Large quantities of pharmaceuticals (PAs) are in use worldwide as feed additives, growth promoters and for prophylactic and therapeutic purposes. Many pharmaceuticals undergo a structural changes in the human or animal bodies. While some of drugs are only moderately or poorly metabolized some of them are mainly excreted in the changed forms. Normally it is assumed that metabolism of native forms of pharmaceuticals leads to decreased toxicity. In some cases, however, metabolites can be more active or exhibit toxicological effects similar to the parent compound. Metabolites might be stable but can also be further transformed in the environment to yield stable transformation forms. Transformation of these compounds (parent forms and metabolites) can occur in environment but also in the environment/technical facilities such as sewage treatment and drinking water treatment plants. Transformation reactions occur in surface waters, sediments, manure and soils through biotic and abiotic systems covering microbial degradation, redox reactions, hydrolysis, or photolysis. All the chemicals generated at the end in the environment (metabolites and degradation products) are commonly named as transformation products (TPs). The formation and environmental presence of transformation products adds further complexity to any chemical risk assessment. Transformation products may contribute significantly to the risk posed by the parent compound (i) if they are formed in a high yield, (ii) if they are more persistent and/or more mobile than the parent compound, or (c) if they are highly toxic. While some of the resulting transformation products are known to be more abundant in the aquatic environment than their parent compounds, the majority of them present there have most likely not even been identified yet. Thus, human and the environment are exposed to a highly variable and unknown cocktail of chemicals.

Usually PAs are designed to exhibit a specific pharmacological action. They may affect not only target organisms (for example, antibiotics may affect bacteria) but may also have unknown effects on environmentally relevant non-target organisms such as unicellular algae, invertebrates, fish and plants. However, in non-target organisms they may produce effects resembling the target effects or the side effects observed in target organisms. For instance, the synthetic oestrogen ethinyloestradiol is well known for its potential to cause endocrine disruptive and reproductive effects: exposure to 5 ng L-1 of ethinyloestradiol delays embryonic development in zebrafish (Danio rerio). It was also observed that the population of the oriental white-backed vulture (OWBV) Gyps bengalensis (one of the most common raptors on the Indian subcontinent) had suffered declines of > 95%, starting in the 1990s. It was found that residues of diclofenac were responsible for the decline in OWBV numbers because renal disease was caused in OWBVs by direct oral exposure and through feeding vultures diclofenac-treated livestock. Certain anticancer drugs and their TPs, which of all PAs, are suspected of posing a specific risk to aquatic non-target organisms, mainly because they act unselectively on all growing cells. Although their consumption is low compared to that of other PAs, it is hypothesized that their mode of action makes all organisms, and eukaryotic ones in particular, vulnerable to damage.

Apart from the above observations, knowledge of the potential effects of pharmaceuticals on the environment is very limited. Over the past few years, therefore, regulations have been formulated regarding the assessment of risks of environmental exposure to drugs (Committee for Medicinal Products for Human Use (CHMP) 2006; Committee for Medicinal Products for Veterinary Use (CVMP, 2000, 2004); European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC, 2008); World Health Organization (WHO, 2011). According to these documents the Environmental Risk Assessment (ERA) process differs for veterinary and human drugs. Nonetheless, it usually starts with an initial exposure assessment (Phase I) that is based on a calculation of the predicted or measured environmental concentration (PEC or MEC respectively). But with some exceptions, a fate and effects analysis (Phase II) is only required when exposure-based threshold values, the so-called action limits, are exceeded in different environmental compartments. Hence, risk assessment, described by the Risk Quotient (RQ), is performed by calculating the ratio of the PEC (or MEC) and PNEC on non-target organisms. If RQ < 1, no further testing is recommended. For example, the recently introduced European guidelines on assessing the risks of human drugs excludes the testing of pharmaceuticals whose PECsurface water is below an action limit of 0.01 µg L-1. The main problem associated with this approach is the fact that no actual sales figures or measured environmental concentrations are at hand when a risk assessment is conducted. Therefore, only crude PEC calculations are performed. Furthermore, the ecotoxicity tests included in Phase II focus on the acute toxicity of only single compounds.

It must be highlighted that pharmaceuticals occur in matrices of natural environmental compartments not as single, isolated drugs but in mixtures. Available literature data demonstrate that the simultaneous presence of several pharmaceuticals in the environment may result in a higher level of toxicity towards non-target organisms than that predicted based on the results of tests for individual active substances. Therefore, accumulated concentrations or synergistic-antagonistic effects need to be considered in mixture toxicity assessment.

In the last decade pharmaceuticals have been regularly detected not only in aquatic but also in terrestrial environments. Although their concentrations in environmental samples are quite low (at the µg L-1 or ng L-1 level), they are continuously being released into ecosystems. Long-term exposure to the compounds may cause chronic toxic effects in non-target aquatic and terrestrial organisms. Therefore chronic toxicity test should be included in the environmental risk assessment of pharmaceuticals.

Therefore, the main aim of this proposal is to assess whether and which groups of pharmaceuticals and their most common transformation products pose very serious hazards to the environment and human health.

The research hypothesis are:

(i) there is a potential high bioactivity (consequently toxicity and ecotoxicity) of several groups of PAs and TPs of thereof while being released and further transformed in the environment

(ii) transformation products could pose even a greater risk than the parent compound.

The main criterion for the selection of compounds determined in the project were: (i) extended use in human and veterinary medicine; (ii) their presence statement in the abiotic components of the ecosystem proved in the frame of previous studies; (iii) potential risk of ecotoxicological effects. Pharmaceuticals belonging to different therapeutic groups like non-steroidal anti-inflammatory drugs, antibiotics, anticancer drugs, steroid hormones, antiparasites have been chosen for the study. For the verification of the proposed research hypothesis a flexible ecotoxicological test battery will be used, including luminescent marine bacteria (Vibrio fischeri), soil bacteria (Arthrobacter globiformis), green algae (such as: Scenedesmus vacuolatus and/or others),

duckweed (Lemna minor), crustacean (Daphnia magna), soil collembolan (Folsomia candida) in order to take into account organisms representing different levels of biological organisation inhabiting both water and soil environments. For the selected transformation products and mixtures cytotoxicity test with rainbow trout (Oncorhynchus mykiss) cell line will be performed in order to evaluate potential toxicity to fish. Analysis of different environmental samples collected from northern Poland using a variety of developed analytical methods like GC-MS, LC-MS, LC-MS/MS, or UHPLC-MS enable identification of selected pharmaceuticals and their transformation products. As a result of the task measured environmental concentrations (MEC) of the target compounds will be also obtained. Special attention will be paid to collect the reliable data on environmental fate of PAs and TPs and their mixtures.

For selected for the study pharmaceuticals and their transformation products reliable data on their exposure levels, the environmental fates and ecotoxicity will be collected. The toxicological assessment of PAs from different groups of applications and their transformation products (metabolites and degradation products) as well as the use of the battery of test organisms at various level of biological organization will lead to a better understanding of the environmental impact of these substances. The toxicological tests will provide accurate insight into the effects of the PAs and TPs examined in this study on organisms representing different trophic levels in terrestrial and aquatic ecosystems. The results of proposed research will enable us to work out precise relationships between structure, physicochemical properties and biological activity of the compounds with broad variety of structures. In addition, the research will yield an assessment of the effects due to the chronic exposure of organisms to trace amounts of pharmaceuticals and their transformation products in the environment. This research will also provide the information necessary for evaluating complex synergistic and/or antagonistic effects of mixtures of PAs and their TPs.

The main results of the project will be presented in the form of publications in international journals and as posters at Polish and international conferences.