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Deriving predicted no-effect concentrations (PNECs) for emerging contaminants in the river Po, Italy, using three approaches: Assessment factor, species sensitivity distribution and AQUATOX ecosystem modelling

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ABSTRACT

Over the past decades, per- and polyfluoroalkyl substances (PFASs) found in environmental matrices worldwide have raised concerns due to their toxicity, ubiquity and persistence. A widespread pollution of groundwater and surface waters caused by PFASs in Northern Italy has been recently discovered, becoming a major environmental issue, also because the exact risk for humans and nature posed by this contamination is unclear. Here, the Po River in Northern Italy was selected as a study area to assess the ecological risk posed by perfluoroalkyl acids (PFAAs), a class of PFASs, considering the noticeable concentration of various PFAAs detected in the Po waters over the past years. Moreover, the Po has a large environmental and socio-economic importance: it is the largest Italian river and drains a densely inhabited, intensely cultivated and heavily industrialized watershed. Predicted no-effect concentrations (PNECs) were derived using two regulated methodologies, assessment factors (AFs) and species sensitivity distribution (SSD), which rely on published ecotoxicological laboratory tests. Results were compared to those of a novel methodology using the mechanistic ecosystem model AQUATOX to compute PNECs in an ecologically-sound manner, i.e. considering physical, chemical, biological and ecological processes in the river. The model was used to quantify how the biomasses of the modelled taxa in the river food web deviated from natural conditions due to varying inputs of the chemicals. PNEC for each chemical was defined as the lowest chemical concentration causing a non-negligible yearly biomass loss for a simulated taxon with respect to a control simulation. The investigated PFAAs were Perfluorooctanoic acid (PFOA) and Perfluorooctanesulfonic acid (PFOS) as long-chained compounds, and Perfluorobutanoic acid (PFBA) and Perfluorobutanesulfonic acid (PFBS) as short-chained homologues. Two emerging contaminants, Linear Alkylbenzene Sulfonate (LAS) and triclosan, were also studied to assess the performance of the three methodologies for chemicals whose ecotoxicology and environmental fate are well-studied. The most precautionary approach was the use of AFs generally followed by SSD and then AQUATOX, except for PFOS, for which AQUATOX yielded a much lower PNEC compared to the other approaches since, unlike the other two methodologies, it explicitly simulates sublethal toxicity and indirect ecological effects. Our findings highlight that neglecting the role of ecological processes when extrapolating from laboratory tests to ecosystems can result in under-protective threshold concentrations for chemicals. Ecosystem models can complement existing laboratory-based methodologies, and the use of multiple methods for deriving PNECs can help to clarify uncertainty in ecological risk estimates.

1. Introduction

Pollution is a major threat to aquatic ecosystems worldwide, impacting water quality and biodiversity and reducing the provision of ecosystem services which valuably contribute to human wellbeing (Millennium Ecosystem Assessment, 2005). Ecological risk assessment (ERA), the estimation of the risk posed by the presence of human-released chemicals to living organisms in ecosystems, is a fundamental step to guide management and inform policy towards sustainable solutions for mitigation of this threat. The basic steps of ERA include hazard identification, effects assessment, exposure assessment and risk characterization, where the main goal of the effects assessment is setting a safe threshold for the concentration of chemicals (Predicted No-effect Concentration, or PNEC), below which no adverse effects on ecosystem structure and functions are expected (De Laender et al., 2013; European Chemicals Bureau, 2003).

The foundation for ERA in the European Union is represented by several standardized procedures adopted for protecting ecosystems. The

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guidance for the implementation of the REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) regulation (REACH, EC, 2006), adopted to improve the protection of human health and environment from the risk posed by chemicals, and the Technical Guidance For Deriving Environmental Quality Standards as part of Common Implementation Strategy for implementation of the Water Framework Directive 2000/60/EC (European Community, 2000), provide a consistent approach to estimate ecologically-safe thresholds in aquatic ecosystems (ECHA, 2008; European Commission, 2011). Based on the abovementioned European regulations, PNECs can be derived in three manners: a deterministic approach based on the use of coefficients called assessment factors (AFs), a statistical approach based on the so-called species sensitivity distribution (SSD), and results from model ecosystems and field studies (European Commission, 2011). The most common approach is the use of assessment factors, where threshold exposure concentrations measured in the laboratory for individual species are extrapolated to populations in real-world ecosystems by dividing them by AFs, whose value depends on the amount and quality of available toxicity data (European Commission, 2011; Lei et al., 2010; Meli et al., 2014). When there is a sufficient amount of ecotoxicological data available for different taxa, the species sensitivity distribution (SSD) method is used instead (European Commission, 2011; Valsecchi et al., 2017). SSD is a cumulative probability distribution fitted to a set of toxicity thresholds for individual species of the ecosystem under the assumption that acceptable effects levels follow a certain distribution as a function of the concentration of the chemical (e.g. normal, logistic, triangle) and that the limited number of tested species is a random sample of the whole ecosystem (De Laender et al., 2013; Gao et al., 2014).

The AF and SSD methods rely on the assumption that ecosystem sensitivity to a given chemical can be related to the status of the most sensitive species, and that protecting ecosystem structure is enough to protect ecosystem functions too (Wright-Walters et al., 2011). However, population dynamics in polluted environments are not only driven by the direct toxicity effects of chemicals on single species, but also by ecological interactions between them and by the influence of abiotic factors (De Laender et al., 2007), therefore community- and ecosystem-level assessments could provide better indications of species' responses to chemicals than individual-level ones (Zhang et al., 2013). To assess ecological interactions, experimental ecosystems (microcosm, mesocosm and field enclosure studies), which can account for both direct and indirect effects of chemicals, have been used (De Laender et al., 2007, 2008a). Nevertheless, these methods are laborious, expensive and time-consuming, and the extrapolability of results to much more complex natural ecosystems, characterized by myriads of ecological interactions, remains uncertain (Lei et al., 2010; Naito et al., 2003; Zhang et al., 2013; Zhang and Liu, 2014). Considering that these methods cannot be used in the routine practice in lower tiers, there is a strong need for alternative approaches to extrapolate single-species effects information to ecosystem-level responses (De Laender et al., 2008b). Ecological models are cost-effective alternatives for ERA of toxic chemicals, providing rapid forecasting analyses, particularly under circumstances where field experiments cannot be conducted or experimental data are lacking (which is generally the case for the contaminants of emerging concern investigated here) (Grechi et al., 2016; Lombardo et al., 2015; Naito et al., 2003; Zhang and Liu, 2014).

Although several ecological models have been developed and reviewed for use in ERA for chemicals (Galic et al., 2010; Lei et al., 2008; Naito et al., 2003), mechanistic effects modelling has not been extensively used for regulatory purposes yet because of the lack of official guidance for models choice, development and use (Galic et al., 2010; Meli et al., 2014). Among the models used in ERA (De Laender et al., 2008b; Galic et al., 2010; Lei et al., 2008; Naito et al., 2003; Pereira et al., 2017; Zhang et al., 2013), the U.S. Environmental Protection Agency's AQUATOX, an aquatic ecosystem model, is one of the relatively few comprehensive and well documented models that have been designed specifically for environmental fate and ecological impact assessment of pollutants. AQUATOX simulates both abiotic and biotic (including trophic) processes as well as lethal and sub-lethal toxicant effects, and so it can depict the propagation of these effects through food webs and ecosystems (Lei et al., 2008; Park and Clough, 2014; Zhang and Liu, 2014).

Over the past decade, serious concerns have been raised regarding the presence of per- and polyfluoroalkyl substances (PFASs) in different environmental media, particularly water, in Northern Italy (Castiglioni et al., 2015; Loos et al., 2008; McLachlan et al., 2007; Squadrone et al., 2015; Valsecchi et al., 2017, 2015). Especially high PFAS concentrations were detected in the river Po when compared to other European rivers (McLachlan et al., 2007), confirming that the Po and its tributaries are highly polluted by different perfluoroalkyl acids (PFAAs) (Castiglioni et al., 2015; Loos et al., 2008; Valsecchi et al., 2015). Water quality management in the Po is a complex issue and matter for research, since this river crosses a densely inhabited, intensely cultivated and heavily industrialized watershed of about 71.000 km^2, representing one of the wealthiest areas of Europe whose human activities exert multiple large pressures on its high biodiversity (Grechi et al., 2016).

This work aims to assess the ecological risk posed by a few selected unregulated and emerging contaminants in the river Po by applying three methods for deriving PNECs: AF, SSD, and a novel method based on AQUATOX modelling. ERA is carried out for four PFAAs, two long-chained (perfluorooctanesulfonic acid PFOS, perfluorooctanoic acid PFOA) and two short-chained ones (perfluorobutanesulfonic acid PFBS, perfluorobutanoic acid PFBA). The AQUATOX model used here quantitatively simulates ecosystem functioning in the final lowland section of the Po River based on the extensive use of well-documented local data, and was previously calibrated against observations (Grechi et al., 2016). The goals of this work are to compute ecologically-safe thresholds (PNECs) for emerging contaminants in the Po River applying the two classical methods and the AQUATOX-based method proposed here, and then to compare the three methods highlighting their advantages and drawbacks for deriving PNECs for emerging contaminants in rivers. To better contribute to the discussion on the tools to use for the future regulation of contaminants of emerging concern, the three ERA methods were also applied to two well-studied personal care products, linear alkylbenzene sulfonate and triclosan, which had already been investigated by Grechi et al. (2016) using the AQUATOX Po model.

2. Materials and methods

2.1. Study area: the River Po and its biota

The Po is the longest river (652 km) in Italy, with the greatest average discharge (1470 $m^3 s^{-1}$). It flows through the entire northern Italy, and its drainage area covers about one fourth of Italy's surface, including the main industrial and most populated areas where nearly one third of all Italian population lives (Valsecchi et al., 2015). In this study the most representative species and taxa present in the lower stretch of the Po River were considered following the selection by Grechi et al. (2016) (Table 1).

The anthropogenic substances discharged to the river from its watershed exert high pressures not only on its water quality and ecological status, but also on downstream ecosystems: the Po freshwater discharge, which summed to those of other smaller Northern Italian river is about 20% of the river runoff into the whole Mediterranean Sea, carries large nutrient loads which caused severe eutrophication in the Adriatic Sea coastal zone some decades ago (Barausse et al., 2011; Vollenweider et al., 1992). The biomonitoring and ecosystem modelling efforts made for this river in the past (Grechi et al., 2016) make it an ideal study case to assess the ecological risk due to emerging contaminants such as PFAAs and to understand how the outcomes of ERA depend on the methodology chosen to quantify ecologically-safe chemicals thresholds.

Table 1
Po River taxa considered in this study.

	Po River taxa
Phytoplankton	Cyclotella
	Chromulina
Zooplankton	*Brachionus calyciflorus*
Macroinvertebrates	Amphipoda (*Echinogammarus*)
	Diptera (*Chironomus*)
	Oligochaeta
	Trichoptera (*Hydropsychidae*)
	Gastropoda
	Odonata - nymphs
Fish	Bleak (*Alburnus alburnus*)
	Chub (*Leuciscus cephalus*)
	Wels catfish (*Silurus glanis*) - adult and juvenile

2.2. Assessed contaminants

2.2.1. PFAAs

Due to their unique physico-chemical properties, PFASs have been widely used in industrial processes, but also represent a health and ecological threat because their high chemical stability and inertness make them resistant to hydrolysis, photolysis and microbial degradation, and consequently highly persistent and widespread in the environment (Squadrone et al., 2015; Valsecchi et al., 2015). PFASs have been employed from the 1950s in various industrial processes and products such as surface treatment of textiles and papers, building paints, cosmetics, insecticides, firefighting foams and fluoropolymer production (Castiglioni et al., 2015; Valsecchi et al., 2015; Zareitalabad et al., 2013). PFASs include thousands of chemicals, but environmental impact assessment studies mainly concentrate on perfluoroalkyl acids (PFAAs), mostly perfluoroalkylsulfonic acids (PFSAs) and perfluoroalkylcarboxylic acids (PFCAs) (Castiglioni et al., 2015; Valsecchi et al., 2015). Being primarily emitted to surface waters, water is the largest reservoir of PFAAs in the environment and the most important medium for their transport (McLachlan et al., 2007; Zareitalabad et al., 2013; Valsecchi et al., 2015).

Data on the presence of four different PFCAs in 14 major European rivers showed that the highest concentrations were detected in the river Po (McLachlan et al., 2007); further monitoring campaigns confirmed that the Po and its tributaries are highly polluted by different PFAAs, sometimes in concentrations above 6000 ng/L (the maximum reported PFAA concentration was 6480 ng/L for PFOA in Valsecchi et al., 2015) (Castiglioni et al., 2015; Loos et al., 2008; Valsecchi et al., 2015).

The European Commission recently added PFOS to the List of priority substances, identifying it as a “priority hazardous substance”, through Directive 2013/39/EC (The European Parliament and the Council of the European Union, 2013), with an annual average concentration of 0,65 ng/L as Environmental Quality Standard (EQS) for inland surface water (freshwater). From June 2013, PFOA is on the Candidate List of substances of very high concern for Authorisation, in accordance with the REACH regulation, as a PBT (persistent, bioaccumulative and toxic) and CMR (carcinogenic, mutagenic and toxic for reproduction) substance, but at the moment, there are still no established environmental thresholds (ECHA, 2013). In 2017, two other PFASs entered this list, Nonadecafluorodecanoic acid (PFDA) and its sodium and ammonium salts (ECHA, 2017a), and Perfluorohexane-1-sulphonic acid (PFHxS) and its salts (ECHA, 2017b), while PFOA and its salts entered the Restricted list (ECHA, 2017c). PFOS and PFOA have been usually detected as the main PFASs in environmental compartments worldwide through the past decades (Zareitalabad et al., 2013; Pierre and Riess, 2015; Valsecchi et al., 2017, 2015; Xiao, 2017), so their ecotoxicology and physico-chemical properties are the most researched among PFASs and they were selected as representatives of the long-chained PFAAs, while PFBA and PFBS were selected as their common short-chained industrial substitutes (Smith et al., 2016).

2.2.2. LAS and triclosan

Linear alkylbenzene sulfonate (LAS) is an anionic surfactant introduced in 1964 as the readily biodegradable replacement for highly branched sulfonates. LAS is one of the most used anionic surfactant detergents, with major domestic applications and minor industrial ones, commonly found in wastewater (Oliver-Rodríguez et al., 2015).

Triclosan (TCS) is an antimicrobial agent frequently used in various personal care products, usually ending unchanged in the aquatic environment after passing through wastewater treatment plants. Contamination by TCS in surface waters has been reported worldwide as an emerging issue considering its high bioaccumulation potential to non-target organisms in aquatic environments.

Among emerging contaminants, both LAS and TCS are well-known and ubiquitous (Grechi et al., 2016; Guo and Iwata, 2017). The overall freshwater PNEC for LAS derived under REACH is 268 µg/L (ECHA, 2017d) and 843 ng/L for TCS (ECHA, 2017e). Since the AQUATOX Po model had already been used to conduct ERA for LAS and TCS (Grechi et al., 2016), both were included in this study to test the novel ecosystem modelling method for deriving PNECs and compare it to regulated methodologies.

2.3. Deriving PNECs with assessment factors

For all selected contaminants, available toxicity data were collected from the ECOTOX EPA database (U.S. EPA, 2000) and scientific literature, and then associated to the taxa found in the River Po to single out the species representative of the local aquatic ecosystem (Xu et al., 2015). Given the overall lack of toxicity data for non-standard test species, a biological read-across approach was applied: species with available toxicity data and belonging to the same taxon or living in similar natural habitats as the Po taxa, with similar size and diet, were selected in the database, then the Po taxa were assigned the same ecotoxicological endpoint values under the assumption of similar biological response to the given chemical (Grechi et al., 2016; Lombardo et al., 2015; Rand-Weaver et al., 2013). Toxicity data for all six contaminants are available in the Supplementary material (Tables S1–S6). Afterwards PNECs were computed following the European Technical Guidance Document (TGD) for deriving EQS, using the Annual Average Quality Standard (AA-$QS_{fw,eco}$) methodology (protection against the occurrence of prolonged exposure) based on direct ecotoxicity for protection of the pelagic species (equivalent to the REACH guidelines for deriving PNECs for freshwater species) (ECHA, 2008; European Commission, 2011). So, different assessment factors were used according to the availability of long- or short-term toxicity data for three different trophic levels (fish, invertebrates - preferably *Daphnia*, algae).

2.4. Deriving PNECs using species sensitivity distribution

The four steps required for effects risk assessment with SSD include screening of toxicity data, selecting the distribution model, fitting the SSD curve, and calculating the values of hazardous concentration (HC) and PNEC to quantify the ecological risk (Gao et al., 2014). To derive PNECs with this method, the available toxicity data for Po and read-across species were analyzed and adapted to the method requirements. Ideally, the dataset for the SSD method should be statistically and ecologically representative of the community of interest and, when the problem is in the lower concentration range, the SSDs used to derive PNECs should be based on chronic ecotoxicity data, preferably no-observed effect concentrations (NOECs). Accordingly, at least eight species covering different taxonomic groups are desirable, with preferably 10–15 NOECs (Amiard and Amiard-Triquet, 2015; ECETOC, 2014; ECHA, 2008). When equivalent ecotoxicological data were available, obtained from the same test conditions on the same endpoint and species, the geometric mean was used as input for calculations (ECHA, 2008). When data were available for multiple time points, the longest time point was used (Tenbrook et al., 2010).

Since chronic toxicology studies were not available for all tested species, acute ecotoxicological data needed conversion to NOECs. Extrapolation was made by using acute-to-chronic ratios (ACRs). For LAS and triclosan, ACRs were accepted from ECETOC (2003), proposing EC50/NOEC = 2 and LC50/EC50 = 1,7 for LAS and EC50/NOEC = 2 and LC50/EC50 = 3,9 for triclosan. ACRs for PFAAs were unavailable in the literature, so they were derived according to ECETOC guidelines (ECETOC, 2003). All species with both acute and chronic ecotoxicological data available for the same PFAA were used for linear regression and the resulting slopes of 1,85 and 1,6 were used as EC50/NOEC and LC50/EC50 ratios for all PFAAs toxicity data conversions.

Both chronic and acute toxicity data were lacking for short-chained PFAAs (PFBA and PFBS), so an estimation based on interspecies correlation was made. To estimate the acute toxicity of PFAAs to untested species that were taxonomically too far for using the read-across approach, the Web-based Interspecies Correlation Estimation (ICE) model was used in the SSD mode for aquatic species (U.S. EPA, 2016a). Web-ICE estimates the acute toxicity of a chemical to a species, genus or family with no test data, by using acute ecotoxicological data available for more commonly tested surrogate species (Raimondo et al., 2010). The outputs of the ICE model were EC50s of all species, then local or read-across species of the Po river were selected (Tables S7 and S8 in the Supplementary material).

SSD curves were derived by using the lognormal statistical distribution, a simple and accepted model, allowing comparison with other studies (Gao et al., 2014; Xu et al., 2015). Curves were fitted using the SSD CADDIS generator (log-probit distribution) developed by U.S. EPA (2016b).

SSDs are used to estimate the concentration that should be protective for the most of the species in the ecosystem. PNEC values are usually taken to correspond to HC5 (5% hazard concentration), the 5th percentile of the distribution, a concentration that should protect 95% of the species in the ecosystem (Amiard and Amiard-Triquet, 2015; European Commission, 2011; Gao et al., 2014). Although SSD modelling explicitly deals with differences in sensitivity between species, and SSDs can be constructed only when data are plentiful, HC5 should be divided by an additional AF to account for remaining uncertainties in the estimated threshold (the highest AF = 5 is the default value) (Amiard and Amiard-Triquet, 2015; ECHA, 2008; European Commission, 2011).

2.5. Deriving PNECs using AQUATOX

2.5.1. The AQUATOX model

US-EPA AQUATOX 3.1 (Park and Clough, 2014) is an integrated ecological and ecotoxicological process-based model intended for use in prospective ERA to predict the fate of nutrients, organic chemicals and toxicants in aquatic ecosystems and their direct and indirect effects on living organisms. In this study, the calibrated Po River AQUATOX model developed by Grechi et al. (2016) was used to predict the fate and effect of different concentrations of LAS, triclosan and PFAAs in a segment of the Po River, stretching from the closing section of the catchment to the beginning of the branching section of the delta, modelled as a continuous stirred-tank reactor. The simulated food web is depicted on Fig. S2 (Supplementary material), and technical information about the ecosystem model parameterization are in Grechi et al. (2016). The impact of each chemical was evaluated in terms of variation of biota biomass density between control (i.e. without pollutants) and perturbed (i.e. with pollutants) model runs. To simulate PFAA behavior, the related AQUATOX sub-model (Park and Clough, 2014) was used (Section 2.5.1.2): to our knowledge, this is its first published application in a peer-reviewed international journal (but see Park et al., 2007, and Rashleigh et al., 2010).

2.5.1.1. Parameters of the chemicals. Input parameters for organic chemicals in AQUATOX, governing their fate and partitioning, include chemicals' properties (i.e. physico-chemical parameters describing fate processes such as ionization, volatilization, hydrolysis, photolysis, sorption and microbial degradation), ecotoxicity data for the modelled organisms, initial concentrations and loading from upstream. The required ecotoxicity endpoints are LC50 and EC50 (on growth and reproduction) for every consumer species, and LC50 and EC50 (on photosynthesis) for every primary producer (Park and Clough, 2014). For our purpose of simulating the constant chronic exposure to selected chemicals, the initial concentration and the inflow concentration (from upstream) were kept equal, and inflow concentration was constant during the simulation. The main physico-chemical and ecotoxicological parameters of LAS and TCS are in the Supplementary material (Tables S21, S23 and S24).

2.5.1.2. PFAA submodel. The characteristics and environmental behavior of PFAAs distinguish them from other organic contaminants, and the increased public interest following their widespread appearance in different environmental compartments, particularly aquatic ecosystems, has led to the development of a PFAA submodel in AQUATOX. The submodel has different inputs regarding physico-chemical parameters, e.g. it does not use the octanol-water partitioning coefficient for bioaccumulation, sorption and in-water mobility predictions, due to PFAAs non-typical lipid partitioning dynamics, their persistence to biodegradation in water or soil, and resistance to hydrolysis and photolysis (Smith et al., 2016). This resulted in a different approach for calculating sorption, biotransformation, bioaccumulation, uptake and depuration in the submodel, and in computing bioaccumulation factors through empirical equations based on chain length and type of terminal functional group. Since PFAAs do not follow the typical pattern of partitioning and accumulation into fatty tissues, but tend to bind to proteins, their kinetics calculations did not include the fraction of lipids in organisms (Martin et al., 2003; Park and Clough, 2014). Sorption is modelled through an empirical approach requiring the organic matter partition coefficient for sediments, calculated by dividing the soil organic carbon–water partitioning coefficient by the fraction of organic matter in detritus, considering that PFAAs sorption depends on the fraction of organic carbon in the sorbent (Asante-Duah, 1995; Park and Clough, 2014; Smith et al., 2016). Sorption to primary producers, simulated in AQUATOX through bioconcentration factors (BCFs) for Algae and for Macrophytes, can also be modelled empirically (Park and Clough, 2014), but in this study BCFs were taken from literature.

The physico-chemical parameters for the chosen PFAAs in AQUATOX are shown in Table S22 (Supplementary material). Species with available toxicity data for PFOS, PFOA, PFBS and PFBA were associated to the taxa modelled in AQUATOX; considering the scarcity of data, a read-across was performed, as well as conversions using ACR and LC50/EC50 ratios depending on data availability. Interspecies Correlation Estimation for PFBS and PFBA was used as described in Section 2.4. PFAAs ecotoxicological parameters for the modelled species are on Tables S25–S28 (Supplementary material).

2.5.2. Deriving ecologically-safe thresholds using AQUATOX

To represent ecosystem seasonality, the Po River model had been stabilized and calibrated by Grechi et al. (2016) for a period of one year, so here every simulation was run for one year, with daily time steps. Moreover, a one-year simulation makes the AQUATOX methodology comparable to regulated ones, as chronic exposure is estimated there as annual average (European Commission, 2011). The objective was to develop a methodology to derive PNEC based on the biomass density (mg_{dry}/L or g_{dry}/m^2) of each modelled taxon, by deciding what is a non-negligible biomass loss negatively affecting the corresponding population(s). The cut-off value proposed by the few available publications dealing with a similar issue (De Laender et al., 2008b, 2008c; Lei et al., 2010; Naito et al., 2003; Zhang et al., 2013; Zhang and Liu, 2014) was based on the claim of Suter (1992) that a 20% reduction is

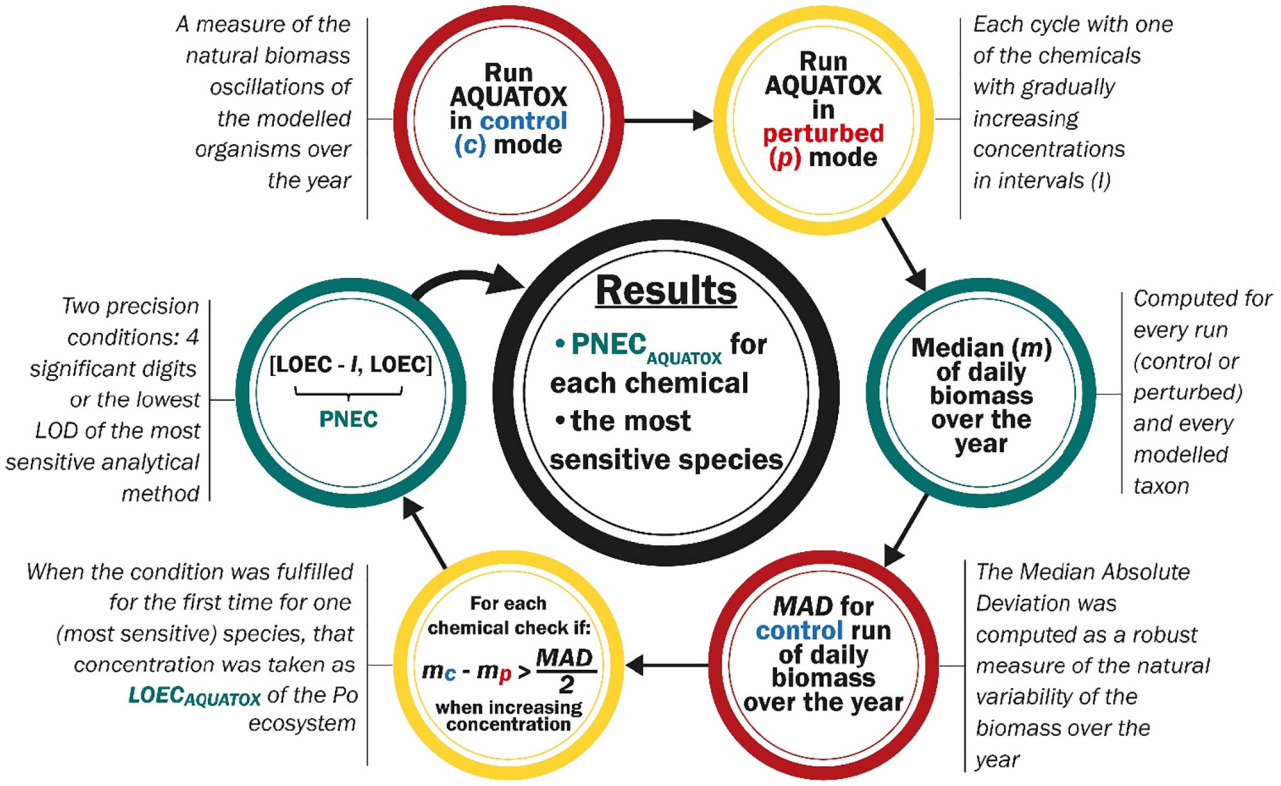

Fig. 1. Scheme of the developed methodology for deriving PNEC through AQUATOX ecosystem modelling.

the minimum detectable difference in population biomass in the field. However, this approach can be criticized since not all populations are equally sensitive to a 20% biomass reduction (which may not affect the viability of one population, but it could lead to local extinction of another, e.g. if its biomass distribution over the year generally displays very low values except for a single, short-lived but large bloom), which is thus arbitrary. Furthermore, we see no reason to define an ecologically safe threshold of biomass change based on field detectability when using a mechanistic model that can keep track of arbitrarily small biomass changes. Therefore, we proposed an alternative methodology to derive a taxon-dependent threshold (Fig. 1):

1) AQUATOX was run in control mode (with no chemicals), taken as a measure of the natural biomass oscillations of modelled organisms over the year. Simulation plots are provided on Fig. S1 (Supplementary material);
2) Perturbed runs (with one chemical: LAS, TCS, PFOS, PFOA, PFBS or PFBA) were performed for gradually increasing chemical concentration, decided in accordance with previously proposed overall PNECs (ECHA, 2017d, 2017e; Valsecchi et al., 2017). Perturbed runs for LAS were from 0 (control) to 500 µg/L with increments of 10; TCS: from 0 (control) to 2 µg/L with increments of 0,05; PFOS: from 0 (control) to 0,01 µg/L with increments of 0,0001 and from 0,01 to 2 µg/L with increments of 0,01; PFOA: from 0 (control) to 3000 µg/L with increments of 200 and from 3000 to 6000 µg/L with increments of 100; PFBS: from 0 (control) to 10 µg/L with increments of 0,5, from 10 to 5000 µg/L with increments of 10 and from 5000 to 30'000 µg/L with increments of 500; PFBA: from 0 (control) to 2000 µg/L with increments of 50;
3) For every run (control or perturbed), the median of daily biomass values over the year was calculated for every modelled taxon. The median was preferred to the mean as a measure of typical annual biomass, being less sensitive to the short-lived blooms characterizing several simulated taxa over the year (Legendre and Legendre, 1998) (Fig. S1, Supplementary material);
4) For the control run, the median absolute deviation of daily biomass values (*MAD*) was computed as a robust measure of the natural variability of the biomass across the year. *MAD* was preferred to the standard deviation due to its insensitivity to outliers and since the yearly biomass distribution could not generally be approximated by a normal distribution (Legendre and Legendre, 1998);
5) The differences between the median biomass of the control (m_c) and the medians of the perturbed runs ($m_{p,i}$, for every ith concentration) were calculated for every modelled taxon and compared to half of *MAD* for the control. So, the decrease in the typical biomass (represented by the median) of a taxon over the year due to the increased toxicant concentration was compared to the natural biomass variability level over the year. On the first occasion that, following an increase in toxicant concentration, the following condition was fulfilled for a taxon

$$m_c - m_{p,i} > \frac{MAD}{2} \quad (1)$$

the decrease in median biomass was considered larger than a natural biomass change for that taxon and, hence, significant: that concentration can be considered as the Lowest Observed Effect Concentration (LOEC). The rationale is that, for taxa with nearly-constant biomass over the year (low *MAD*), even a small biomass decrease indicates a significant deviation from usual conditions, while for naturally highly-oscillating taxa (high *MAD*), only a large biomass decrease represents a significant deviation given the characteristic variability;

6) Following REACH (ECHA, 2008) and TGD guidelines (European Commission, 2011), and the perspective that an ecosystem can be considered as sensitive as its most sensitive species, the LOEC of the most sensitive taxon according to the simulations was taken as the LOEC of the Po river ecosystem;
7) After finding LOEC, the highest concentration not fulfilling Eq. (1) was sought in the interval [LOEC - I_j, LOEC], where I_j is the

increment of the j^{th} contaminant, and that highest concentration was taken as the Po ecosystem PNEC. Two conditions were applied to decide the precision in searching for PNEC. One was the limit of the detection (LOD), i.e. the current precision of the most sensitive analytical method for determining each chosen contaminant in surface water. The other was a maximum of four significant digits for PNEC.

3. Results and discussion

3.1. Assessment factor method

To derive PNEC for LAS, two long-term results (NOECs) from species representing two trophic levels (fish and/or *Daphnia* and/or algae) were available, implying the use of AF = 50. Such AF was applied to the lowest NOEC of *Crytophycophyta* (140 μg/L), yielding PNEC = 2,8 μg/L. In comparison to the overall freshwater PNEC = 268 μg/L given by ECHA (2017d), the PNEC for the Po river ecosystem was two orders of magnitude stricter.

For triclosan, long-term results (NOECs) from at least three species (fish, *Daphnia*, algae) representing three trophic levels were available, leading to AF = 10. The lowest NOEC referred to *Chlamydomonas* sp. (0,015 μg/L), so PNEC in the Po was 0,0015 μg/L after dividing by AF, again stricter than the overall freshwater PNEC given by ECHA (0,843 μg/L) (ECHA, 2017e).

The same condition of three available long-term results was met for both PFOS and PFOA, so AF = 10 was applied to the lowest NOECs. For PFOS, the lowest NOEC was 49 μg/L (*Chironomus tetans*), so PNEC = 4,9 μg/L was computed for the Po. The recent PFOS EQS dossier (European Commission Subgroup on Review of the Priority Substances List, 2011) estimated a freshwater PNEC of 0,65 ng/L, and Qi et al. (2011) indicated an aquatic toxicity threshold for PFOS from 0,61–6,66 μg/L. NOEC for *Brachionus calyciflorus* was used for PFOA (4000 μg/L), leading to PNEC = 400 μg/L for the Po. The overall PNEC for freshwater derived using mesocosm studies on male plasma concentrations of the fish *Pimephales promelas* (Valsecchi et al., 2017) was 30 μg/L for PFOA, but given the uncertain ecological relevance of this endpoint and the unclear implications for population mortality, growth or reproduction, we did not consider this test result as applicable and did not include it in Table S4 (Supplementary material).

Without extrapolation of results by using Interspecies Correlation Estimation, AF would have been the only feasible method for deriving PNEC for PFBS and PFBA. For PFBS, two long-term results (NOECs) from species representing two trophic levels (fish and/or *Daphnia* and/or algae) were available. However, the additional criterion that the trophic level of the NOECs includes the trophic level of the lowest acute L(E)50 was not met: in this case, the application of AF = 100 to the lowest L(E)50 is recommended by TGD if the lowest L(E)50 is lower than the lowest NOEC. EC50 = 450'000 μg/L for *Danio rerio* (read-across species substitute for *Gobio gobio*) was used for deriving PNEC for PFBS in the Po, which was 4500 μg/L. The overall PNEC proposed by Valsecchi et al. (2017) for PFBS was 372 μg/L, the difference mainly coming from the use of AF = 1000 on different species. For PFBA, AF = 1000 was used, as at least one short-term L(E)50 from each of the three trophic levels was available. There were no available long-term toxicity data for any of the Po species, and the lowest LC50 was 110'000 μg/L (*Brachionus calyciflorus*), giving PNEC = 110 μg/L after dividing by AF. The same value for the overall PNEC for PFBA was proposed by Valsecchi et al. (2017).

3.2. SSD method

SSD curves with the corresponding HC5s are in Fig. 2, while statistical details are in Tables S9–S20 (Supplementary material). To derive PNECs, AF = 5 was applied to all HC5s, being no reasons to reduce AFs according to the TGD guidelines (European Commission, 2011). The resulting PNECs for the Po River ecosystem were 42,33 μg/L for LAS, 0,0026 μg/L for TCS, 15,95 μg/L for PFOS, 1065,70 μg/L for PFOA, 5665,84 μg/L for PFBS and 1417,83 μg/L for PFBA.

3.3. AQUATOX model

LOECs and PNECs for each contaminant in the Po River ecosystem determined using AQUATOX are on Table 2. Fig. 3 shows the yearly biomass variations in the control and perturbed modelling scenarios for the most sensitive organisms indicated in Table 2. For all chemicals, PNECs were determined with four significant digits (LODs were much lower) except PFOS, whose value was determined using the smallest known LOD, which is 0,00003 μg/L (Gallen et al., 2014).

By modelling physical, chemical, biological and ecological processes, AQUATOX allowed us to reconstruct the fate of the chemicals in the Po ecosystem. To do this, we computed a mass balance for each chemical in the model. For all chemicals, the input to the modelled system took place through water advection from upstream, washout of the chemical mass with the outflowing water being the only loss from the system with the exception of LAS, where microbial degradation between 10 and 35% of the total mass loss per day occurred. Both of these model features are consistent with literature, PFAAs being resistant to degradation under environmental conditions (HERA, 2013; Smith et al., 2016), but inconsistent with laboratory experiments showing that TCS is subject to photolysis (Health Canada, 2012). The modelled mass of the chemicals in the system was computed as the difference between input and loss. Modelling results showed that more than 98% of the mass of the chemicals was dissolved in the water on every daily time step but for TCS, whose mass in the water varied between 78% and 100% as a consequence of its uptake by microalgae, up to 9% in the growing season, and of its almost- constant mass in animals ranging from 7 to 9% of the total mass in the system over the year. This result can be explained by the high bioaccumulation of TCS, particularly in fish (Table S24, Supplementary material). While BCFs were entered manually for LAS and TCS, BCFs were estimated by the chain-length and functional group dependent equations for PFAAs, and were equal for all organisms in the food web for each PFAAs. The computed BCFs were 1482 L/kg for PFOS, 4,4 L/kg for PFOA and less than 0,03 L/kg for both PFBS and PFBA, approximately correspondent to the average of the few available experimentally-derived values (Smith et al., 2016). Regarding the total modelled chemicals mass in biota, most of the TCS and LAS mass was present in the primary producers (algae), while PFAAs were almost entirely in animals, especially PFOS with almost 99%. In agreement with the used BCFs most of the total modelled mass of LAS and TCS in animals was contained in fishes, while for PFAAs most of the mass was predicted in primary and secondary consumers (i.e. Rotifers, Oligochaeta, Caddisfly, Odonata).

3.4. Comparison of the methods

The computed PNECs differed across contaminants and depended on the methods employed to derive them (Table 3). The AF method always gave the lowest, most precautionary PNEC but for PFOS, for which the AQUATOX-derived PNEC was very small (the ratio of $PNEC_{AF}$ and $PNEC_{SSD}$ to $PNEC_{AQUATOX}$ was about 200'000 and 700'000, respectively). However, $PNEC_{SSD}$ and $PNEC_{AF}$ were generally of the same or similar order of magnitude, with their ratio ranging from 1,3–15. AQUATOX tended to give the highest, least precautionary PNEC except for PFOS, as mentioned, and PFBA, for which $PNEC_{AQUATOX}$ was slightly lower than $PNEC_{SSD}$ (for PFBA, $PNEC_{AQUATOX}$:$PNEC_{SSD}$ = 0,78, $PNEC_{AQUATOX}$:$PNEC_{AF}$ = 10): in all other cases, the ratio of $PNEC_{AQUATOX}$ to $PNEC_{AF}$ or $PNEC_{SSD}$ ranged from 2,6–167 and from 2 to 96, respectively. However, $PNEC_{AQUATOX}$ and $PNEC_{SSD}$ were generally of the same order of magnitude (the only exceptions were TCS and PFOS), unlike when comparing AQUATOX to the AF method.

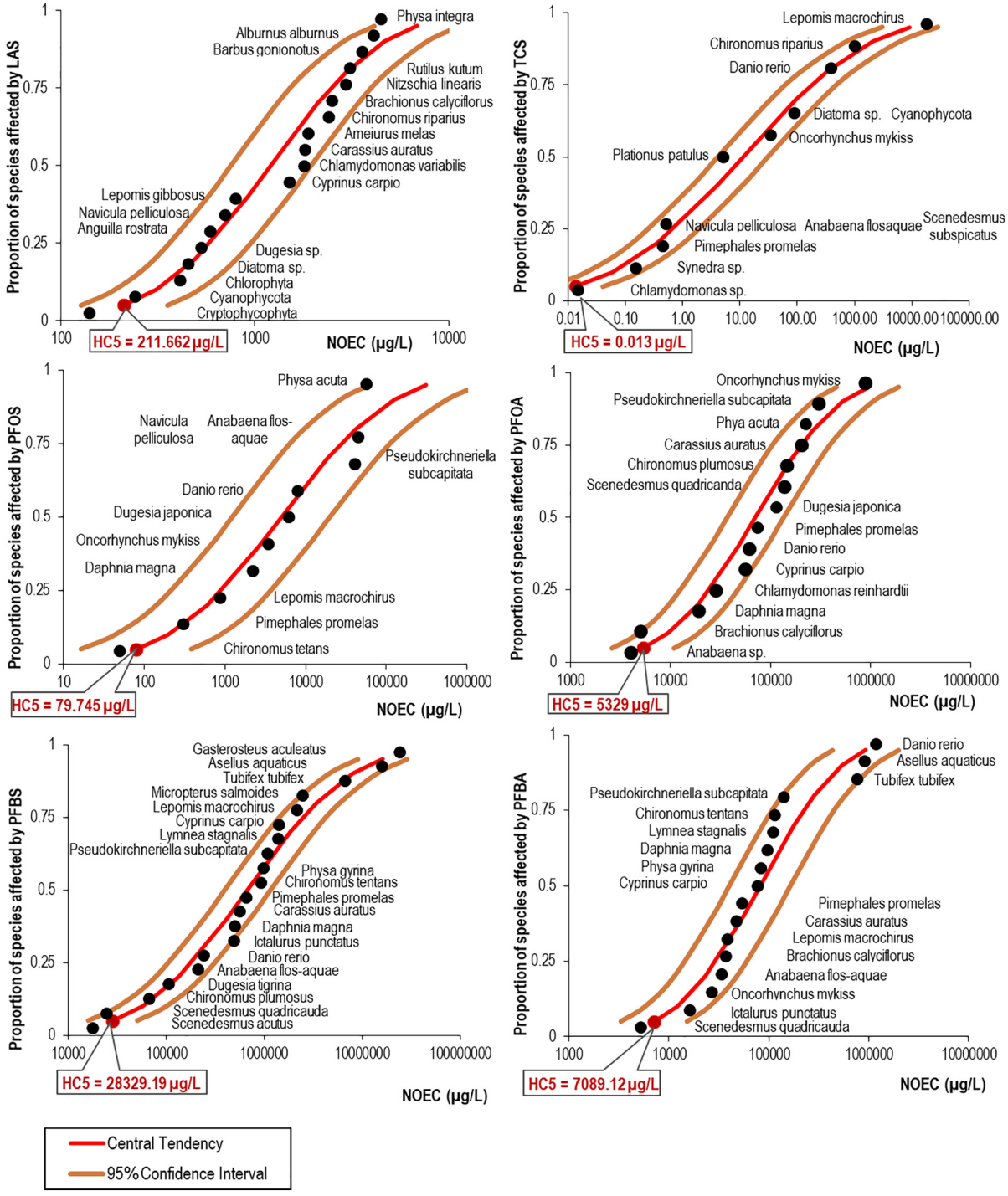

Fig. 2. Fitted SSD curves for long-term toxicity data (NOECs) of the Po river taxa, for each contaminant.

Table 2
LOECs and PNECs resulting from the AQUATOX ERA methodology.

	LAS	TCS	PFOS	PFOA	PFBS	PFBA
LOEC (µg/L)	190	0,30	0,0001	2600	12'000	1150
PNEC (µg/L)	187,0	0,2502	0,000023	2546	11'620	1102
Most sensitive taxon	Caddisfly, Trichoptera	Caddisfly, Trichoptera	Caddisfly, Trichoptera	Caddisfly, Trichoptera	Odonata	Caddisfly, Trichoptera

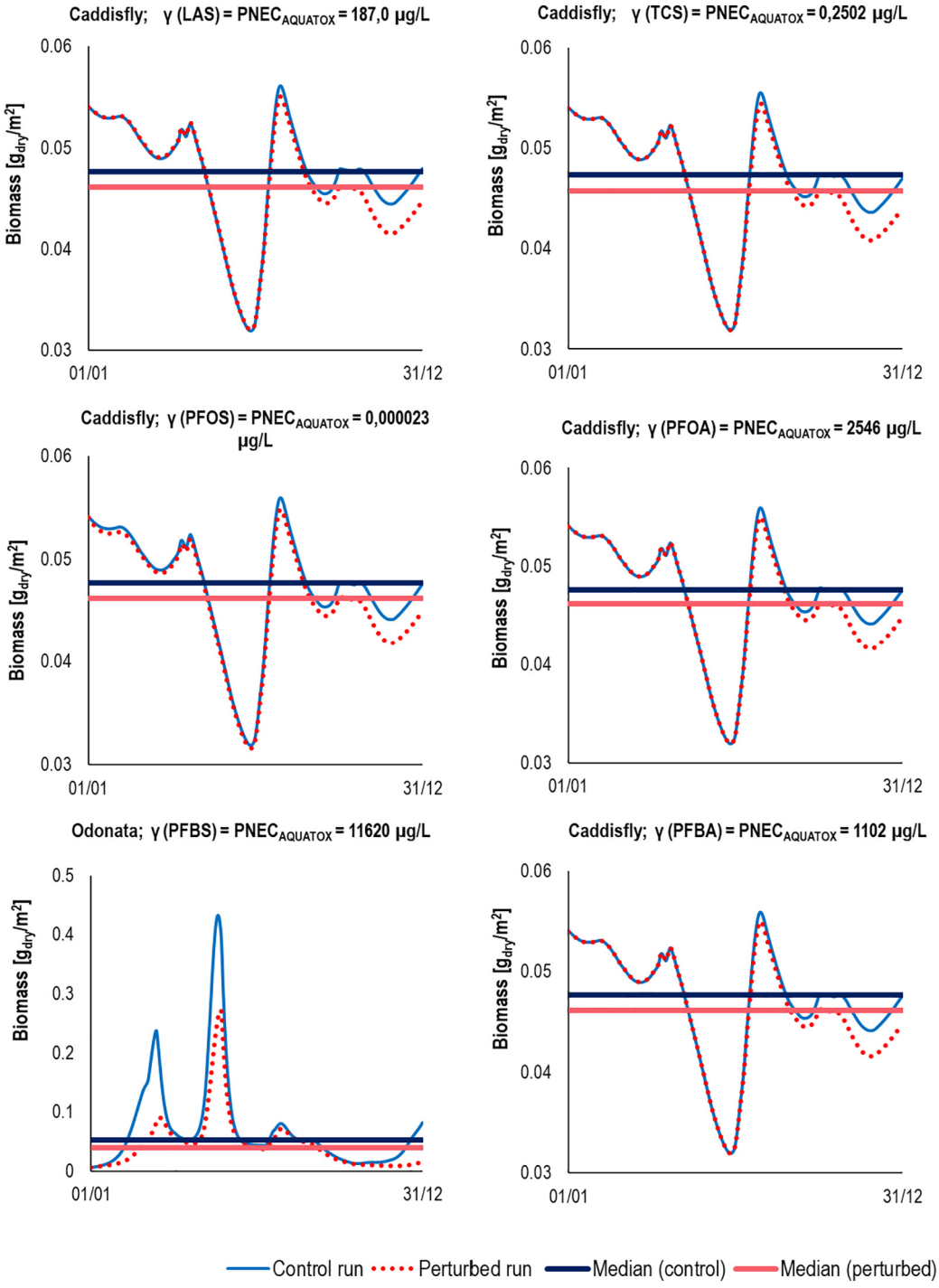

Fig. 3. Control and perturbed biomass changes in AQUATOX over the simulated year, for contaminant concentration equal to the PNEC derived using AQUATOX.

Table 3
Comparison of the derived PNECs for all contaminants and methods.

	LAS	TCS	PFOS	PFOA	PFBS	PFBA
$PNEC_{AF}$ (µg/L)	2,8	0,0015	4,9	400	4500	110
$PNEC_{SSD}$ (µg/L)	42,33	0,0026	15,95	1065,70	5665,84	1417,83
$PNEC_{AQUATOX}$ (µg/L)	187,0	0,2502	0,000023	2546	11'620	1102

While it is intuitive that the AF method is the most precautionary, being based on the protection of the most sensitive species and on the use of very large factors when there is scarcity of data (particularly for chronic toxicity), there are many explanations for the other differences in the order of magnitude and (sometimes) rank of the estimated PNECs across methodologies and contaminants. One is the different data needs of the three methods: for example, extrapolation was employed to fulfill the data requirements of SSD and AQUATOX (either just as ACRs or using both ACRs and ICE for PFBS and PFBA), and consequently not entirely the same ecotoxicological data were used in all methods (Sections 2.3, 2.4 and 2.5). Another explanation is the submodel that AQUATOX uses to model internal toxicity (Park and Clough, 2014) which may introduce additional, hard-to-assess bias in the comparison. Finally, the three methods are based on radically different assumptions: the AF and AQUATOX methods aim to protect the most sensitive species (and with it, the whole community), SSD just a major fraction of the

community, and both AF and SSD methods are based on laboratory-derived toxicity data but ignore sub-lethal effects of chemicals as well as ecological interactions between species, and between species and abiotic factors (European Commission, 2011; Wright-Walters et al., 2011). AQUATOX simulates the impact of a chemical not only in terms of lethal toxicity (a direct effect), but also of sublethal toxicity (e.g. how the presence of a toxicant can impair physiological rates such as growth, consumption or reproduction) and indirect effects (Ulanowicz, 2009) triggered by the input of that chemical (e.g., predator-prey interactions). So, it simulates the mediating role of ecological processes, such as predation, which can propagate the effect of toxicity to other taxa in the ecosystem in noticeable and even counterintuitive manners (Grechi et al., 2016; Lombardo et al., 2015; Niu et al., 2016; Zhang and Liu, 2014). In AQUATOX the different toxicity modes of action of contaminants may also become relevant, as they determine which biological processes or functional groups are most impacted and how toxicity effects propagate throughout the food web (Lombardo et al., 2015). The level of aggregation of the food web modelled in AQUATOX, which does not simulate just single species, and the few taxa selected for modelling (out of the many found in the Po river) are also a potential, well-known source of bias in food web models whose impact is however difficult to quantify without complex investigations (Abarca-Arenas and Ulanowicz, 2002; Pinnegar et al., 2005; Ulanowicz, 2009).

Parallels can be drawn between our AQUATOX-based approach and traits-based approaches recently introduced into ERA (Rubach et al., 2011), proposing that the ecotoxicological effects of a chemical determining the vulnerability of a population can be linked to species traits, where a trait can be defined as a "phenotypic or ecological character of an organism, generally measured at the individual level, but often applied as the mean state of a species". AQUATOX can simulate several ecological and ecotoxicological processes and factors, such as population growth, ingestion, trophic position and food preference, habitat choice, dispersal, toxicokinetics processes, etc. which can be related to traits (see Rubach et al., 2011). For example, bioaccumulation modelling in AQUATOX requires mean individual wet weight and lipid fraction, which are key traits determining the sensitivity of species to the bioaccumulation of chemicals (Baird and Van den Brink, 2007; Park and Clough, 2014; Rubach et al., 2011). Thus, AQUATOX represents a tool for quantitatively testing the strength of the relationship between species traits and vulnerability to chemicals in complex, ecologically realistic conditions, keeping in mind the well-known limitations of ecological models, which are simplified descriptions of real processes and ecosystems, and whose reliability depends on the quantity and quality of input data (Jorgensen and Bendoricchio, 2001).

Field studies such as microcosms and mesocosms have been used as evidence for evaluating the accuracy of SSD and other ERA methodologies. The use of SSD should yield a more precautionary estimation of risk than field studies (Belanger et al., 2017). Since AQUATOX simulates ecological interactions and sub-lethal chemical effects which take place in riverine communities, we expect that PNECs based on field studies are closer to $PNEC_{AQUATOX}$ than to PNECs derived using SSD and AF methodologies, if the model can properly simulate the relevant features of the modelled ecosystem. For the chemicals tested in this work, PNECs based on field studies have been derived only for LAS. One work investigating C12-LAS through a 56-day experimental stream mesocosm study reported a NOEC of 0,268 mg/L (Belanger et al., 2002), this data was then normalized using QSARs resulting in PNEC = 0,27 mg/L (McDonough et al., 2016). The above NOEC was also used for calculating an overall freshwater PNEC = 268 μg/L (NOEC/AF, with AF = 1) by ECHA (Belanger et al., 2002; ECHA, 2017d). $PNEC_{AQUATOX}$ for the Po River is indeed closer to this number in comparison to $PNEC_{AF}$ and $PNEC_{SSD}$, suggesting that our modelling approach to ERA is ecologically sound.

Although ecosystem models have been used for predicting ecologically-safe thresholds such as NOECs (De Laender et al., 2008b, 2007; Naito et al., 2003) for different pollutants in various ecosystems, to our knowledge AQUATOX was used for deriving NOECs only in Baiyangdian Lake, China (Zhang et al., 2013; Zhang and Liu, 2014), and for deriving PNECs only for three chlorophenols in Taihu Lake, China (Lei et al., 2010). Lei et al. (2010) found a PNEC rank, $PNEC_{SSD} > PNEC_{AQUATOX} > PNEC_{AF}$, not confirmed by our findings, since we report such rank only for one out of the six tested chemicals, and for four other chemicals we found $PNEC_{AQUATOX} > PNEC_{SSD} > PNEC_{AF}$. This difference is probably due to our alternative PNEC derivation methodology in AQUATOX and the fact that Lei et al. (2010) focused on a single group of chemicals. All the mentioned applications, except that of Zhang and Liu (2014), did not rely on a large amount of local ecological data or did not include a quantitative model calibration, which is a key step in ecological modelling (Jorgensen and Bendoricchio, 2001). Moreover, all these authors followed the approach based on a 20% threshold for biomass decrease, i.e. the lowest detectable biomass decrease in the field according to Suter (1992), to highlight significant impacts.

Indeed, an important issue in our comparison of ERA methodologies is that the AQUATOX estimates of PNEC also depend on the significant biomass decrease threshold defined for each taxon, which we related to its natural fluctuations. Although our choice represents an advancement with respect to the current literature, which relies on an arbitrary (in our opinion) 20% biomass decrease (De Laender et al., 2008c, 2008b; Lei et al., 2010; Naito et al., 2003; Suter, 1992; Zhang et al., 2013; Zhang and Liu, 2014), more research is needed to unambiguously define what an non-negligible biomass decrease is: other definitions are possible and their implications could be explored through comparative work. For example, from a conservation perspective it could be better to focus on the annual biomass minimum of the most sensitive species over the year, which is a better indicator of extinction risk in the case of endangered species than median biomass. In a human-centered vision, a non-negligible biomass decrease could be an ecologically significant one, capable of directly or indirectly impairing the provision of the desired level of ecosystem services by the river, e.g. a fish population decline which makes recreational fishing no longer feasible. We acknowledge that the choice of the coefficient 2 in the threshold *MAD*/2, meant to make this threshold smaller than natural fluctuations (*MAD*), is arbitrary too: our initial idea was to define this coefficient based on inter-calibration between the AQUATOX method and the AF and/or SSD methods, but such purpose was abandoned since AQUATOX and SSD derived PNECs were already of the same order of magnitude for 4 contaminants out of 6 and for the other 2 contaminants the difference was marked, so an inter-calibration would have simply complicated interpretation without being informative. We recommend that future studies carry out an uncertainty analysis regarding the value of such coefficient and try to define it depending on the quality of ecological/ecotoxicological parameters used to build the model (analogously to an assessment factor) and on the biology of the population under scrutiny. For example, a larger protective coefficient should be adopted for highly-variable populations tending to experience very low biomass minima and hence more prone to local extinction if impacted by a toxicant, thus linking such coefficients to population traits and stochasticity. Indeed, AQUATOX is a deterministic model, but natural populations experience stochastic variations in abundance, e.g. due to environmental variability or demographic stochasticity (Lande, 1993), which can potentially drive them to extinction even under conditions not predicted to be unfavorable by AQUATOX. Future developments of our work should explore how risk estimates change when the effect of stochasticity on population dynamics is modelled, e.g. through the built-in AQUATOX routine (Park and Clough, 2014).

In comparison to lethal toxicity, understanding the impact of sub-lethal toxicity and indirect effects, that are accounted for in AQUATOX unlike in the AF and SSD methods, on riverine biota dynamics is not straightforward. The interpretation of what drives biomass changes in AQUATOX is complicated by the high model complexity resulting from

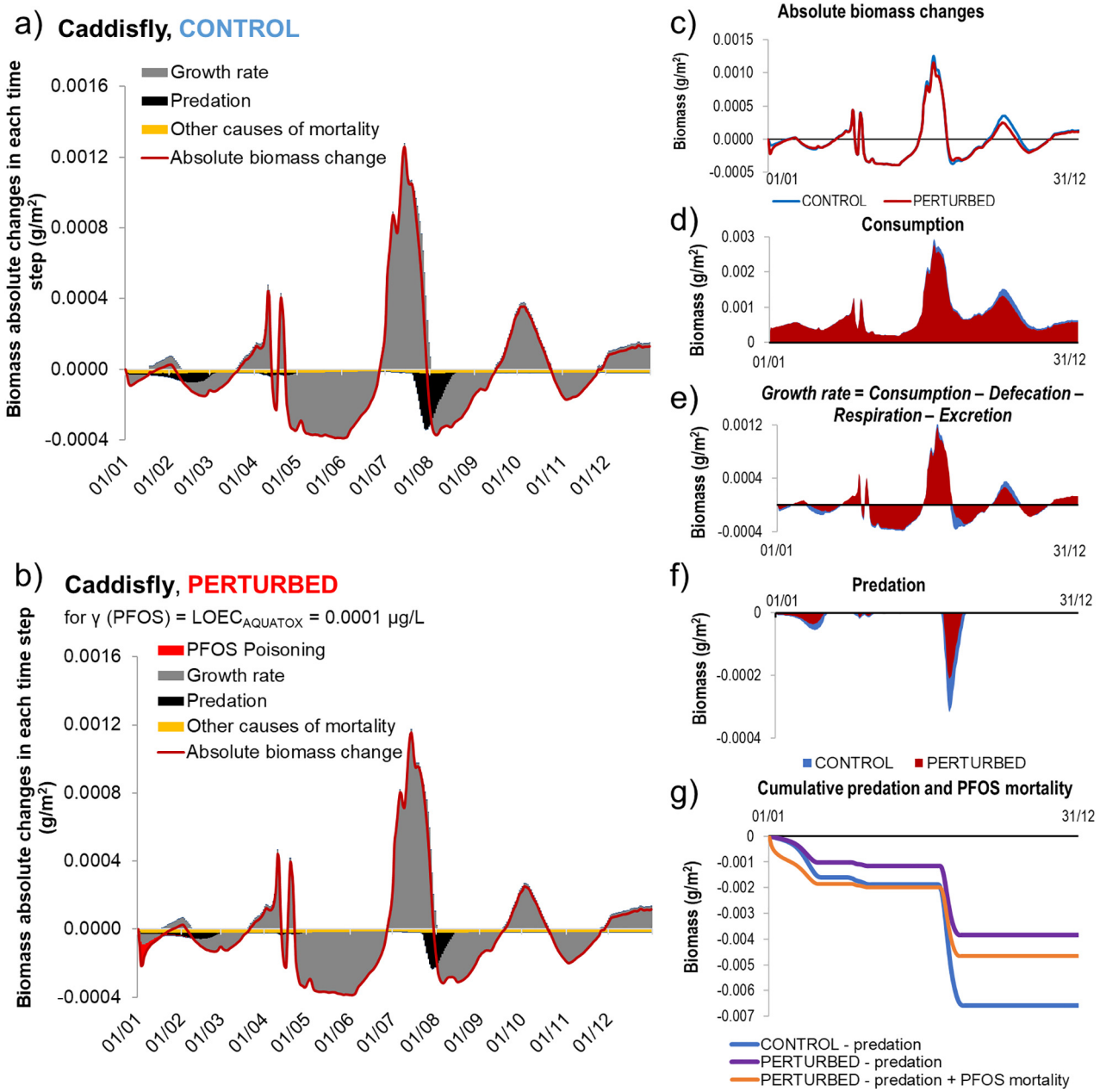

Fig. 4. Modelled Caddisfly process rates in the control and perturbed PFOS simulations with AQUATOX. The absolute biomass change curve shows biomass differences between consecutive days (the model time step is daily) for a) control and b) perturbed simulations. The contributions of the most significant processes are shown as differently colored areas. Subplots c–f compare process rates, expressed as biomass change, for the control and perturbed simulations. Subplot g) shows the total contribution of predation-induced biomass decrease in the control scenario and of predation and PFOS poisoning in the perturbed scenario, expressed as cumulative biomass on each time step.

the large amount of parameters and simulated processes and interactions (Lombardo et al., 2015; Niu et al., 2016), and requires an in-depth and time-consuming analysis of modelling outputs, an exercise that was carried out here only for PFOS, being not the main goal of the paper. Indeed, the PNEC and LOEC derived for PFOS (Tables 2 and 3) were several orders of magnitude smaller than those computed using the AF and SSD methods, highlighting this chemical as an interesting case. So, for PFOS, the rates of processes affecting biomass changes were computed for the most sensitive organism (Caddisfly, according to the AQUATOX methodology) using model outputs such as consumption and predation rates, poisoning rates, and others, for each daily time step (Park and Clough, 2014). In this way, we could approximately reconstruct the changes in biomass (increase or decrease) ascribable to each process over time. The weight of direct effects related to lethal toxicity on biomass variation was assessed based on the percentage of poisoned organisms, while sublethal toxicity and indirect effects were evaluated by looking at biomass variations related to consumption and predation mortality. Moreover, rates of PFOS uptake and depuration were also noted and used to support the evaluation of the direct and indirect effects. All computations were made for the control scenario as well as for a PFOS concentration equal to LOEC (Fig. 4). At the beginning of the perturbed simulation, PFOS poisoning caused a marked decline in Caddisfly biomass, due to the rapid uptake through gills (Fig. 3, Fig. 4a, b), pointing out to a direct toxicity effect. Direct lethal effects are modelled depending on the internal toxicant concentration and on BCF, LC50 and the Weibull shape parameter as a measure of the mortality spread; all these variables and parameters strongly contributed to the mortality of Caddisfly since it has the lowest LC50 in

comparison to the other organisms (particularly invertebrates), high BCF, consequently one of the highest internal concentrations in animals, and a higher Weibull shape parameter (Christensen, 1984; Park and Clough, 2014) (Supplementary material Tables S21–S28). Sublethal effects are modelled based on the EC50 values for growth and reproduction, also relying on the Weibull shape parameter and the internal toxicant concentration and can generate both direct effects and indirect effects on the ecosystem through reduced predation and increased production of detritus (Park and Clough, 2014). A reduced consumption in comparison to the control, ascribable both to a lack of preys (an indirect effect of the toxicant) and to the sublethal effect of PFOS on ingestion, appeared to be the main cause of the Caddisfly biomass decrease in the perturbed simulation in summer-autumn with respect to the control, leading to reduced growth. However, a lower biomass decrease from mid-July to mid-August because of a release from predatory mortality in the perturbed simulation points out the presence of indirect effects which are beneficial for Caddisfly, a counterintuitive result already noticed in other river ecosystem simulations (De Laender et al., 2007; Grechi et al., 2016; Lombardo et al., 2015; Naito et al., 2003; Zhang et al., 2013). In conclusion, Fig. 4 shows that PFOS can influence the population dynamics of Caddisfly, at least in the model, through both direct and indirect effects with varying intensity over the year.

3.5. Advantages and disadvantages of the methods

Compared to the AF and SSD methods, the AQUATOX methodology proposed here has the advantage of summarizing both lethal and sublethal toxicity effects and all biomass changes resulting from indirect effects having predominance in different parts of the year, all in one criterion, which takes into account the peculiarities of the population dynamics of each taxon, measured as biomass fluctuations over the year through the *MAD* estimator. It also has the capability of simulating the effect of chemical mixtures and of the concurrent action of multiple human stressors associated with water quality (discharges of chemicals, nutrients, organic substance, etc.). Thus, AQUATOX has the potential of being more ecologically realistic. Furthermore, it has all the advantages of ecological models as management and scientific tools (Jorgensen and Bendoricchio, 2001), such as the capability of making predictions (e.g. in the assessment of possible management scenarios), of being usable to test ecological hypotheses (e.g. cause-effect relationship regarding the input of a chemical and changes in the ecosystem), and of highlighting weaknesses in our knowledge of the studied system (e.g. the need for certain biomonitoring data; Grechi et al., 2016; Zhang and Liu, 2014), without high costs and effort. On the other hand, a good quality model requires good quality data for calibration and validation (Jorgensen and Bendoricchio, 2001), which can be challenging to find in the case of river ecosystem models and their use for ERA, limiting their application to regulatory risk assessment: river ecosystem models have high data requirement in comparison to what is collected in current biomonitoring programs, especially if one aims to distinguish the impact of a chemical on biota from those of other chemicals, human pressures and natural forcings (Grechi et al., 2016; Lei et al., 2008; Lombardo et al., 2015). The potential bias on results given by food web aggregation, discussed in Section 3.4, should also be mentioned. In the case of the Po, the available biomonitoring data allowed Grechi et al. (2016) to construct a good quality ecosystem model which they successfully stabilized and calibrated against mean annual biomass values for food web compartments (calibration is a fundamental good practice in modelling, rarely done in AQUATOX; Lombardo et al., 2015), and then used to highlight that realistic exposure concentrations of LAS and triclosan likely have a negligible impact on riverine biota under the tested scenario (exposure to single chemicals). Also, the simulations carried out here highlighted that sublethal toxicity and ecological relationships are potentially important in the Po, e.g. they made us identify a very precautionary protective threshold concentration when modelling PFOS. On the other hand, the biomonitoring data used by Grechi et al. (2016) to construct the Po model were not of sufficient quality to faithfully reproduce all seasonal dynamics in the river ecosystem; in general, the Po model has not been validated enough to tell if its higher ecological realism translates to a more trustable ERA with respect to the AF and SSD methods in this work. A possible partial solution, beyond the scope of this paper, would be to carry out an uncertainty analysis to test the robustness of AQUATOX outputs to different model parameterizations.

Unlike ecological modelling, the AF and SSD methods have the important advantage of being standardized approaches. In particular, the AF method is well-tested, simple and applicable with limited ecotoxicological datasets, which is often the case with new chemicals. On the other hand, the estimated PNECs can show large uncertainty, since the extrapolation from the individual to the population level is being made only with a single, simplistic factor (Gao et al., 2014; Grechi et al., 2016; Lombardo et al., 2015; Meli et al., 2014), which does not consider that the standard conditions of laboratories, where toxicity data are derived, radically differ from those in the ecosystem under consideration. In natural ecosystems, environmental fluctuations and ecological interactions can exert a large influence on population dynamics, affecting their response to the input of a chemical in a complex manner and acting together with additional human stressors (including other chemicals), and also sublethal toxicity can play an important role. This issue leaves open the question whether AFs result in over- or under-protective PNECs (Meli et al., 2014). Also, the AF methodology strongly depends on the amount of available data (Belanger et al., 2017): when applying the AF method to the Po, we chose not to consider all the available ecotoxicological data for the tested chemicals, but only data for species relevant for the Po ecosystem (i.e. species found there or similar to them). It is hard to say how this choice affected the computed $PNEC_{AF}$: on the one hand, we possibly omitted previously-tested species showing high sensitivity to the tested chemicals from the analysis, on the other hand the inclusion of more species in the analysis could have led to select less precautionary AFs (e.g., because more trophic levels would have been covered). Thus, more ecotoxicological data for species relevant for the local ecosystem are needed to make the AF methodology more reliable in the case of the Po.

Such issues apply also to SSD: although this method can provide larger statistical confidence compared to the AF method (Lei et al., 2010), as it focuses on toxicity effects on the whole community, it assumes that the sensitivity of a community can be computed from a set of independent species sensitivities obtained from single-species toxicity tests, entirely ignoring ecological interactions between species (De Laender et al., 2008a; Grechi et al., 2016; Lei et al., 2010; Naito et al., 2003). SSD requires more ecotoxicological measures than the AF method, which in addition should be statistically and ecologically representative of the investigated community, something difficult to achieve. The SSD approach used here aimed, at least, to reflect the local conditions of the Po, by fitting toxicity data only for species relevant for that ecosystem and avoiding mixing data for species which do not belong to the same community as done in regulatory applications where the SSD method is generally used for deriving an overall PNEC applicable to different ecosystems (Belanger et al., 2017). To address the drawbacks of the SSD approach, research is ongoing: for example, computing separate SSDs for reproduction, growth and mortality makes this approach more ecologically sound, avoiding the mix of different endpoints (Beaudouin and Pery, 2013), and novel approaches are being developed like field-based SSD (using field data based on population abundance and biomass), hierarchical SSD (addressing data gaps in taxa diversity through knowledge of how sensitivity relates to taxonomic distance between species) and trait-based SSD (using groupings based on traits instead of species) (Belanger et al., 2017).

4. Conclusions

Here, ecologically-safe thresholds for LAS, triclosan and four perfluoroalkyl acids were evaluated by deriving PNECs for the Po River ecosystem through the assessment factor method, the species sensitivity distribution and a novel methodology relying on the process-based ecosystem model AQUATOX. These methodologies sometimes provided similar results, but in other cases PNEC estimates were quite different. The case of PFOS suggests that by taking indirect ecological effects and both lethal and sublethal toxicity into account, higher risk than expected is possible: the PNEC resulting from AQUATOX is 3.6% of the accepted annual average EQS for freshwater (The European Parliament and the Council of the European Union, 2013). Thus, the use of multiple, complementary methods for PNEC derivation seems useful to clarify uncertainties in ecological risk estimates and ensure higher confidence in results. Our PNEC estimates for PFAAs could surely be improved, e.g. with the availability of more ecotoxicological data, needed to avoid ICE extrapolation, and, in the case of AQUATOX, by using measured BCFs and not those modelled using chain-length dependent equations. An ecological risk assessment for PFAAs mixtures can be implemented using AQUATOX and appears as a logical future step, given the simultaneous presence of different substances in the groundwater and surface water contamination in northern Italy.

This work shows that including ecological relationships and chemical sublethal toxicity in ERA through models can give a fuller picture of the concentration–response relationship in ecosystems, potentially resulting in a more ecologically-relevant risk assessment, provided that good quality data are available for model construction, calibration and validation, and that the uncertainty of model predictions is properly acknowledged and investigated. Ecosystem models could be a useful tool in planning mesocosm studies and for pre-evaluation in the assessment of chemical impact on ecosystems as a whole. Nonetheless, more work needs to be done to standardize modelling approaches and provide guidelines for their application, before their full inclusion in regulatory risk assessment. Also, to satisfy the expectations of policy and decision-making and allow standardized use, ERA models should not be too complicated or provide hard-to-interpret results, two requirements which clash with the fact that ERA models should be complex enough to realistically depict a wide range of ecological scenarios.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2018.06.017.

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