous environmental samples. [w:] Insights on Environmental Changes (Eds. T. Zielinski, K. Pazdro, A. Dragan-Górska, A. Weydmann), GeoPlanet: Earth and Planetary Sciences, 14, 1-16, ISBN 978-3-319-03683-0, Springer International Publishing, Szwajcaria.

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5.2. Scientific and research achievement before obtaining the PhD degree

My scientific-research achievements gained before obtaining the PhD degree in chemical sciences were related to the realization of my PhD thesis (in the years 2007-2015) and was aimed at providing important data on analytics [U1-U2], environmental fate [U3-U4] and ecotoxicity [U5] of one therapeutic group of pharmaceuticals – sulfonamides. It also included the publication of two review papers on the basic problems related to the analysis of the residues of pharmacueticals, including antibiotics, in environmental samples [U6-U7].
In addition, in cooperation with other research teams, methods for determining glutathione and identifying acridine derivatives using LC-MS [U8-U9] have been developed.

List of supplementary publications published before obtaining the PhD degree:

[U1] **A. Białk***, J. Kumirska, R. Palavinskas, P. Stepnowski, 2009. Optimization of multiple reaction monitoring mode for the trace analysis of veterinary sulfonamides by LC–MS/MS. Talanta 80, 947–953. **IF_{2009} = 3,290**

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[U3] **A. Białk-Bielińska**, S. Stolte, M. Matzke, A. Fabiańska, J. Maszkowska, M. Kołodziejska, B. Liberek, P. Stepnowski, J. Kumirska, 2012. Hydrolysis of sulphonamides in aqueous solutions. Journal of Hazardous Materials, 221-222, 264-274. **IF_{2012} = 3,925**

[U4] **A. Białk-Bielińska***, J. Maszkowska, W. Mrozik, A. Bielawska, M. Kołodziejska, R. Palavinskas, P. Stepnowski, J. Kumirska, 2012. Sulfadimethoxine and sulfaguanidine: Their fate in natural soils. Chemosphere, 86, 1059-65. **IF_{2012} = 3,137**

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[U9] K. Krzemiński, A. Roshal, B. Zadykowicz, **A. Białk-Bielińska**, A. Sieradzan, 2010. Chemiluminogenic properties of 10-Methyl-9-(phenoxycarbonyl)acridinium Cations in Organic Environments. Journal of Physical Chemistry, 114, 10550–10562. **IF_{2010} = 2,732**

5.3. Further scientific plans

Despite the fact, that there have been already many scientific published on the presence and threats posed by the residues of pharmaceuticals in the environment, this subject is still not sufficiently known. Therefore, in the future I intend to continue the started research tasks related to the presence of transformation products of these medicinal substances in the environment. This will allow to understand the scale of the problem associated with the presence of drugs and indirectly also degradation products and/or metabolites in the environment. This research task will pose the continuation of the project, that I was the Project Leader (MNiSW 0012/IP3/2015/73, 2012-2017) and will be implemented as part of a research project in which I am currently taking part as the Investigator (NCN UMO-2015/17/B/NZ8/02481, 2016 - 2019).
Bearing in mind the above-mentioned main goal (apart from the research tasks included in the aforementioned projects), I intend to further improve my analytical skills and attempt to develop analytical methodologies for determining even more analytes during one chromatographic run (multi-analysis), including native forms of medicines, their transformation products and isotope-labeled standards as well as the application of the ultra-high performance liquid chromatography coupled with mass spectrometry (UHPLC-MS) technique. This will allow to shorten the time of analysis and carry out routine monitoring analysis in order to obtain more information on the degree of environmental pollution by pharmaceuticals and their transformation products. The

application of such a technique would follow the principles of Green Analytical Chemistry by shortening the analysis time, saving energy and the amount of solvents used and waste produced, as well as reducing the analyst's working time. In this context, I intend to conduct analyses in two modes, the so-called: target analysis and non-target analysis. For this reason, I plan to apply ultra-high performance liquid chromatography in combination with a high-resolution mass spectrometer (UPLC-Q-TOF), which will allow primarily to identify various substances present in the given samples. It would be extremely interesting to check whether it would be possible to determine certain standard profiles of these polar pollutants in various types of environmental samples, which apart from the use of the UPLC-Q-TOF technique, would require additional spectroscopic techniques (NMR, IR) and chemometric methods. This would enable cataloging and quantification of as many chemical compounds as possible in the analyzed samples and monitoring changes in these profiles as a result of different incidents.

In addition, it is still fascinating for me to look for an answer to the question *Does the presence of pharmaceuticals, and hence their transformation products, pose some risks in the environment? T*herefore, I intend to continue the research on the assessment of the environmental fate and toxicity of these compounds towards various organisms, with particular emphasis on studies on the determination of their mixture toxicity and long-term effects. I would also like to extend the research on the toxicity assessment of mixtures not only to the pharmaceutical transformation products, but also to other common environmental contaminants, so that these model studies will refer to real environmental conditions to a greater extent. In addition, for selected substances (with high sorption potential), toxicity to selected soil organisms will also be tested. There is a great need to conduct such research, because the information on this topic is very limited.

Finally, I would also like to continue the ongoing research focused on the searching for the efficient methods for removing these environmental pollutants from different matrices, including the evaluation of the toxicity of the resulting byproducts.

5.4. Statement of the total bibliometric data

Data from 30.01.2018	A. Before PhD	B. After PhD	C. Included in the scientific achievement	Total (A+B)
Hirsch Index *(Web of Science)*			**14**	
Scientific publications in journals listed in Journal Citation Reports (JRC)	7	36	12	**43**
Scientific publications in journals not listed in JRC	2	7		**9**
Total Impact Factor (IF)	**19,079**	**138,906**	**50,494**	**157,985**
Number of citations (*Web of Science*)				
• without self citations			142	**427**
• with self citations			151	**503**
Position in the publications included in my scientific achievement				
• fist author			4	
• corresponding author			8	
• last author			4	
• other			1	
Book chapters	0	4		**4**
Oral presentations during national and international conferences				
• as presenting author	1	3		**4**
• as co-author of the oral presentation	0	17		**17**
Number of posters	43	61		**104**
Involvement in the realization of international and national projects				
• as Principal Investigator	0	1		**1**
• as Investigator (Expert)	2	4		**6**

Anna Białk-Bielińska