



Issues, challenges, directives, and limitations concerning the improvement of environmental risk assessment of pharmaceutically active compounds

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ABSTRACT

Nowadays, when tons of different chemicals including pharmaceutically active compounds (PhACs) are known to be released into the environment, applying adequate risk assessment relating to the protection of the ecosystem is very important. A broad body of scientific papers expresses a growing demand for improvement of the method(s) for ecological/environmental risk assessment (ERA). Although certain issues about ERA often emerge in the community, most of them cannot be considered as real problems and its methodology was developed keeping several limitations in mind. Nevertheless, the current approaches can be improved in order to better serve the intended purposes. For example, there is a lack of an integrated, manageable ecotoxicological database. It is not uncommon for basic, but extremely important, influencing factors such as time of exposure, interactions between different compounds, and characteristics of different habitats to be ignored. Discussing under the basic regulatory framework used in the EU, this correspondence paper deals with these and other examples to present the current features of ERA, identify gaps in process and application, and propose possible improvements/directives.

1. Introduction

In the last three decades, different risk assessment methods and guidelines regarding chemical substances that endanger the ecosystem have shown an extensive proliferation resulting in ERA becoming a topic that people are widely interested in (Bartell, 1996; De Zwart and Posthuma, 2005; US Environmental Protection Agency, 1989; Zhou et al., 2019). In recent years, a broad body of scientific papers has expressed a growing demand for reform of the method(s) for ERA (Martin et al., 2019; Tannenbaum, 2020). A few years ago, the Centre for Ecological Research, Balaton Limnological Institute (Tihany, Hungary) also started to deal in-depth with the science of ERA regarding the concentrations of PhACs detected in surface waters (Kondor et al., 2021; Maasz et al., 2021; Molnar et al., 2020). In our opinion, sometimes it is difficult to take a stand on a given methodical step of ERA since, for example, there are different regulatory frameworks (e.g., the Directive 2001/83/EC and the Regulation (EC) 726/2004 in EU; National Environmental Policy Act of 1969 in USA; Canadian Environmental Protection Act of 1999) and many articles do not even perform ERA in a way that suits their country/government (Lee and Choi, 2019). Furthermore, certain problems and contradictions often emerge in the community regarding ERA (e.g., applicability of the worst-case scenario, see Section 2.5.), however, when considered carefully, most of them are neither real problems nor contradictions. In this correspondence article, our goal is not to criticize published assessments in general but to discuss assessments under a regulatory framework. Our aim is to identify the limitations of ERA as it is, what it could be like, and the gap between the two. Since we basically utilize the regulatory framework/procedure of ERA used in EU (EMA, 2018; Lee and Choi, 2019), we will present our concerns and thoughts accordingly.

2. Presentation of the concerns

2.1. Selection of PhACs to be assessed

Currently, the EU operates a tiered approach for ERA (reviewed in Lee and Choi (2019)). The first step is the determination of concentration of the given PhACs by analytical measurements (measured environmental concentrations [MEC]) or calculations (predicted environmental concentration [PEC], EMA, 2018) giving preference, in our opinion, to frequent and continuous sampling over prediction.

Basically, if the MEC or PEC value of a given PhAC is < 10 ng/L, it is considered to be of no risk to the ecosystem. Otherwise, a potential risk is assumed and effects need to be further analysed. To note, irrespective of MEC and/or PEC, lipophilic drugs with octanol/water partition coefficient ($\log K_{OW}$) > 4.5 also need to be further investigated because of their ability to bioaccumulate (EMEA/CHMP, 2006). The approach considers also performing ERA on some another PhACs that may affect invertebrate and vertebrate species at concentrations lower than 10 ng/L. These compounds include mainly the different synthetic steroidal compounds (e.g., estrogens) that have been shown to affect the different aquatic species even at very low concentrations (e.g., Runnalls et al., 2013; Svgruha et al., 2020). However, the continuous and long-term presence of other kinds of PhACs at concentrations lower than 10 ng/L, especially in mixture, may also be able to affect invertebrate and vertebrate species. Taking these compounds into consideration would make ERA a higher tier and more accurate.

At least, it would be necessary to create a priority lists of PhACs approved for marketing based on a quantitative ranking approach that considers the amount of prescriptions, metabolisms, efficiency of removal in waste water treatment plant, and multiple toxic endpoints, in order to identify compounds that can potentially pose a risk to the ecosystem (Dong et al., 2013).

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2.2. Management of ecotoxicological data

The problem of ecotoxicological data management appears in many articles dealing with ERA, however it should be noted that they were written not specifically according to EU guidelines. The collection of raw ecotoxicological data (e.g., EC50, LC50, NOEC, HC5) is required for the determination of the certain so-called predicted no effect concentrations (PNEC) of PhACs. Different ecotoxicological values for individual PhACs can be found when going through the literature in part due to them being carried out using a wide variety of different endpoints, test durations, and species. However, the practice of testing PhACs in several ways is understandable because different PhACs take effect through a variety of mechanisms in vivo, including growth, mortality, reproduction, development, behavior, molecular, cellular, and tissue level alterations. Another problem is that some predictions are solely based on toxicological data between chemical compounds with structural/physicochemical similarity (e.g., according to read-across approach, adverse outcome pathways, modeled EC50s from ECOSAR database) (Ankley et al., 2010; ECETOC, 2012; Patlewicz et al., 2013; Sanderson et al., 2004) instead of being derived from actual experimental data of the particular PhAC. Of course, we agree that given the high number of PhACs and the fact that testing living organisms brings about serious ethical concerns and is severely limited because it is not defensible, in some cases robust read-across or robust modeling can be used to substitute animal testing. However, in their present form, modeled data needs to be treated with a high degree of uncertainty (e.g., many read-across cases fail to demonstrate toxicokinetic and toxicodynamic similarities (Escher et al., 2019)). These problems mean that, if the data are inappropriate (e.g., the wrong endpoint or test animal has been used, the experimental technique was not sufficiently rigorous) or the data are based wholly or partly on non-robust modeling, ERA estimates could be wrong by even orders of magnitude. The solution is presumably found in the improvement of modeling that could enhance the robust across-chemical extrapolation and in the appropriate management of ecotoxicological data; to note, attempts have already been made in this direction (e.g., CAFE database and software which is applicable to plot a species sensitivity distribution [SSD] curve from ecotoxicological data of PhACs too.) (Bejarano et al., 2016). Table 1 demonstrates with the example of estradiol (E2) that using raw ecotoxicological data from various sources leads to different PNEC values, thus ultimately gives divergent risk levels even when calculating with the same MEC or PEC.

2.3. Habitat specificity

Expanding on the points made above, in many cases, ERA is not adapted to the ecosystem of the given habitat, i.e. the risk levels are calculated using test results of species which do not occur at the study area. The sensitivity of species from different habitats can vary widely. Usually, experiments based on the widely recommended OECD guidelines utilizing standard test organisms (e.g., *Daphnia magna*), while very useful for comparing different chemicals in a highly standardized way in a laboratory, do not necessarily indicate how a different type of organism in a different habitat would respond to the same chemicals. The ERA could become more accurate by involving habitat specificity, i.e. use of native or even more invasive species of a given habitat instead of organisms recommended by OECD guidelines. However, it must be pointed out that such efforts mostly may not be feasible, due to time, methodological or ethical constraints. Besides, a more meaningful ERA can be achieved by addressing the habitat to be investigated (based on measurable data such as spatial extent and characteristic rates of ecological processes (Suter and Norton, 2019)).

2.4. Duration of exposure

Although the tiered approach of ERA used in EU contains environmental fate assessment for PhACs (e.g., adsorption-desorption,

Table 1
Representative comparison of ERA results of E2 (estradiol is a frequent and widely investigated PhAC in aquatic ecosystems) based on different ecotoxicological data sources. EC50: half maximal effective concentration, NOEC: no observed effect concentration, HC5: hazardous concentration for five percent of the species, SSD: species sensitivity distribution, AF: assessment factor (5–1000; depending on available ecotoxicological data), PNEC: predicted no effect concentration, maxMEC: maximum measured environmental concentration, RQ: risk quotient.

PhAC	Note	Ref	Ecotoxicological data										Level of risk				
			Based on acute test results					Based on chronic test result						Based on SSD	AF	PNEC [ng/L]	RQ = maxMEC/PNEC (if assumed maxMEC=1 ng/L)
			EC ₅₀ (algae) [ng/L]	EC ₅₀ (daphnid)	EC ₅₀ (fish)	EC ₅₀ (fish)	NOEC (algae)	NOEC (daphnid)	NOEC (fish)	NOEC (fish)	HC5						
E2	Modeled data from ECOSAR database <i>Melostira varicans</i> - Enhancing cell growth <i>Daphnia magna</i> - Sex differentiation <i>Oncorhynchus mykiss</i> - Semen volume CAFE database (>5 freshwater species)	Sanderson et al. (2004) Julius et al. (2007) Brennan et al. (2006) Routledge et al. (1998); Lahnsteiner et al. 2006 Molnar et al. (2020)	8.00E+05	2.77E+05	4.40E+04	4.40E+04	n.d.	n.d.	8.00E+04	n.d.	n.d.	n.d.	n.d.	1.00E+03	4.40E+01	2.27E-02	low
			n.d.	n.d.	n.d.	n.d.	n.d.	8.00E+04	n.d.	n.d.	n.d.	n.d.	n.d.	1.00E+01	1.00E-01	1.00E+01	high
			n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.00E+04	n.d.	n.d.	n.d.	n.d.	5.00E+00	2.00E+00	5.00E-01	medium

biodegradability), it assumes a general, relatively continuous exposure for the aquatic environment through wastewater treatment plant effluents. For this, the approach generally does not apply to short-term testing but the PNEC values are calculated based on a standard set of chronic toxicity data (Lee and Choi, 2019). However, one can expect situations (e.g., a major malfunction in wastewater treatment or a large music festival (Maasz et al., 2021)) that may significantly change the expected relatively continuous environmental concentration of the given PhACs resulting in outstanding concentration values (e.g., an immediate increase in the concentration of PhACs in the particular surface water by this acute pollution). Based on this example, there may be situations when generally preferred long-term toxicity results (NOEC) are not suitable/reasonable; and in this case, short-term toxicity results (EC50, LC50) need to be applied to the calculation of ERA to make it more realistic. Besides, Fig. 1 represents that ignoring time dependence of contamination (i.e. if one considers only a single time point) will lead to miscalculation: a constant, an occasional, and a fluctuating load can result the same maximum of MEC (maxMEC) - and so the same risk level when further calculating with the given PNEC. Of course, we accept that temporal, financial, and methodological constraints hinder the continuous sampling of a given habitat to include temporality of contamination. From the perspective of temporality, a predictive ERA also could be improved by applying toxicokinetic-toxicodynamic models, such as General Unified Threshold model for Survival (GUTS), that take the time-course of processes leading to toxic effects into consideration (Baudrot et al., 2018; Jager et al., 2011).

2.5. Overestimation based on the worst case scenario

The most common thing that is often considered as a problem or contradiction is that ERA is mostly calculated as a worst-case scenario based on the maxMEC of PhACs, which can cause overestimation of the real risk (Zhou et al., 2019). However, it should be pointed out that for the purpose of most ERAs (e.g., few available ecotoxicological data), particularly for regulatory purposes, the aim is precisely to overestimate in order to set a broad safety margin. That is to say, worst case scenarios and overestimations are not an issue since the overestimation is there with intention. Nevertheless, to make ERA a higher tier, besides presenting the worst-case scenario, the general status based on the time dependence of PhACs concentration or at least on mean MECs also should be researched and used when adequate. This would also consider

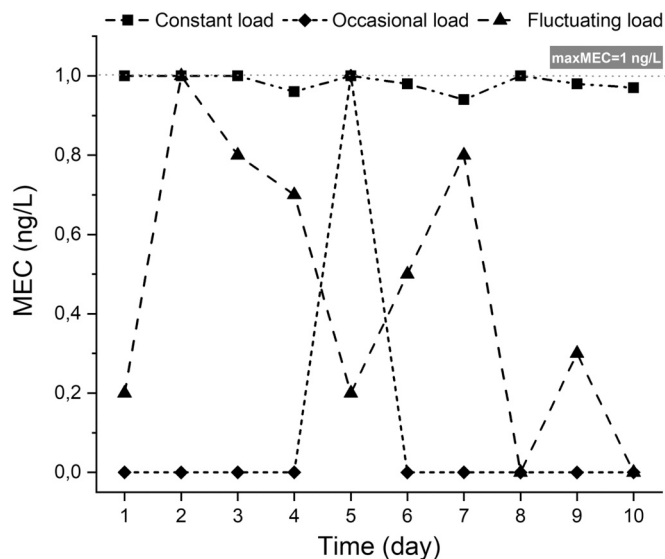


Fig. 1. Representation of change of the environmental load depending on the time in 3 different ways. Despite the various characteristic shape of the curves, all 3 load types can result the same maxMEC (1 ng/L).

the continuously changing environmental conditions (e.g., temperature, rainfall, UV radiation, etc.) that influence the MECs. However, a new scale of the RQ values (and so the risk levels) may be required to evaluate the general status properly. It should be noted that this approach is also influenced by uncertainties, for example, some PhACs are consumed all the days of the year, others are related to season diseases, furthers are occasionally. In this regard, timing and frequency of sampling influences the output of ERA.

2.6. Ignored mixture effects

ERA is primarily performed on single PhACs and ignores the probability of mixture effects. However, PhACs are known to never occur as a single compound but in multi-component/highly complex mixtures of chemicals in the aquatic environment (Liu et al., 2011; Maasz et al., 2019). A well-known toxicological phenomenon that a chemical mixture - regardless of the chemical composition, the exposed organism, and the considered biological endpoint - shows always a higher toxic effect than the individual effect of each of its components (reviewed by Kortenkamp et al. (2009)). That is to say, a mixture may induce a significant toxic effect, even if all individual components are placed at such low concentrations that individually do not have a significant toxicity. For example, a recent proof-of-principle study exposed pairs of fish to five synthetic steroid PhACs and demonstrated a significant mixture effect when each compound in the mixture was present at a concentration which on its own would not result a significant effect (Thrupp et al., 2018).

In consequence, ERAs of single PhACs ignoring possible effects of mixtures might systematically underestimate the real risks for the ecosystem. Despite this being obvious, ERA has been relying basically on investigation of the toxicity of single compounds for a long time. Here, we would like to emphasize that as the number of different mixtures of PhACs in the ecosystem can be considered essentially infinite, applying model predictions is indispensable. Of course, one can expect compounds with similar modes of action (MoA) to affect additively according to the conception of dose addition when present as a mixture, and indeed this has been established numerous times to be the case (e.g., Arrhenius et al., 2004; Brian et al., 2007; Hass et al., 2007). However, precisely because of the large number of drugs, one can also count on the presence of a mixture consisting of PhACs with diverse MoAs (e.g., will affect the same apical endpoint but do so via different pathways and/or different apical endpoints) in a given habitat (Thrupp et al., 2018). Clearly, performing ERAs that also take the interactions of several PhACs into consideration ("cumulative exposure assessment") is one of the greatest challenges in ecotoxicology. To note, approximate formulas such as concentration and response additivity model have already been developed for the complex mixture toxicity, however, they assume that there are no interaction between the PhACs present together, in the other words, that they do not influence each other's uptake, distribution or metabolism (De Zwart and Posthuma, 2005; Backhaus and Faust, 2012). For this reason, and because the experimental evidences are missing, the results of mixture risk assessment cannot be accepted with great certainty.

3. Conclusion

Existing ERA system of EU for human pharmaceuticals has played significant roles in enhancing environmental awareness of the pharmaceutical industries and in protecting environmental health. We agree with a broad body of scientific papers that the ERA procedures can be improved in order to better serve the intended purposes. The most urgent tasks are the improvement of modeling that could enhance the robust cross-chemical extrapolation, the improvement of management of ecotoxicological data, and the development of more appropriate formulae for mixture effect assessment. We hope that these thoughts will provide motivation for the scientific community to set about reforming

ecological risk assessment and that these suggestions can be helpful for countries which consider the development of ERA systems for human pharmaceuticals.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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