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Monitoring Primary Effects of Pharmaceuticals in the Aquatic Environment with Mode of Action-Specific in Vitro Biotests

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The continuously growing and aging world population and the intensified livestock farming worldwide are expected to raise the global consumption and the number of human and veterinary pharmaceuticals on the market in the future. As a result, pharmaceuticals that are incompletely removed by wastewater treatment will occur in higher concentrations and in immense multiplicity in the water cycle. Except for synthetic steroid hormones, environmental monitoring of pharmaceuticals still remains to be based upon analytical chemistry of single substances which is incapable to encompass entities of pharmaceutical compounds with, for example, the same mode of action (MOA) at once, and thus will soon be overstrained by the sheer number of compounds, metabolites, and new product developments. For all the major classes of pharmaceuticals, we will therefore need MOA-based cell culture assays that report on the immediate interaction of compounds with their specific molecular target and, thus, on possible effects on organisms in the environment.

The topic's relevance is without doubt. Globally, pollution by pharmaceuticals has been identified as a matter of concern by environmental policy. Recently, a nomination dossier was submitted to the United Nations that proposed 'environmentally persistent pharmaceutical pollutants' as an emerging policy issue under the framework of the 'Strategic Approach to International Chemicals Management'. Last year, representatives of 24 countries and representatives of industry and academia agreed on the necessity to establish systematic and global monitoring programs on pharmaceuticals as a basis for a constant evaluation of potential risks to man and the environment.[1] Moreover, in the last amendment of the European Water Framework Directive, the European Commission was mandated to develop a strategy to reduce possible environmental impacts of pharmaceuticals. As a consequence, diclofenac, 17β-estradiol and 17α-ethinylestradiol are discussed to be placed on a watch list for which EU-wide monitoring data must be gathered. According to the EU, new pharmaceuticals have to undergo an environmental risk assessment (ERA) before marketing approval. For several pharmaceuticals, potential risks have been already identified and, in contrast to human medication, marketing authorization can be denied or restricted for veterinary medicinal products (VMP). A major drawback of the current approach for pharmaceutical ERA, however, is the lack of suitable data on exposure and ecotoxicological effects for the vast majority of the "old" active ingredients in pharmaceuticals which were already on the market before the requirement for an ERA was introduced into legislation. In addition, long-term effects resulting from chronic exposure of nontarget organisms and the potential of mixture effects as well as interactions with biotic and abiotic confounding factors are far from being well understood.

As an advanced tool for an ERA, different strategies of pharmaceutical prioritization have been proposed among which the most promising ones incorporate ecotoxicity data that are plausibly linked to specific modes of action (MOA). Furthermore, for monitoring purposes, MOA-based tools have been identified (e.g., in the U.S. Tox21 and ToxCast

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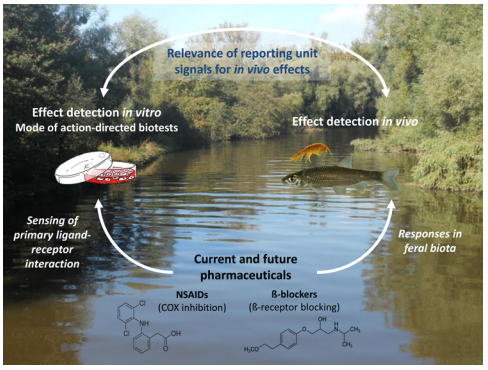

Figure 1. Pharmaceuticals may, phenomenologically, exert effects in nontarget biota and pose environmental risks. The vast number of these compounds and their metabolites makes it impossible to routinely screen their abundance by analytical chemistry. MOA specific life cell biosensing could trace primary action of pharmaceutical classes and attribute in vivo effects to them.

programmes) as auspicious instruments that can provide information on potential pharmaceutical class-related effects in environmental samples. A detailed study giving numerous examples of application of MOA-based techniques to environmental samples has been published recently.[2] Nevertheless, all these approaches have focused on nonpharmaceutical compounds with the exception of synthetic steroid hormones.

However, MOA-based techniques are within reach also for other classes of pharmaceuticals. Theoretical approaches to predict interactions of human pharmaceuticals with receptors of wildlife species have already been developed.[3] Even though a high diversity of biota with different physiology is potentially affected in the environment, the biochemical function of receptors remains rather phylogenetically conserved within a clade, at least among vertebrates. In vitro assays based on highly conserved signal transduction pathways including ligand-receptor interactions are, therefore, supposed to be relevant for a high number of vertebrate species, including fish. Although the use of such MOA-based in vitro assays for environmental monitoring is already common practice for nonpharmaceutical compounds or sex steroids, for other classes of pharmaceuticals such in vitro tests have not left a mark in ecotoxicology yet. This is the more surprising because target-based assays focusing on receptor-ligand binding, coupled to the generation of measurable signals have been, historically, the mainstay of substance screening in pharmacological research and development.

In this context, the most recent developments make use of the enormous potential of genetically encoded fluorescent sensors expressed by recombinant cell lines comprising a sensing unit that recognizes its interaction with a chemical and a reporting unit which indicates the sensing unit's state and, accordingly, leads to immediate fluorescence signal changes[4] - in contrast to reporter gene assays which generate a signal at the downstream end of a long signal transduction pathway allowing side effects by interfering substances at every step along this cascade. Adapting such methods to construct cell lines that generate immediate fluorescent signals following a pharmaceutical-target interaction should therefore substantially advance MOA-directed analysis of the primary impact of pharmaceuticals in environmental samples. Own current research revealed a fluorescence resonance energy transfer (FRET)-based cell line created to monitor β-adrenoreceptor binding to provide an optical signal for β-blockers in the concentrations range of the lowest observed effect concentration (LOECs) reported for the most sensitive biota (10 to 100 nmol/L).[5]

Of course, the transfer of these cell culture technologies from the "clean" conditions of active component screening to the "dirty" composition of environmental samples will likely pose challenges for an appropriate processing of these samples. Furthermore, receptor activation as a primary effect of environmental compounds needs to be validated by biomarkers and population-relevant endpoints in environmentally relevant biota including fish, crustaceans, and sediment-dwelling organisms for the same environmental situation — a labor-intensive but achievable task (Figure 1). Consequently, novel MOA-directed in vitro assays on primary pharmaceutical action will serve as both compound class-selective and effect-oriented tools, which bridge the still existing gap between the analytical chemistry of pharmaceuticals in waters and sediments and their in vivo effects in an elegant way. They need to be urgently implemented in environmental monitoring programmes and the routine assessment of environmental quality.

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Notes

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