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A risk based assessment approach for chemical mixtures from wastewater treatment plant effluents

Saskia Finckh^{a,b,*}, Liza-Marie Beckers^c, Wibke Busch^d, Eric Carmona^a, Valeria Dulio^e, Lena Kramer^d, Martin Krauss^a, Leo Posthuma^{f,g}, Tobias Schulze^a, Jaap Slootweg^f, Peter C. Von der Ohe^h, Werner Brack^{a,b}

^a Department of Effect-Directed Analysis, UFZ – Helmholtz Centre for Environmental Research, Leipzig, Germany

^b Institute of Ecology, Evolution and Diversity – Goethe University, Frankfurt am Main, Germany

^c Department of Aquatic Chemistry, BfG – Federal Institute of Hydrology, Koblenz, Germany

^d Department of Bioanalytical Ecotoxicology, UFZ – Helmholtz Centre for Environmental Research, Leipzig, Germany

^e Institut National de l'Environnement Industriel et des Risques (INERIS), Verneuil-en-Halatte, France

^f RIVM, National Institute for Public Health and the Environment, P.O. Box 1, 3720 BA Bilthoven, the Netherlands

^g Department of Environmental Science, Faculty of Science, Radboud University, Nijmegen, the Netherlands

^h German Environment Agency (UBA), Dessau-Roßlau, Germany



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ABSTRACT

In this study, 56 effluent samples from 52 European wastewater treatment plants (WWTPs) were investigated for the occurrence of 499 emerging chemicals (ECs) and their associated potential risks to the environment. The two main objectives were (i) to extend our knowledge on chemicals occurring in treated wastewater, and (ii) to identify and prioritize compounds of concern based on three different risk assessment approaches for the identification of consensus mixture risk drivers of concern. Approaches include (i) PNEC and EQS-based regulatory risk quotients (RQs), (ii) species sensitivity distribution (SSD)-based hazard units (HUs) and (iii) toxic units (TUs) for three biological quality elements (BQEs) algae, crustacean, and fish.

For this purpose, solid-phase extracts were analysed with wide-scope chemical target screening via liquid chromatography high-resolution mass spectrometry (LC-HRMS), resulting in 366 detected compounds, with concentrations ranging from < 1 ng/L to > 100 µg/L. The detected chemicals were categorized with respect to critical information relevant for risk assessment and management prioritization including: (1) frequency of occurrence, (2) measured concentrations, (3) use groups, (4) persistence & bioaccumulation, and (5) modes of action. A comprehensive assessment using RQ, HU and TU indicated exceedance of risk thresholds for the majority of effluents with RQ being the most sensitive metric. In total, 299 out of the 366 compounds were identified as mixture risk contributors in one of the approaches, while 32 chemicals were established as consensus mixture risk contributors of high concern, including a high percentage (66%) of pesticides and biocides. For samples which have passed an advanced treatment using ozonation or activated carbon (AC), consistently much lower risks were estimated.

1. Introduction

Awareness on the presence of large numbers of emerging contaminants (ECs) (Daughton, 2005) in surface and wastewaters, as well as the performance of techniques to analyse them by liquid chromatography high-resolution mass spectrometry (LC-HRMS), has rapidly increased over the last decade (Brack et al., 2019b). More than 200,000 chemicals are listed in the Classification and Labelling (C&L) Inventory of New and

Existing Substances in the EU (ECHA, 2021). When released into the environment, this results in complex mixtures of contaminants in European waste- and surface waters, requiring a holistic approach to water quality monitoring, assessment and management (Posthuma et al., 2019a) to meet the ambitions of the European Water Framework Directive (WFD) (European Commission, 2000). While environmental monitoring has long been focused on nonpolar persistent organic pollutants, the last two decades of research have seen increasing attention

* Corresponding author at: Department of Effect-Directed Analysis, UFZ – Helmholtz Centre for Environmental Research, Leipzig, Germany.
E-mail address: saskia.finckh@ufz.de (S. Finckh).

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paid to the large number of polar organic pollutants. A specific focus was given to pharmaceuticals and personal care products that were considered particularly relevant (Daughton and Ternes, 1999; Ternes, 1998) and European surveys were launched to screen for polar compounds, typically addressing 30–50 targets including synthetic chelating agents, surfactants, dispersants, biocides and corrosion inhibitors, personal care products, pharmaceuticals and pesticides (Loos et al., 2009; Reemtsma et al., 2006). Since then, the number of detected chemicals in the water cycle continuously increased, with 125 chemicals out of 156 polar organic contaminants detected in wastewater treatment plant effluents (WWTP) in 2013 (Loos et al., 2013). Recently, a method for wide-scope screening for more than 2000 ECs in wastewater samples has been developed, which allowed to detect 280 out of 2248 analysed compounds in WWTP effluents from the Danube catchment (Alygizakis et al., 2019), as well as 315 out of 2316 analysed compounds in one single WWTP effluent from Athens, Greece (Gago-Ferrero et al., 2020).

The increasing number of detected chemicals in surface and wastewater samples also enhanced the requirement to better characterize these chemicals regarding use groups, frequency of detection, detected concentration ranges, and environmental fate (Drakvik et al., 2020), and to prioritize compounds according to toxicity, hazards, fate and risks (Diamond et al., 2011; von der Ohe et al., 2011). Water pollutants have been assessed and prioritized for different criteria including the frequency of occurrence (Alygizakis et al., 2019), measured environmental concentrations (Gros et al., 2017; Loos et al., 2013), persistence (Blum et al., 2017; Cousins et al., 2019; Gros et al., 2017), the potential for bioaccumulation (Blum et al., 2017; Gros et al., 2017), and toxic risks (von der Ohe et al., 2011). The latter were expressed based on (1) risk quotients (RQs) of measured (MEC) or predicted environmental concentrations (PEC) and predicted no-effect concentrations (PNEC) or environmental quality standards (EQS) (Alygizakis et al., 2019; Gros et al., 2017; Markert et al., 2020; Rodriguez-Mozaz et al., 2020; von der Ohe et al., 2011; Zhou et al., 2019), (2) toxic pressure quantification for species assemblages based on species sensitivity distributions (SSDs) and mixture modelling (Munz et al., 2017; Posthuma and de Zwart, 2006), or (3) toxic units (TUs) for selected biological quality elements (BQEs) of the WFD, such as algae, crustaceans and fish (Malaj et al., 2014; Markert et al., 2020). Within the NORMAN network for emerging contaminants, a prioritization approach based on the frequency and spatial extent of exceedance of PNECs by MECs has been developed (von der Ohe et al., 2011) and continuously advanced (Brack, 2015).

Risk assessment of chemicals and mixtures based on RQs applies ratios of MECs and a measure for the toxic potency of the individual compounds, represented by a PNEC or a legally binding EQS under the WFD, of which the latter is preferably being used if available. PNECs are derived for a specific compartment or receptor at risk using the lowest agreed effect concentration (acute LC_{50s} or preferably chronic NOECs). The EQS values are derived for each chemical as an overall threshold which is intended to protect all receptors (i.e. aquatic life, predators via secondary poisoning, and human health via the consumption of fishery products and drinking water) by taking into account all exposure routes (e.g. water, sediment and biota). Several different types of receptors and associated quality standards (QS) are considered and the lowest of these values is set as the overall EQS (European Communities, 2011; Scientific Committee on Health, 2018). Data availability for deriving a PNEC or an EQS can vary. The WFD defines two types of EQS: the annual average EQS (AA-EQS) used here, which is derived from chronic toxicity data, and the maximum acceptable concentration EQS (MAC-EQS), which is derived from acute toxicity data. A compound-specific assessment factor (AF) below 5 for mesocosm and SSD data, between 10 and 100 in case of chronic and 1000 in case of acute data, can be applied to account for uncertainty associated with the amount of data, intra- and interlaboratory variation, biological variance and not tested taxa, short-term to long-term extrapolation, as well as laboratory to field extrapolation (European Communities, 2011; Scientific Committee on Health, 2018). The RQ-based approach has an obvious link to regulatory frameworks,

such as the WFD. Provided that all chemicals found in a sample are accounted for, RQs below 1 imply that the 'sampling site' can be considered 'sufficiently protected', with a high degree of certainty that human health or aquatic life are unaffected by any of the detected mixture components. RQ summation is applied as a pragmatic approach for mixture risk evaluations, although it poses a potential logical interpretation problem, if the resulting mixture risks are based on toxicity values from different species with different AFs (Kortenkamp et al., 2019).

Multi-species approaches based on SSDs target mixture impacts *a priori* on the entire aquatic community. The basis of SSDs are multiple NOEC or EC_x values for as many species as possible, which allow to calculate a statistical measure of the toxic pressure of single compounds, compound groups or whole mixtures towards species assemblages, which are expressed as (multi-substance) potentially affected fractions of species, (ms)PAF (Fonseca et al., 2020; Munz et al., 2017; Posthuma and de Zwart, 2012). This does, however, not take species interactions (e.g. predator–prey interactions, displacement of niche species) into account. For each compound, a hazard unit (HU) can be calculated as intermediate step, which is based on the ratio of MECs and concentrations for which a specific fraction of species is affected (commonly the hazard concentration for 5% of species, referred to as HC5). In this case, AFs are not applied. Given available data (Posthuma et al., 2019), the HU approach can be used to discriminate chronic impacts (HU_{ch} based on EC_{x,s} from 0 to 20% or NOECs) from acute risks (HU_{ac} based on EC_{x,s} from 30 to 70%), using chronic or acute test data collections, respectively. HU summation and an exceedance of 1 could be used as an indication of the likelihood that the mixture can impact the local species community exposed to the sample.

The BQE-specific TU approach (von der Ohe et al., 2009) is based on the concept of concentration addition (CA) (Sprague, 1970), and is calculated as the quotient of MECs and a respective measured or predicted effect concentration (EC) for algae, crustaceans and fish, etc. Analogous to HUs, and in contrast to RQs, no compound-specific AFs are applied to the ratio. The use of acute effects, however, makes it more difficult to define a threshold at which effects are not acceptable any more, which may vary from BQE to BQE. Moreover, taxonomic-specific effects may be overlooked if only one standard test species per BQE is used. This approach is often used for group-specific risk assessments, to identify the compounds causing observed or predicted impairment in the aquatic community. Following the CA model as applied by Backhaus and Faust (2012), TU summation is used for the assessment of mixture risks to different organism groups of interest, such as the BQEs considered in the classification of ecological status and impacts under the WFD (Kandie et al., 2020; Machate et al., 2021; Markert et al., 2020; Munz et al., 2018; von der Ohe et al., 2009). By correlating the loss of sensitive species from algal, invertebrate and fish communities, as for example indicated by the species at risk (SPEAR) index for invertebrates (von der Ohe and Liess, 2004), threshold levels for acute toxic risks (0.1 for all BQEs), and chronic risks (0.02 for algae, 0.001 for crustacean and 0.01 for fish) are applied, which should not be exceeded (Malaj et al., 2014).

All three approaches are frequently applied and have provided insights into individual and mixture risks; they helped prioritize chemicals with major contributions to these risk metrics as demonstrated in the above cited studies. However, often, it remained largely unclear why a specific approach has been selected and how the results compare to other approaches and thus, how robust the prioritization of environmental chemicals and mixtures actually is. Comparative assessments using different approaches, based on real-world mixtures, are largely lacking.

Thus, the current study aims are (1) to extend the knowledge on chemicals occurring in WWTP effluents across Europe based on LC-HRMS target screening of 499 ECs in 56 WWTP effluents, and to characterize the detected chemicals regarding critical information relevant for risk assessment and management, including frequency of occurrence, concentration ranges, use groups, persistence and modes of action, (2) to identify and prioritize compounds of concern involving the PNEC-based

RQ metric, the SSD-based HU metric and the BQE-specific TU metric, and (3) to evaluate mixture risks of the WWTP effluents and the potential positive effect of applying advanced wastewater treatments via ozonation or activated carbon (AC) on the occurrence of compounds and related estimated risks.

2. Material and methods

2.1. Sampling, extraction, and sample storage

In total, 56 effluent samples were taken from 52 European WWTPs located in 15 different countries: Germany (17), Switzerland (6), Czech Republic (5), Spain (5), Croatia (2), Greece (3), Netherlands (3), Romania (2), Slovenia (2), Sweden (2), Austria (1), France (1), Hungary (1), Serbia (1) and Slovakia (1). Different capacities and conventional treatment technologies, as well as two advanced treatment technologies are included. One sample was taken at a WWTP with AC treatment (EU019). Three WWTPs applying ozonation were sampled before and after this treatment step, providing two samples each (incl. ozonation: EU032, EU128, and EU130). One of these WWTPs had already been sampled before the upgrade, resulting in a total of 56 (52 + 4) samples, which are treated as independent samples in the data set. In general, all samples were taken over a period of 1½ years from August 2017 to April 2019. The precise date is provided in the supporting information (Table S1). At each site, 50 L of treated effluent water were collected, using on-site large volume solid phase extraction (LVSPE) (Schulze et al., 2017; Vålitalo et al., 2017). In brief, the samples were pre-filtered (Sartopure GF + MidiCap, 0.65 µm separation size, Sartorius) and extracted using cartridges filled with 10 g of hydrophobic polystyrene divinylbenzene copolymer (Chromabond HR-X, Macherey-Nagel). The latter were conditioned with 200 mL ethyl acetate (LC-MS grade) followed by 200 mL methanol (LC-MS grade) and 100 mL water (LC-MS grade) before sampling. In the laboratory, the cartridges were dried with nitrogen and afterwards freeze-dried to remove remaining water. The cartridges were eluted with 100 mL ethyl acetate, 100 mL methanol, 100 mL methanol with 1.0 vol% formic acid (98–100%, p.a., Merck), and 100 mL methanol with 2 vol% of 7 N ammonia in methanol (Sigma-Aldrich). In addition, 13 traveling blanks were prepared by extracting 1 L of LC-MS grade water by LVSPE and transporting/processing them together with the sampling cartridges. All extracts were finally re-dissolved in LC-MS grade methanol at a relative enrichment factor (REF) of 1000 (i.e. 50 mL) and stored at –20 °C until further analysis.

2.2. Chemical target screening

2.2.1. Selection of target compounds

The list of the 499 target substances (Table S2) was compiled based on an extensive literature review and includes chemicals previously detected in the water cycle, but also novel compounds that were recently identified in water samples with non-targeted and effect-directed approaches (Beckers et al., 2020; Muschket et al., 2018). Various criteria were considered, such as substance properties, fate, behaviour in the environment, occurrence and measurability by LVSPE and LC-HRMS. In addition, the selection was intended to cover important application areas, which are sources for WWTP contaminants, such as the medical sector (pharmaceuticals) and the agricultural sector (pesticides).

2.2.2. LC-HRMS analysis

For analysis, 100 µL of LVSPE samples (REF 1000) were transferred into a 2-mL autosampler vial with a 200-µL conical glass insert and 10 µL of an internal standard mixture containing 38 isotope-labelled compounds (1 µg/mL) (Table S3), 30 µL of methanol (LC-MS grade) and 60 µL of water (LC-MS grade) were added. Travel blanks were treated in the same way as LVSPE samples. The sample and travel blank aliquots were analysed in four batches in a wide-scope target screening by LC-HRMS, including calibration aliquots and instrumental blanks. An example

sequence is presented in the supporting information (Table S4). The matrix-matched calibration was prepared from filtered water from a pristine stream (Wormsgraben) in the upper Harz Mountains. To this end, 1-L aliquots were spiked with mixtures of all target compounds at 15 levels ranging from 0.1 to 5000 ng/L. These calibration standards were extracted based on a laboratory-scale SPE method using 200 mg of HR-X sorbents and the same solvents for elution as for the LVSPE method. The calibration aliquots for analysis were prepared the same way as the sample aliquots. Instrumental blanks were prepared with the same composition (methanol:water 70:30) and run for 4–6 replicates in each sequence.

For the measurement, a reversed-phase LC separation on a Thermo Ultimate 3000 LC and an injection volume of 5 µL were used. HRMS data was acquired in full scan combined with data-independent mode, using a quadrupole-Orbitrap MS (QExactive Plus, Thermo Scientific) with electrospray ionization (ESI), and separate runs in positive and negative mode. To quantify compounds which have been measured at concentrations > 5000 ng/L in the first run, extracts were diluted 50-fold using LC-MS grade methanol. The diluted samples (REF20) were analysed with the same calibration standards and LC method on a different LC-HRMS device (LTQ Orbitrap XL, Thermo Scientific) in full scan mode. Further information on settings and instrument parameters are described in detail in Beckers et al. (2020).

2.2.3. Data processing and evaluation

The obtained Thermo.raw files were converted to the mzML format using ProteoWizard (v. 3.0.18265) (Chambers et al., 2012) and processed using the software MZmine 2.38 (Pluskal et al., 2010) for peak picking, deconvolution, alignment, gap filling and peak annotation as detailed in Beckers et al. (2020). The in-house R package MZquant (Schulze et al., 2021a) was used for the semi-automated quantification of compound concentrations. A generalized additive model was used for calibration, which was trimmed to the detection range with at least four data points (calibration levels) included. Some compounds were checked manually, using the vendor software TraceFinder (version 4.1, Thermo Scientific). These were either quality controls (QCs) or compounds, which could not be reliably annotated automatically based on previous experience. In total, 44 out of 56 samples were analysed using the complete mix of 499 standard compounds (Mix-2), while the first 12 samples were analysed with a previous mix (Mix-1), containing 365 compounds (Table S2). The new compounds from Mix-2 were subsequently added by retrospective analysis of the LC-HRMS files, together with a newly measured Mix-2 calibration line, considering the retention time (RT) and the mass-to-charge ratio (m/z) (Wagner, 2020). Method detection limits (MDLs) were determined on the basis of US-EPA guidelines (US-EPA, 2011). To account for the impact resulting from different handling of non-detects, concentrations below the MDL were set to zero (dataset A) or to MDL/2 (dataset B). The latter was used as the default dataset in this paper, if not stated differently. An overview of the final concentrations (dataset A & B) of the 56 WWTP effluent samples is presented in the supporting information (Tables S5, S6), and is also available on PANGAEA (Finckh et al., 2022).

Data analysis and basic visualization were performed in Microsoft Excel 2013 and Inkscape (version 1.1). Boxplots were created in GraphPad Prism (version 8.4.3). Stacked barplots and heatmaps were created in R. (version 1.2.1335).

2.3. Compound characterization

2.3.1. Use group assignment

Initially, all analysed target compounds including respective transformation products (TP) were assigned to their primary use group according to three main categories: (1) pharmaceuticals; (2) pesticides & biocides, among others comprising a wide range of different herbicides (H), insecticides (I), fungicides (F) and biocides (B); and (3) others. Those compounds, which were detected at least once, were further

specified according to the following sub-categories: (1) pharmaceutical and pharmaceutical TP, (2) plant protection product (PPP), legacy pesticide, biocide and pesticide & biocide TP, and (3) industrial chemical, plastic additive, surfactant, food ingredient, rubber additive, PFC, UV filter, corrosion inhibitor, sweetener, dye, repellent, stimulants, bitter, flame retardant and TPs. Information was researched from different data sources, i.e. PubChem (National Library of Medicine, 2021) and the C&L Inventory (ECHA, 2021). When a compound is knowingly used for different purposes, an individual choice was made, deciding on the main area of application.

2.3.2. Biodegradation and bioaccumulation potential

The biodegradation and bioaccumulation potential were assessed based on a scoring system of five categories, for both the predicted half-life time and the bioconcentration factor (BCF), respectively (Gros et al., 2017). Category 1 (red) represents the highest priority, while increasing numbers (from yellow to green) indicate a lower priority (Table 1). Both parameters were retrieved from the CompTox Chemical Dashboard (Williams et al., 2017), and rely on the OPERA models (Mansouri et al., 2018). In addition, the biodegradation was also described by “removal rates” in % (influent vs. effluent, conventional wastewater treatment) from a review study on the fate of organic micropollutants (Margot et al., 2015).

2.3.3. Mode of action

According to the conceptual understanding of the modes-of-action (MOA) of chemicals presented in Busch et al. (2016), a database query was performed. Then, all detected compounds were annotated by their specific MOAs, which were retrieved from PubChem (National Library of Medicine, 2021), Drugbank (Drugbank, 2021; Wishart et al., 2018), PPDB (University of Hertfordshire, 2021), and Wikipedia (Wikimedia Foundation, 2021). In case of ambiguous information, individual expert assignment of a compound to the best-known or the environmentally most relevant MOA was done. Following a plausibility check on these preliminary results, a literature review on uncertain and missing compounds was added to fill as many gaps as possible. Finally, the specific MOAs were further categorized into higher-level MOA groups (an updated MOA database will be published separately) (Table 2).

2.4. Environmental risk assessment

Environmental risk of a chemical is considered present if the risk metric exceeds an effect threshold for a species or species assemblage. The risk metrics were consistently calculated as the quotient of exposure (measured environmental concentration, MEC) and hazard (toxic potency indicator). Risks are ranked higher with increasing degree of exceedance.

As part of this study, three risk metrics were applied (Table 3), with different final interpretation: (1) the RQ metric applying regulatory EQS and PNEC values, often considering single species endpoints from one of three trophic levels, (2) the SSD-based HU metric, using chronic toxicity data to statistically derive a measure of species assemblage impacts, and (3) the TU metric, based on acute toxicity data for three different BQEs (i.e. algae, crustacean and fish impacts). All toxicity values of the detected compounds are listed in the supporting information (Table S7),

Table 1

Scoring system for prioritization. Categorization according to Gros et al. (2017). The respective data can be found in the supporting information (Table S7).

Score	1	2	3	4	5
Frequency of detection	>0.75	>0.5	>0.25	>0	0
Biodegradation (predicted half-life time in days, DT50 _{pred} /d)	>180	>60	>37.5	>15	<15
Bioaccumulation (predicted bioconcentration factor, BCF _{pred})	>10000	>1000	>100	>10	<10

Table 2

Modes of action (MOAs) per use group category. The respective data can be found in the supporting information (Table S7).

#	MOAs pharmaceuticals	#	MOAs pesticides & biocides	#	MOAs others
1	Unknown MoA	15	Unknown MoA	35	Unknown MoA
2	Neuroactive	16	Photosynthesis inhibition	36	Neuroactive
3	Cardiovascular system	17	Lipid metabolism	37	Cell and DNA protection
4	Antibiotic	18	Neuroactive	38	Lipid metabolism
5	Antiinflammatory	19	Sterol biosynthesis inhibition	39	Nucleic acid damage
6	Sterol biosynthesis inhibition	20	Respiration inhibition	40	Endocrine
7	Antihistamine	21	Protein biosynthesis inhibition	41	Olfactory/Gustatory system
8	Endocrine	22	Synthetic Auxin	42	Respiration inhibition
9	Nucleic acid biosynthesis inhibition	23	Mitosis, Cell cycle	43	Cardiovascular system
10	Metabolic system	24	Chitin biosynthesis inhibition	44	Metabolic system
11	Lipid metabolism	25	Glycolysis inhibition	45	Nucleic acid biosynthesis inhibition
12	Mitosis, Cell cycle	26	Nucleic acid biosynthesis inhibition	46	Sterol biosynthesis inhibition
13	Folic acid biosynthesis inhibition	27	Carotenoid biosynthesis inhibition	47	Synthetic Auxin
14	Synthetic Auxin	28	Cell wall synthesis inhibition		
		29	Endocrine		
		30	Hormone biosynthesis inhibition		
		31	Metabolic system		
		32	Nucleic acid synthesis		
		33	Respiration inhibition		
		34	Vitamin K biosynthesis inhibition		

and are also available on PANGAEA (Finckh et al., 2022).

2.4.1. Risk quotient

Risk quotients (RQs) were calculated by dividing the MEC of a compound *i* by the corresponding PNEC_{*i*} or EQS_{*i*} (including compound-specific AFs between 2 and 1000) downloaded from the NORMAN Ecotoxicology Database (NORMAN Network, 2021):

$$RQ_i = \frac{MEC_i}{EQS_i \text{ or } PNEC_i} \quad (1)$$

According to the database description, the lowest PNECs are preferably based on experimental eco-toxicity data. In case of no or

Table 3
Overview of the applied risk metrics.

Risk parameter	RQ	HU _{ch}	TU _{algae}	TU _{crust}	TU _{fish}
Name	Risk Quotient	Hazard Unit (chronic)	Toxic Unit (BQE = algae)	Toxic Unit (BQE = crustacean)	Toxic Unit (BQE = fish)
Description	“Regulatory approach”	“Community/biodiversity-level approach”	“Trophic-level-specific approach”	“Trophic-level-specific approach”	“Trophic-level-specific approach”
Toxicity data	<ul style="list-style-type: none"> Environmental quality standard (EQS) Predicted no-effect concentration (PNEC) 	<ul style="list-style-type: none"> No-observed & lowest-observed-effect concentration (NOEC & LOEC) maximum acceptable toxicant concentration Effect concentration (EC_x) from 0 to 20% Note: Compounds without SSD were excluded from the risk assessment 	<ul style="list-style-type: none"> Effect concentration (EC_x or LC_x) of 50% towards algae 	<ul style="list-style-type: none"> Effect concentration (EC_x or LC_x) of 50% towards crustacean 	<ul style="list-style-type: none"> Effect concentration (EC_x or LC_x) of 50% towards fish
Assessment Factor/ Risk threshold	Compound-specific assessment factors, included in PNEC & EQS values (AFs = 2–1000)	Risk threshold defined by SSD-based potentially affected fraction of species (PAF = 0.05)	Algae-specific (chronic) risk threshold of 0.02 (F _{algae} = 50)	Crustacean-specific (chronic) risk threshold of 0.001 (F _{crust} = 1000)	Fish-specific (chronic) risk threshold of 0.01 (F _{fish} = 100)
Availability of toxicity data (of the 366 detects)	<ul style="list-style-type: none"> 157 experimental PNECs 203 predicted PNECs 6 no PNEC available 	<ul style="list-style-type: none"> 108 available SSDs 258 no SSDs available 	<ul style="list-style-type: none"> 111 measurement-based ECs 255 predicted ECs 	<ul style="list-style-type: none"> 99 measurement-based ECs 267 predicted ECs 	<ul style="list-style-type: none"> 116 measurement-based ECs 250 predicted ECs
Formula	$RQ = \frac{MEC}{EQS \text{ or } PNEC}$	$HU_{ch} = \frac{MEC}{HC5_{SSD} (PAF = 0.05)}$	$TU_{algae} = \frac{MEC}{EC_{50,algae}} (\times 50)$	$TU_{crust} = \frac{MEC}{EC_{50,crust}} (\times 1000)$	$TU_{fish} = \frac{MEC}{EC_{50,fish}} (\times 100)$

insufficient empirical endpoints, QSAR predictions were used to estimate a provisional P-PNEC value to allow for a first screening.

Mixture toxicity was expressed by RQ_{sum}. Assuming CA, all available RQ_{EQS,i} values and the RQ_{PNEC,j} values were summed up:

$$RQ_{sum} = \sum_{i=1}^n RQ_i \quad (2)$$

If RQ_{sum} is below 1, the risk metric indicates a sufficient safety of the sample.

2.4.2. Hazard units

Hazard units (HUs) were based on species sensitivity distributions (SSDs) and calculated by dividing the MEC of a compound *i* by the concentration where 5% of the species of a model community are impacted (HC5_{SSD,i}), corresponding to a potentially affected fraction (PAF) of 0.05.

$$HU_i = \frac{MEC_i}{HC5_{SSD,i} (PAF_i = 0.05)} \quad (3)$$

The SSDs were based on all available ecotoxicity data as described in Posthuma et al. (2019b). In the case of the chronic HU_{ch} these are NOEC, LOEC, EC0, EC5, EC10, and EC20 values, as well as maximum acceptable toxicant concentrations, while the acute HU_{ac} are based on sublethal (EC_x) or lethal (LC_x) endpoints from 30% to 70%. SSDs were constructed based on available ecotoxicity data. SSD parameters and SSD-quality scores were obtained from the supporting information in Posthuma et al. (2019b). That publication presents SSDs for a large number of compounds, with a four-digit SSD-quality scoring system. For the present study we aimed to focus on compounds with a robust SSD, to avoid the potential bias that may occur for low-data SSDs in the format of a haphazard occurrence of a data-driven ‘shallow’ slope. On the basis of practical experiences, data-poor compounds can have such a slope, and this biases environmental assessment as they yield a high toxic pressure read-out at a low exposure concentration. In practice, the present study focused on compounds for which the SSD was constructed from data on at least 5 taxa from which a subset had at least 10 taxa, for which the occurrence of bias was found low and very low, respectively.

The impact of chemical mixtures on the community level was expressed as sum of HU_i values, separate for chronic and acute toxicity, assuming CA.

$$HU_{sum} = \sum_{i=1}^n HU_i \quad (4)$$

In this study, we focused on chronic toxicity values, which are more comparable to the protective regulatory RQ metric than the acute toxicity values. The threshold of HU_{ch, sum} = 1 is similar to the 95%-protection level of SSDs (i.e. the HC5), which are sometimes used to derive PNECs and EQSs. The same applies to the mixture, so that 95% of the species are considered to be protected up to a HU_{ch, sum} of 1.

2.4.3. Toxic units

Toxic units (TUs) were calculated according to Malaj et al. (2014), Sprague (1970), by dividing the MEC of a compound *i* by the respective acute effect concentrations (i.e. EC₅₀ or LC₅₀) for algae, crustacean, and fish, respectively, resulting in three TU values (TU_{algae}, TU_{crust}, TU_{fish}) per compound.

$$TU_{BQE,i} = \frac{MEC_i}{EC_{50,BQE,i}} \quad (5)$$

EC₅₀ or LC₅₀ data were selected in the following order: (1) Experimental data retrieved from the US-EPA ECOTOX database (US-EPA, 2021) or, if no experimental data were available, (2) predicted LC₅₀/EC₅₀ values using the ECOSAR type baseline toxicity model for the BQEs fish, daphnia and green algae in Chemprop 6.7.1 (UFZ Department of Ecological Chemistry, 2021). For (1), a statistical value was calculated based on real measurements. In brief, this effect concentration is the 5th percentile of all effect concentrations available per BQE. The different effect categories included mortality, growth inhibition, population and movement inhibition. In addition, only the data sets with “short-term effects” (≤ 120 hours or ≤ 5 days) were considered. If the measured or predicted ecotoxicological data exceeded the predicted water solubility by more than half a log unit, the measured or predicted ecotoxicological value was replaced by the predicted water solubility. Further details on the effect data acquisition procedure are published in Schulze et al. (2021b). The dataset is available on zenodo (Schulze, 2022).

Mixture risks were calculated by summation of all TU_i per BQE of all detected target compounds, yielding the TU_{sum} based on the CA model.

$$TU_{sum} = \sum_{i=1}^n TU_i (\times F_{chronic,BQE}) \quad (6)$$

All WWTP effluent samples and the detected compounds were evaluated and prioritized according to the exceedance of the TU_{sum} risk thresholds suggested by Malaj et al. (2014): chronic risk for algae (TU_{algae}_{sum} = 0.02), crustacean (TU_{crust}_{sum} = 0.001), and fish (TU_{fish}_{sum} = 0.01). These thresholds are empirically based for invertebrates (Beketov et al., 2013; Schäfer et al., 2012) and rely on acute to chronic ratios for algae and fish (Ahlers et al., 2006). In order to apply the same threshold of 1 as for the other metrics a BQE-specific chronic risk factor F_{chronic} was applied to each TU: F_{chronic,algae} = 50, F_{chronic,crust} = 1000, F_{chronic,fish} = 100.

2.4.4. Identification of mixture components of concern

For all risk metrics, the same pragmatic approach was applied to identify drivers of mixture risks. RQ, TU and HU values of the mixture components were summed up individually, starting with the lowest value (i.e. compound with the lowest calculated risk) and ending with the highest value (i.e. compound with the highest calculated risk). Those compounds that in the sum triggered an exceedance of the threshold of 1 were classified as “mixture components of concern”. Components occurring above a risk threshold of 10, reflecting a tenfold effluent dilution in the receiving waters, were prioritized as “mixture components of high concern”. In a consensus approach, all chemicals prioritized by at least two risk metrics as mixture components of concern were ranked as “consensus mixture components of concern”. Those chemicals that were identified by at least two metrics as mixture components of high concern were ranked as “consensus mixture components of high concern”.

3. Results

3.1. Detected compounds and use groups

All analysed and detected compounds are summarized in Table S5 (dataset A) and Table S6 (dataset B), respectively. In addition, all results are available on PANGAEA (Finckh et al., 2022). In the following section on the results, dataset B (Table S6) was used as the default of all figures, where concentrations below the MDL were set to MDL/2.

Within the set of 56 European WWTP effluent samples, 366 out of 499 analysed target compounds were detected in at least one sample (Fig. S1, left). In most effluents, between 200 and 250 compounds were detected (Fig. S1, right); 107 compounds occurring almost ubiquitously with a detection frequency of at least 90%, while 53 compounds were detected in less than 10% of the samples and 18 compounds were detected only once. Compounds were assigned to their specific use groups based on the information available from databases (Drugbank, Pubchem, PPDB, and Wikipedia) and from literature. The list of detected

target compounds includes a variety of pharmaceuticals and pesticides, but also surfactants, food, plastic and rubber additives, per- and poly-fluoroalkyl substances (PFAS), UV filters and corrosion inhibitors, summarized in a group called “others”. In total, 111 pharmaceuticals, 96 pesticides and 98 other parent compounds were detected, complemented by 12, 39 and 10 transformation products (TPs), respectively (Fig. 1, right). Within the three use group categories (pharmaceuticals, pesticides and others), the fractions of detected versus analysed compounds were 79%, 69% and 73%, respectively, and thus quite similar (Fig. 1, left).

Generally, a compound may be used for many purposes. To avoid complex multiple entries, compounds were categorized based on the best-known use or the main field of application. Pesticides were further classified according to their registration as plant protection products (PPPs, approved according to regulation (EC) 1107/2009), biocides (not approved as PPPs but used as biocides), and legacy pesticides (no current approval/approval expired). However, the legal provisions are not always clear, as some substances are subject to exemptions at national level for certain areas of application. Diazinon, for example, has been banned as a biocide in the EU since 2011 (Directive 98/8/EC), but it may still be used for veterinary purposes. As a check of the content declarations of animal products showed, it is indeed used for this purpose, which is why it is remaining in the category for biocides.

3.2. Concentration ranges of measured compounds

Concentrations of the detected target compounds range from less than 1 ng/L up to more than 10⁵ ng/L. Concentration ranges of the top 30 compounds by the 95th percentile of the concentration (MEC95) are displayed as a boxplot (Fig. 2). Among them are ten detected target compounds with median concentrations above 1 µg/L: The sweeteners sucralose (15.3 µg/L) and acesulfame (1.9 µg/L), the corrosion inhibitors 1H-benzotriazole (3.6 µg/L) and 5-methyl-1H-benzotriazole (1.8 µg/L), certain well known pharmaceuticals, such as hydrochlorothiazide (2.3 µg/L), metformin (1.6 µg/L), diclofenac (1.4 µg/L), and the TP N-formyl-4-aminoantipyrine (1.0 µg/L), as well as the industrial chemical 4'-aminoacetanilide (1.7 µg/L) and the UV-filter phenylbenzimidazole sulfonic acid (1.3 µg/L). Further compounds of high detected concentrations are the pharmaceuticals gabapentin-lactam, telmisartan, N-acetyl-4-aminoantipyrine, tramadol, furosemide, candesartan, valsartan and lamotrigine. The compilation is completed with cyclohexylamine, benzophenone-4, hexa(methoxymethyl)melamine (HMMM), m-xylene-4-sulfonic acid, cyclamate, 2-(methylthio)benzothiazole, and the pesticide 2,4-dichlorobenzoic acid.

Very high maximum concentrations (Fig. S2) were retrieved for several industrial chemicals, including hexa(methoxymethyl)melamine

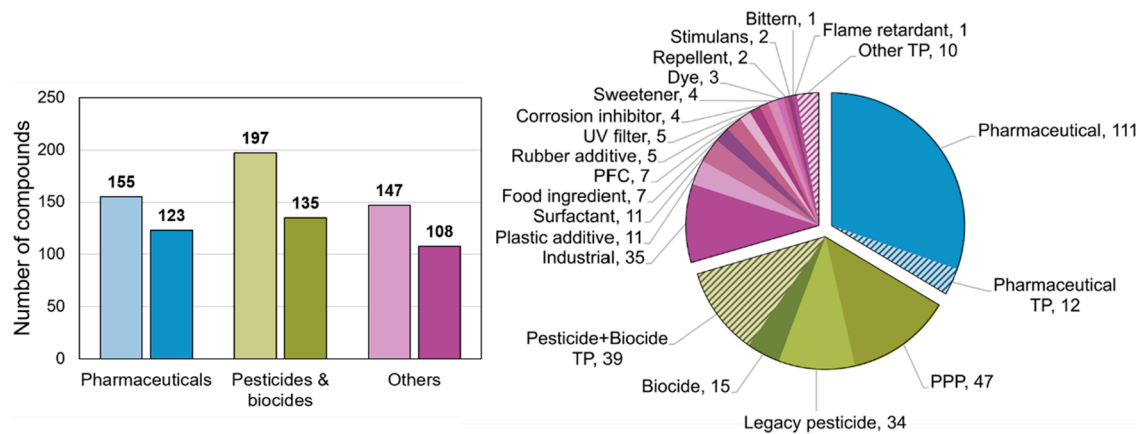


Fig. 1. Quantitative LC-HRMS screening results. Left: number of analysed (light coloured bar) versus detected (dark coloured bar) target compounds per use group category. Right: pie-chart of the detected target compounds per use group category: pharmaceuticals (blue), pesticides & biocides (green), others (purple). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

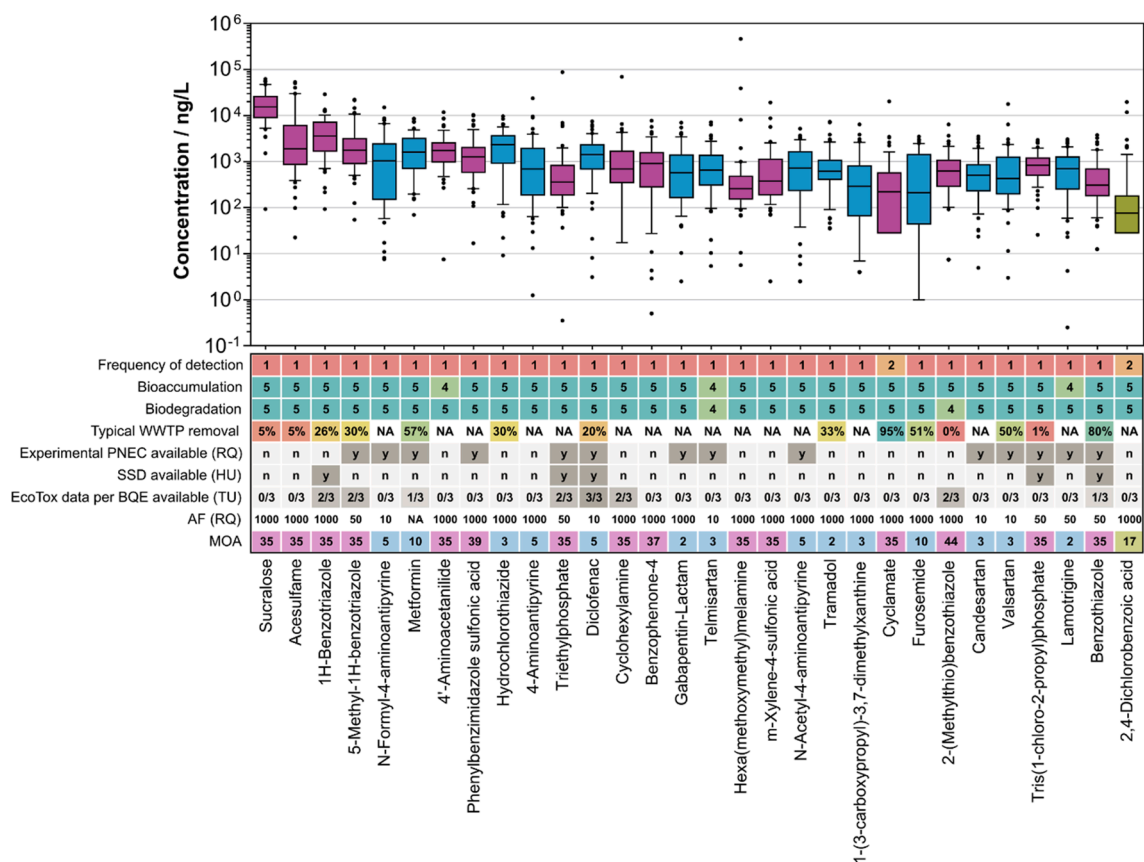


Fig. 2. Top 30 compounds by MEC95 (based on dataset B; NA = MDL/2). Boxplot hinges are representing the 25th and 75th percentile, and the upper/lower whiskers: $\pm 1.5 \cdot \text{IQR}$ (interquartile range). The centre line represents the median concentration. Information on the MOA categories are presented in Table 2. Experimental PNECs include regulatory-adapted EQS, as well as country specific EQS or proposals. The respective data can be found in the supporting information (Table S6).

(HMMM) (461 $\mu\text{g/L}$), tetrapropyl ammonium (117 $\mu\text{g/L}$), triethylphosphate (88 $\mu\text{g/L}$), cyclohexylamine (70 $\mu\text{g/L}$), 2,4-dichlorobenzoic acid (20 $\mu\text{g/L}$), m-xylene-4-sulfonic acid (19 $\mu\text{g/L}$), tetraglyme (19 $\mu\text{g/L}$), and aminoacetanilide (12 $\mu\text{g/L}$). The second-highest maximum concentration was found for the hypnotic and anaesthetic drug secobarbital (150 $\mu\text{g/L}$); the related sedative pentobarbital (14 $\mu\text{g/L}$) was detected in the same effluent.

3.3. WWTPs with advanced treatment via ozonation or AC

Within the set of 56 WWTP effluent samples, four samples have passed an advanced treatment via ozonation (3) or AC (1), resulting in lower measured concentrations. For each ozonation sample, an additional sample upstream of the advanced treatment was taken. For AC, no additional sample was taken. In the six ozonation-related samples, a total of 262 compounds were detected (Fig. S3, left). For 39% (103/262) of the detected compounds, a concentration reduction by at least half was observed (Fig. S3, right). If considering the fact that certain compounds were present and reduced only in two or one WWTP, this ratio increases to 64% (167/262) and 89% (233/262), respectively. Examples for compounds with very high reductions (less than 5% remaining) and high initial average concentrations are: phenazone, N-acetyl-4-aminoantipyrine, clarithromycin, carbamazepine, diclofenac, benzophenone-4, trimethoprim, N-formyl-4-aminoantipyrine, and azithromycin. The effect of ozonation on high and low concentrated compounds was rather similar, as visualized in the second heatmap on scaled concentrations of compounds reduced in all three samples by more than 50% (Fig. S3, right).

3.4. Persistency and bioaccumulation potential of detected compounds

The compounds detected in our study were screened for persistency and bioaccumulation potential, representing hazard criteria in EU REACH regulation (REGULATION (EC) No 1907/2006). Due to a lack of consistent databases on experimental data, the predicted half-life time ($\text{DT}_{50, \text{pred}}$) as indicator for persistency and the predicted bioconcentration factor (BCF_{pred}) as indicator for the bioaccumulation potential were applied, both calculated with OPERA (Mansouri et al., 2018). According to this approach, 49% of all detected compounds and 90% of the top 30 compounds by the MEC95 (Fig. 2), respectively, have a predicted BCF_{pred} below 10 represented by score 5 for the lowest priority regarding the bioconcentration potential. For 85% of the detected compounds and all top 30 compounds, the BCF_{pred} were below 100 (scores 4 or 5), while only 2% of all detected compounds exhibit a predicted BCF_{pred} above 1000 (scores 2 and 1).

Predicted half-life times of 88% and 93% of all detected compounds and the top 30 compounds (Fig. 2), respectively, are below 15 days, indicating chemicals that belong to the least persistent group (score 5). For 95% of the detected compounds and for all top 30 compounds, DT_{50} s below 37.5 days (score 4) were predicted.

To enhance discriminative power, the persistency scoring was complemented with experimentally determined removal rates in WWTPs compiled by Margot et al. (2015). These values were available for 14 out of the top 30 compounds by MEC95 (Fig. 2). According to this information, the artificial sweeteners sucralose (5%) and acesulfame (5%), the plastic additive and flame retardant tris(1-chloro-2-propyl)phosphate (1%) as well as 2-(methylthio)benzothiazole (0%) are only poorly degradable. The pharmaceutical hydrochlorothiazide (30%),

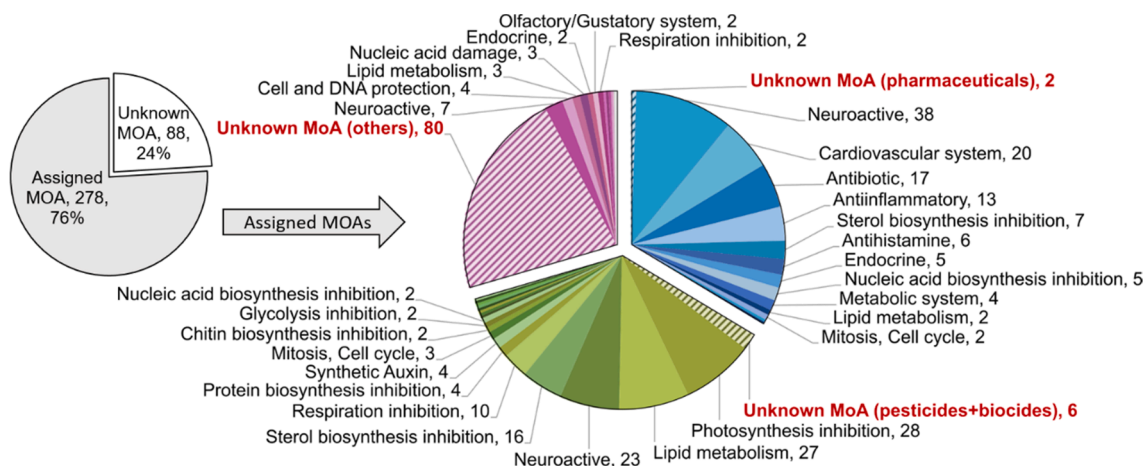


Fig. 3. Mode of action information for detected compounds. Left: fraction of assigned and unknown MOAs. Right: pie-chart of higher-level MOA information of the detected target compounds according to the three main use groups; pharmaceuticals (blue), pesticides & biocides (green), others (purple). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

diclofenac (20%), and tramadol (33%) add to this list of rather persistent wastewater components. Cyclamate (95%), and benzothiazole (80%), in comparison, are considered to be highly degradable compounds.

3.5. Modes of action of detected compounds

In our study, 278 of the 366 detected compounds (76%) could be assigned to at least one specific MOA (Fig. 3, left). The list contains 130 specific MOAs (Fig. S4), grouped in 27 higher-level categories (Fig. 3, right), in which MOAs relevant for different taxa are considered. Within the group of pharmaceuticals 13 MOA categories could be discriminated, with compounds which interact with the nervous system being the most prominent category (38), followed by compounds acting on the cardiovascular system (20), antibiotics (17) and anti-inflammatories (13), including 3, 4, 2 and 2 TPs, respectively. For pesticides and biocides, 19 MOA categories were identified, led by photosystem inhibitors (28) and followed by disruptors of the lipid metabolism (27), neuroactive chemicals (17) and sterol biosynthesis inhibitors (16), including 2, 3, 3 and 1 TPs, respectively. Since both pharmaceuticals and pesticides are designed to have a specific effect on biota, only few of them are lacking MOA information (2 and 6, respectively). In case of the third use group “others”, almost three quarter of the chemicals (74%) lack MOA information. This group includes plastic or rubber additives, corrosion inhibitors and surfactants, among others, but mainly industrial compounds of unknown use groups (35). For 40% of the top 30 compounds by MEC95, no specific MOA was found (Fig. 2).

3.6. Availability of toxicity data for detected compounds

Environmental risk assessment of contamination strongly relies on reliable toxicity data. Experimental PNEC values from the NORMAN Ecotoxicology Database, based on experimental endpoints (incl. regulatory-adopted EQS values or (*ad-hoc*) proposals), were available for 43% of the detected target compounds (Fig. S5, Table S7), while PNECs of the remaining compounds were predicted (55%) or not available (2%). Fractions of 47% of the top 30 compounds by MEC95 are covered by experimental PNECs (Fig. 2). In the case of HU_{ch} for 108 of the 366 detected chemicals (29%) chronic SSDs were available and considered sufficiently reliable (Fig. S5, Table S7). This corresponds to 17% of the top 30 MEC95 compounds (Fig. 2). In the largest experimental toxicity database (US EPA ECOTOX DB), experimental EC_{50} values needed for the TU calculation for all three BQEs were only available for 60 of the 366 detected compounds (16%). For 48 compounds (13%) there were measured values for two BQEs, and for 50 compounds (14%) there were

measured values for only one BQE. For 208 compounds (57%) no measured toxicity data was available (Fig. S5, Table S7). This implies that for 70% of the total of 1098 assessments (366 detects multiplied by 3 BQEs), only predicted effect concentrations based on QSARs for baseline toxicity have been used, neglecting specific effects. For the top 30 compounds ranked by their MEC95, only one compound (diclofenac) was supported by experimental EC values for all BQEs, for 17% of the compounds two BQEs were covered and for 7% only for one BQE experimental data could be found (Fig. 2).

3.7. Risk assessment according to RQ, TU and HU

3.7.1. Availability of toxicity data of highly ranked compounds

For 47% of the top 30 compounds selected by the 95th percentile of the MEC/PNEC ratios (RQ95), experimental PNECs are available (Fig. 4), while 53% of the PNECs are predicted. For 57% of the top 30 compounds, an AF of 1000 is considered. When ranking compounds according to the 95th percentile of MEC/ EC_{50} ratios (TU95), 87%, 87% and 73% of the top 30 compounds for algae, crustacean and fish, respectively, could be assessed based on measured effect concentrations (Figs. 6-8). High-risk chemicals with lacking experimental toxicity data included the quaternary ammonium compounds (QACs) benzyltrimethyltetradecyl ammonium, didecyltrimethyl ammonium, benzyltrimethyltetradecyl ammonium and didecyltrimethyl ammonium, which are mainly used as biocides for surface disinfection, the pharmaceuticals telmisartan, celecoxib, lamotrigine, clotrimazole and secobarbital, and the industrial compounds HMMM, tris(2-ethylhexyl)phosphate and tris(2-ethylhexyl)phosphate. For 40%, 70%, 67% and 60% of the compounds prioritized based on RQ95, TU95_{algae}, TU95_{crust.} and TU95_{fish}, chronic SSDs were available (Figs. 4 & 6-8) allowing for HU-based prioritization. For the remaining compounds highly ranked by RQ95 or TU95, no SSDs were available; consequently no HU_{ch} value could be calculated for them. Therefore, the list of the top 30 compounds according to the 95th percentile of HU_{ch} ($HU95$) (Fig. 5) does not include the above mentioned QACs, vancomycin and HMMM, among others. SSDs were always missing when no or not sufficient experimental toxicity data were available for RQs and TUs.

3.7.2. Individual risk assessment of detected compounds

The list of top 30 compounds, ranked by RQ95 (Fig. 4) start with the pharmaceuticals, vancomycin, diclofenac and azithromycin, of which the first is prioritized based on a predicted PNEC. In addition, highly ranked compounds include the industrial compounds HMMM, perfluorooctanesulfonic acid and 2-(methylthio)benzothiazole, the

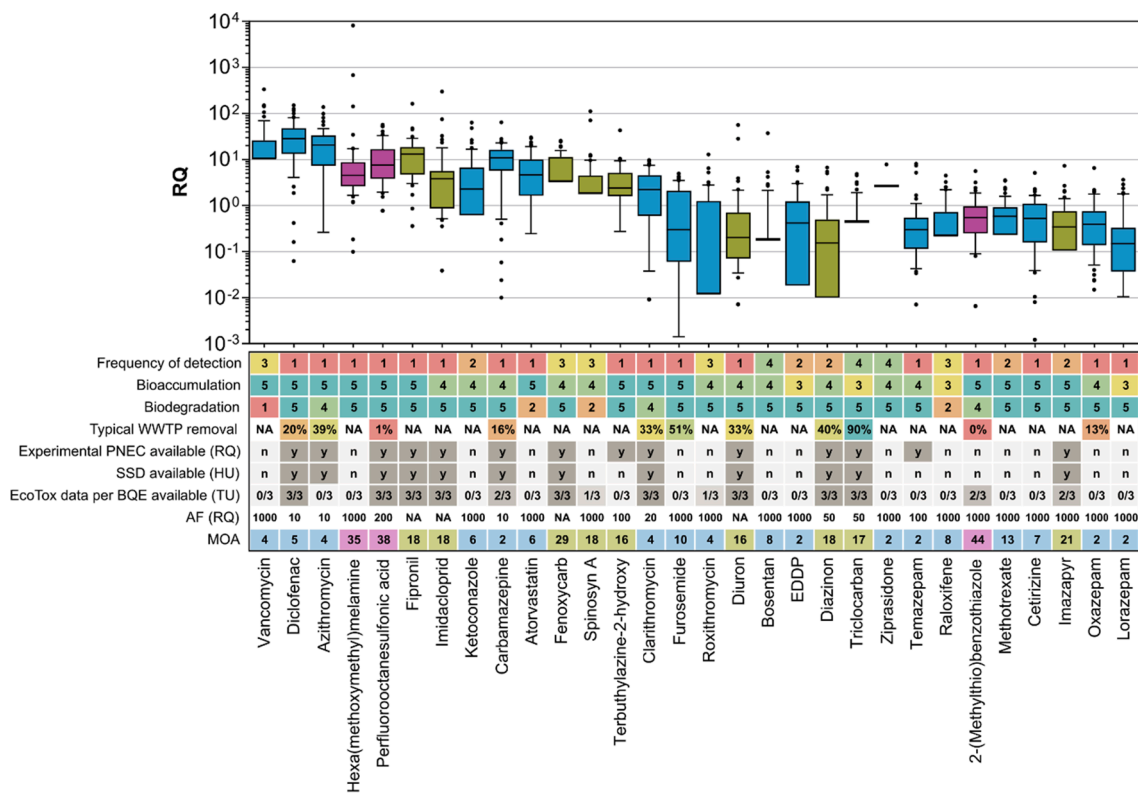


Fig. 4. Top 30 compounds by RQ95 (based on dataset B; NA = MDL/2). Boxplot hinges are representing the 25th and 75th percentile, and the upper/lower whiskers: ±1.5*IQR (interquartile range). The centre line represents the median concentration. Information on the scores (rows 1–3) are presented in Table 1. Information on the MOA categories are presented in Table 2. Experimental PNECs include regulatory-adapted EQS, as well as country specific EQS or proposals. The respective data can be found in the supporting information (Table S8).

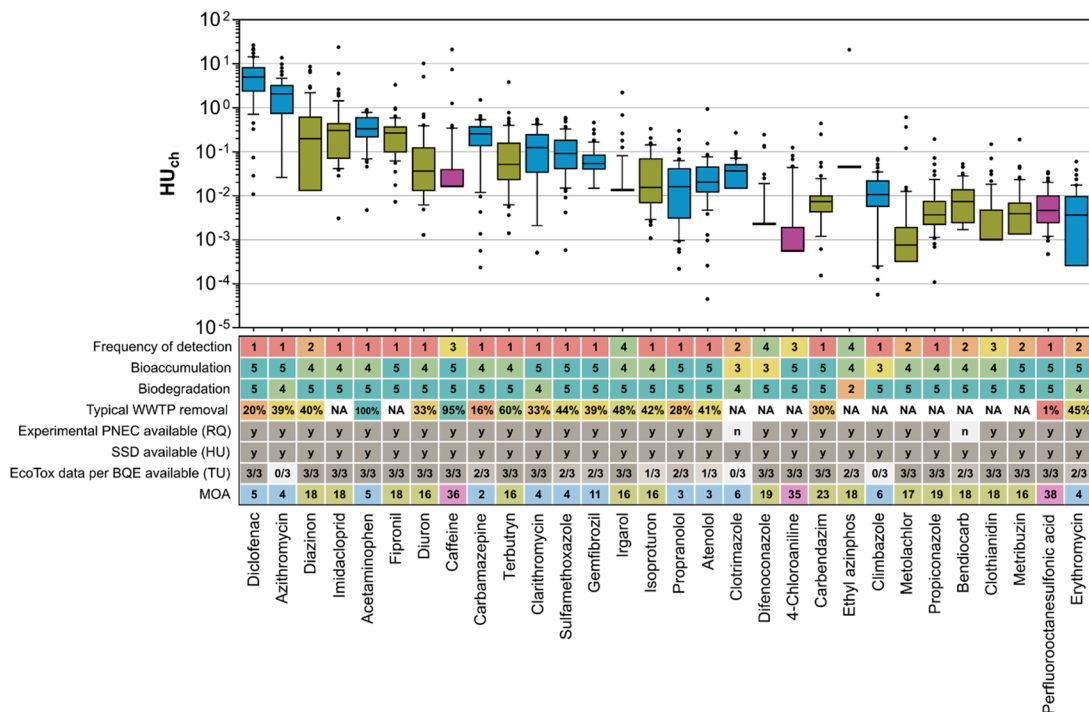


Fig. 5. Top 30 compounds by HU95 (based on dataset B; NA = MDL/2). Boxplot hinges are representing the 25th and 75th percentile, and the upper/lower whiskers: ±1.5*IQR (interquartile range). The centre line represents the median concentration. Information on the scores (rows 1–3) are presented in Table 1. Information on the MOA categories are presented in Table 2. Experimental PNECs include regulatory-adapted EQS, as well as country specific EQS or proposals. The respective data can be found in the supporting information (Table S9).

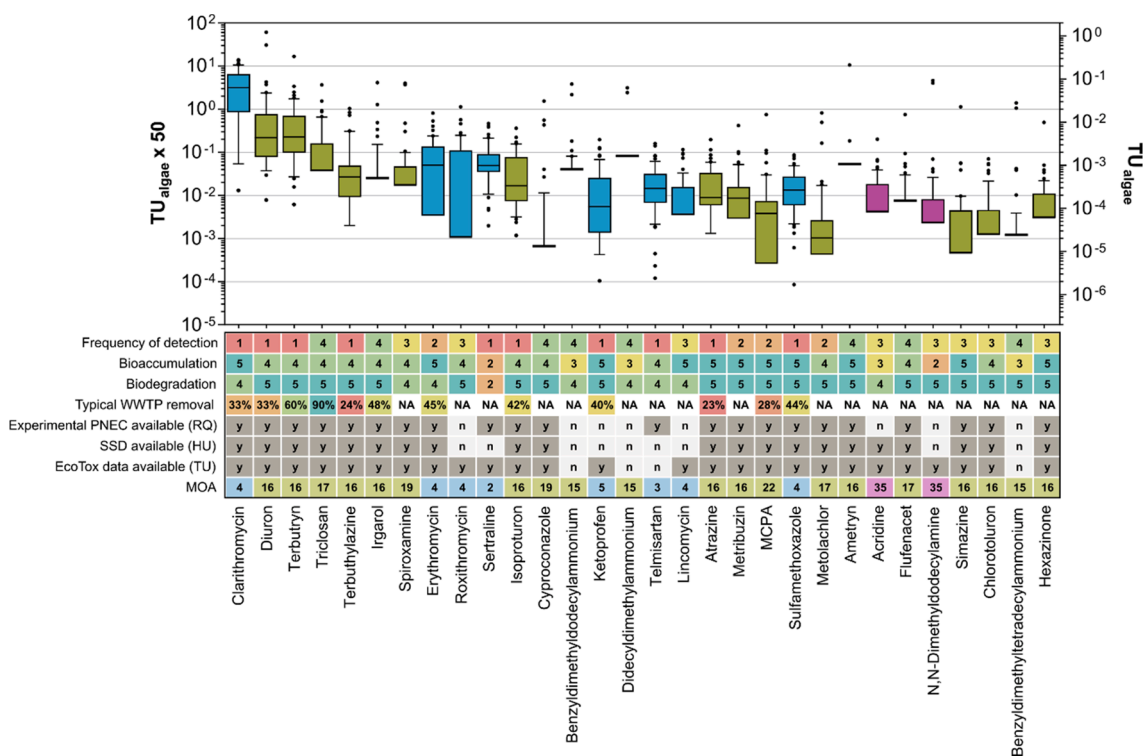


Fig. 6. Top 30 compounds by TU95_{algae} (based on dataset B; NA = MDL/2). Boxplot hinges are representing the 25th and 75th percentile, and the upper/lower whiskers: ±1.5*IQR (interquartile range). The centre line represents the median concentration. Information on the MOA categories are presented in Table 2. Experimental PNECs include regulatory-adapted EQS, as well as country specific EQS or proposals. The respective data can be found in the supporting information (Table S10).

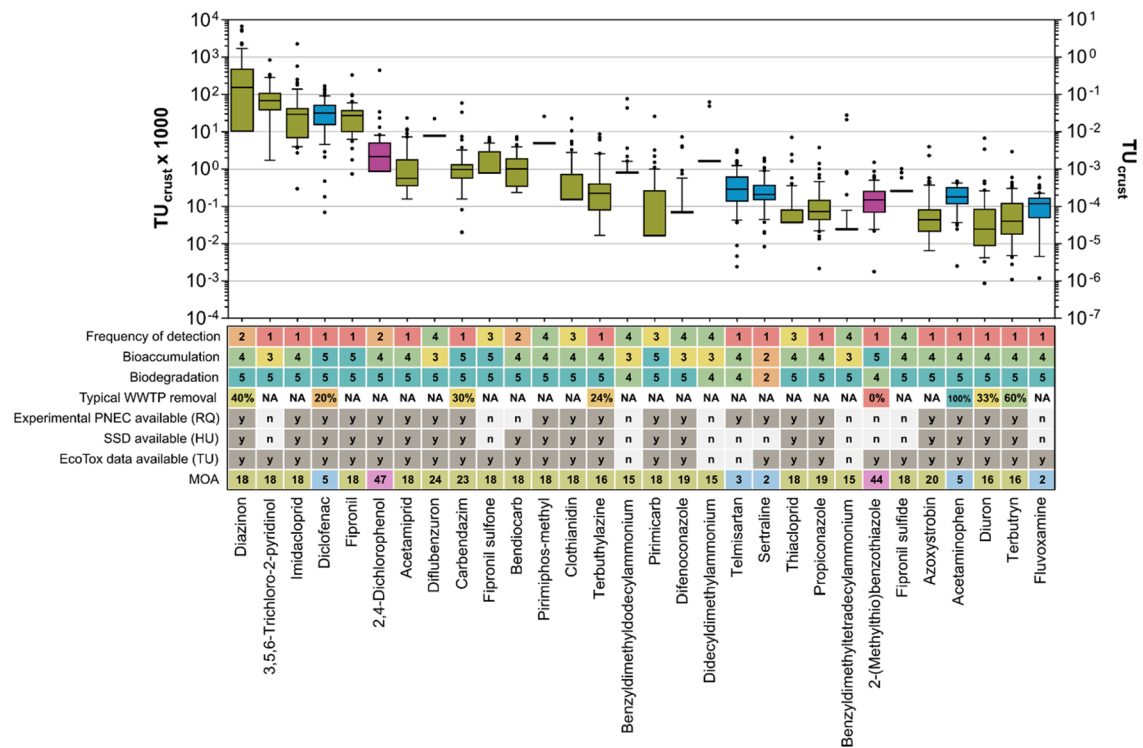


Fig. 7. Top 30 compounds by TU95_{crust} (based on dataset B; NA = MDL/2). Boxplot hinges are representing the 25th and 75th percentile, and the upper/lower whiskers: ±1.5*IQR (interquartile range). The centre line represents the median concentration. Information on the MOA categories are presented in Table 2. Experimental PNECs include regulatory-adapted EQS, as well as country specific EQS or proposals. The respective data can be found in the supporting information (Table S11).

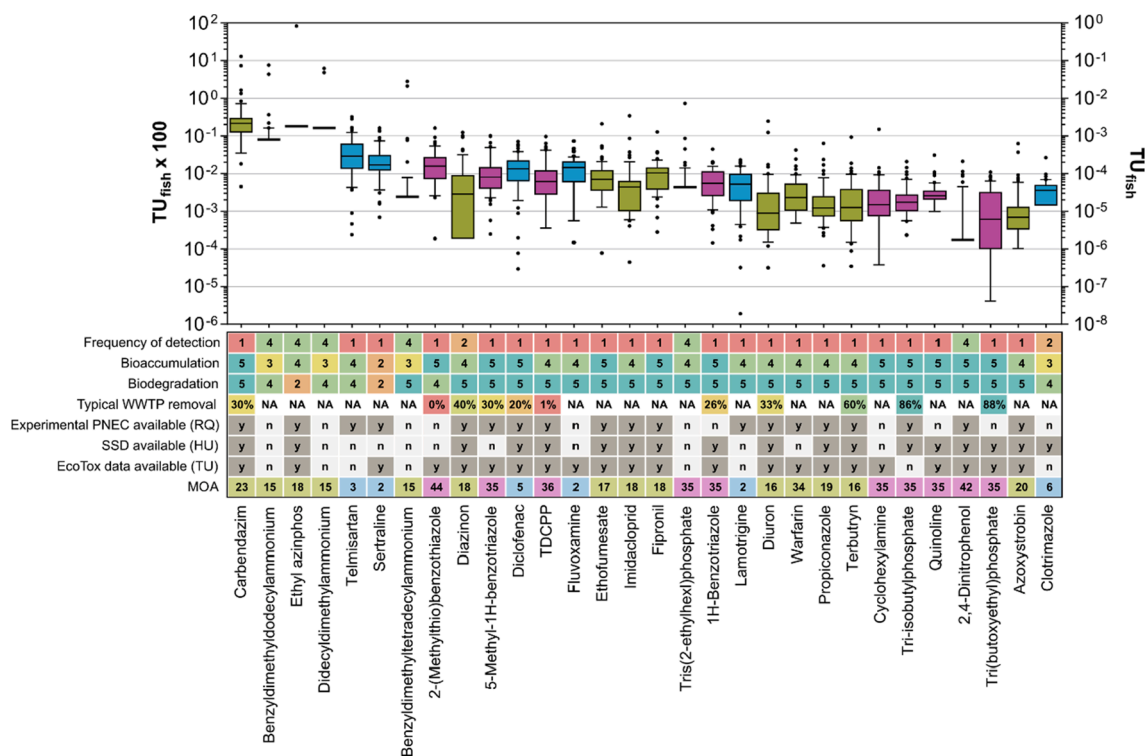


Fig. 8. Top 30 compounds by $TU_{95_{fish}}$ (based on dataset B; NA = MDL/2). Boxplot hinges are representing the 25th and 75th percentile, and the upper/lower whiskers: $\pm 1.5 \cdot IQR$ (interquartile range). The centre line represents the median concentration. Information on the scores (rows 1–3) are presented in Table 1. Information on the MOA categories are presented in Table 2. Experimental PNECs include regulatory-adapted EQS, as well as country specific EQS or proposals. The respective data can be found in the supporting information (Table S12).

pesticides fipronil (I), imidacloprid (I), fenoxycarb (I), spinosyn A (I), terbuthylazine-2-hydroxy (H-TP), diuron (H), diazinon (I), triclofuran (B) and imazapyr (H), and further pharmaceuticals such as ketoconazole, carbamazepine, atorvastatin, clarithromycin, furosemide, roxithromycin, bosentan, EDDP, ziprasidone, temazepam, raloxifene, methotrexate, cetirizine, oxazepam and lorazepam.

According to the assessment based on HU_{95} (Fig. 5), the greatest chronic impact is expected from the pharmaceuticals diclofenac and azithromycin, followed by several pesticides including diazinon (I), imidacloprid (I), and further down also fipronil (I), diuron (H), terbuthylazine (H), irgarol (H), isoproteron (H), difenoconazole (F), carbendazim (F), ethyl azinphos (I), metolachlor (H), propiconazole (F), bendiocarb (I), clothianidin (I) and metribuzin (H). Additionally, the pharmaceuticals acetaminophen, carbamazepine, clarithromycin, sulfamethoxazole, gemfibrozil, propranolol, atenolol, clotrimazole, climbazole and erythromycin, as well as other compounds such as caffeine, 4-chloroaniline and perfluorooctanesulfonic acid were in the top risk group.

Based on TU_{95} , the greatest risk for algae (Fig. 6) was observed for the antibiotic clarithromycin, followed by several herbicides including diuron, terbuthylazine, terbuthylazine and irgarol, the biocide triclosan, the fungicide spiroxamine, as well as the pharmaceuticals erythromycin, roxithromycin and sertraline. All three top contributors including clarithromycin, diuron and terbuthylazine were detected with high frequency. Greatest TU_{95} -based risks to crustaceans (Fig. 7) are associated with the insecticides diazinon, 3,5,6-trichloro-2-pyridinol and imidacloprid, further down followed by fipronil, acetamiprid and diflubenzuron, complemented by the pharmaceutical diclofenac and the industrial chemical 2,4-dichlorophenol. The seven top-ranked compounds also exhibit high detection frequencies (scores 1 or 2). For fish, the highest TU (Fig. 8) are exhibited by the fungicide carbendazim, different QACs, the insecticide ethyl azinphos and the pharmaceuticals telmisartan and sertraline. Here, only carbendazim and the two pharmaceuticals are characterized by a high detection frequency. The QACs, which were

often ranked very high, were detected in few WWTP effluents; the underlying TU s were based on predicted EC_{50} values (baseline toxicity). In general, among the top 30 compounds for fish, a great diversity of compound classes and use groups were found.

For the top 30 compounds according to the 95th percentile of RQ, HU_{ch} , TU_{algae} , TU_{crust} , and TU_{fish} , the number of compounds with unknown MOAs (MOA 1, 15 or 35) is 1, 1, 5, 3 and 10 (Figs. 4–8), respectively. Compared to the previous ranking by concentration (MEC95) with 12 compounds of unknown MOA (Fig. 2), these numbers are much lower. Among the compounds with specific MOA information, but without measured toxicity data for any BQE were phenylbenzimidazole sulfonic acid (MOA 39 “nucleic acid damage”), hydrochlorothiazide, 1-(3-carboxypropyl)-3,7-dimethylxanthine, candesartan and valsartan (MOA 3 “cardiovascular system”), benzophenone-4 (MOA 37 “cell and DNA protection”), gabapentin-lactam and tramadol (MOA 2 “neuroactive”). For few transformation products, including N-formyl-4-aminoantipyrine and 4-aminoantipyrine (TPs of dimethylaminophenazone, MOA 5 “antiinflammatory”) N-acetyl-4-aminoantipyrine, and 2,4-dichlorobenzoic acid (TP of spirodiclofen, MOA 17 “lipid metabolism”), only the MOA of the parent compounds is known.

3.7.3. Mixture risk assessment of detected compounds

Mixture toxicity estimates for the different metrics are shown as bar plots (Figs. 9–13). The numbers above each bar indicate the number of compounds contributing to the exceedance of the chronic risk threshold of 1 (bold line); all numbers and names of these components of concern are listed in Tables S13 and S14, respectively. Using RQ summation, all samples exceeded the threshold of 1 with the highest mixture risk of 8400 (EU009), exceeding by almost 4 orders of magnitude (Fig. 9), while the lowest risk was found for EU124 and the advanced treatment sites (EU032, EU128, EU019, and EU130) with a degree of exceedance of 33 to 91. Between 49 (EU124) and 183 (EU018) compounds contributed to the exceedance of the risk threshold.

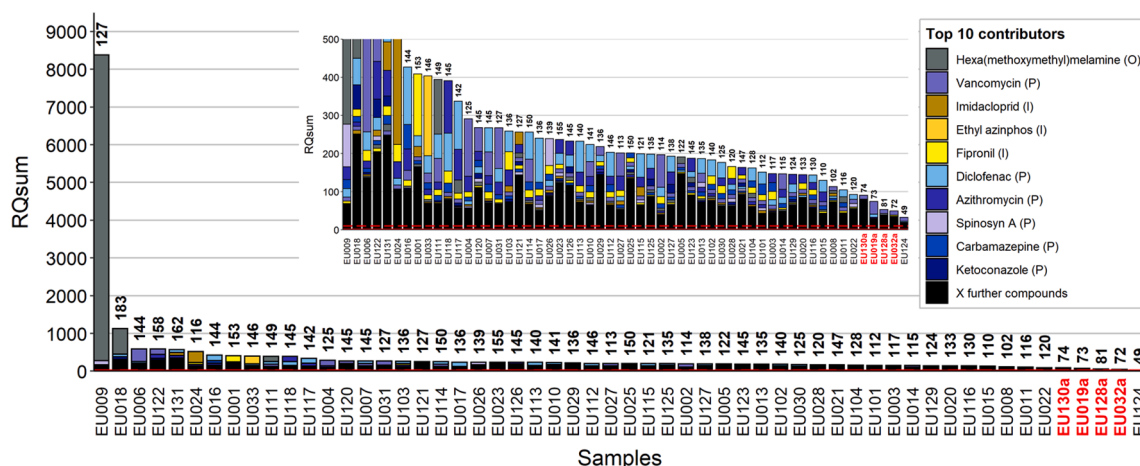


Fig. 9. Risk driving compounds according to RQsum (based on dataset B; NA = MDL/2). The bars represent RQsum for each sample. The coloured stacks within the bars represent the compound-specific RQs of the top 10 contributors by maximum RQ (over all samples), and are sorted by increasing values. Further compounds are aggregated in the black coloured stacks at the bottom of each bar (“X further compounds”). The bold line indicates the risk threshold of 1. The dashed line indicates the risk threshold incl. a dilution factor of 10. Samples from WWTPs with advanced treatment are highlighted in red (suffix “a”). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

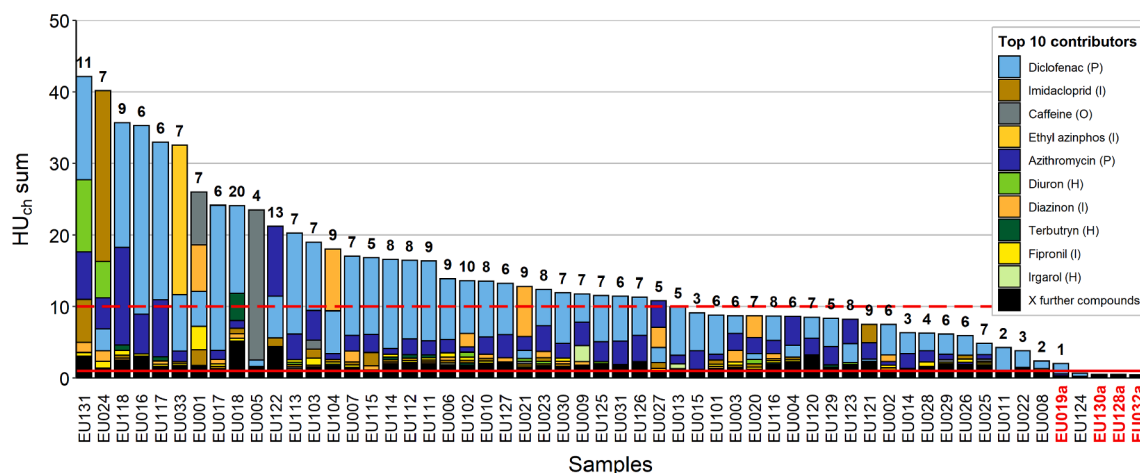


Fig. 10. Risk driving compounds according to HU_{ch}sum (based on dataset B; NA = MDL/2). The bars represent HU_{ch}sum for each sample. The coloured stacks within the bars represent the compound-specific HU_{ch}s of the top 10 contributors by maximum RQ (over all samples), and are sorted by increasing values. Further compounds are aggregated in the black coloured stacks at the bottom of each bar (“X further compounds”). The bold line indicates the risk threshold of 1. The dashed line indicates the risk threshold incl. a dilution factor of 10. Samples from WWTPs with advanced treatment are highlighted in red (suffix “a”). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The calculated HU_{ch}sum of the different samples covered a range of two orders of magnitude (Fig. 10). In many WWTP effluents, diclofenac was the major risk driver, followed by azithromycin and diazinon. Examples for more site-specific risk drivers are the insecticides imidacloprid (EU131, EU024), ethyl azinphos (EU033) and fipronil (EU001), the herbicides diuron (EU131, EU024), terbutryn/prometryn (co-eluting compounds) (EU018) and irgarol (EU009) as well as the food ingredient caffeine (EU001, EU005). All samples except the three treated with ozonation (EU032, EU128, EU130) and EU124 exceeded the risk threshold, with one (EU019) to 20 (EU018) contributing compounds.

Based on BQE-specific TU_{sum}, 91%, 100% and 54% of the WWTP effluents exceed the risk threshold maximum by a factor of 71, 6900 and 84 for algae, crustaceans and fish, respectively (Figs. 11–13), covering two to nearly three orders of magnitude. The maximum number of compounds contributing to the exceedance was 20, 52 and 7 compounds (all in EU018) for the three BQEs. Diazinon and imidacloprid predominate mixture risks to crustaceans, while major contributors to TU_{sum}algae were clarithromycin and some herbicides. Similar to the other

risk metrics, TU_{sum} of effluents from the treatment plants with advanced treatment (ozonation and AC) were the lowest, with only slight exceedance for crustaceans.

4. Discussion

4.1. Detected compounds

Compared to the most comprehensive study on ECs in treated wastewater from one WWTP effluent (Gago-Ferrero et al., 2020), the number of detected compounds could be further enhanced from 315 to 366. Compared to four of the most recent and largest wide-scope target studies (Alygizakis et al., 2019; Gago-Ferrero et al., 2020; Munz et al., 2017; Nickel et al., 2021), we quantified 218 additional compounds not yet detected, which include 51 pharmaceuticals, 84 pesticides and biocides and 83 other compounds. Some of these compounds have been detected in more than one third of the samples and are prioritized by at least two metrics later in the consensus prioritization (Tables 3, S14), including HMMM (56/56), 7-diethylamino-4-methylcoumarin (55/56),

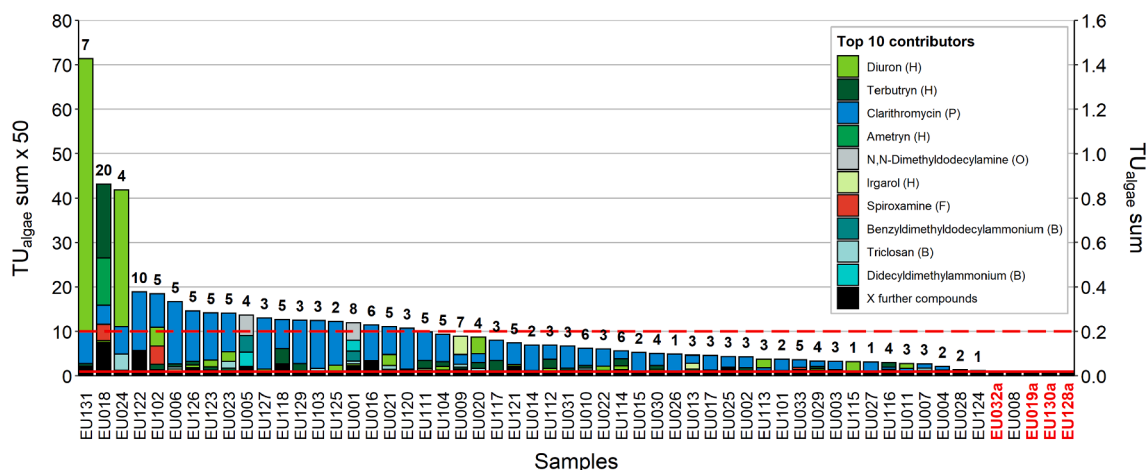


Fig. 11. Risk driving compounds according to $TU_{\text{algae sum}}$ (based on dataset B; $NA = MDL/2$). The bars represent $TU_{\text{algae sum}}$ for each sample. The coloured stacks within the bars represent the compound-specific TUs of the top 10 contributors by maximum TU (over all samples), and are sorted by increasing values. Further compounds are aggregated in the black coloured stacks at the bottom of each bar (“X further compounds”). The bold line indicates the risk threshold of 1. The dashed line indicates the risk threshold incl. a dilution factor of 10. Samples from WWTPs with advanced treatment are highlighted in red (suffix “a”). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

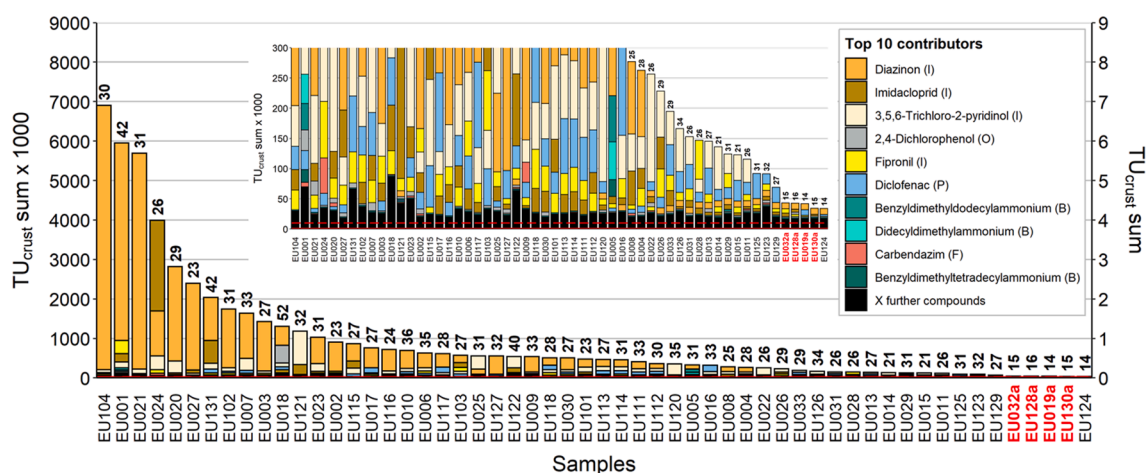


Fig. 12. Risk driving compounds according to $TU_{\text{crust sum}}$ (based on dataset B; $NA = MDL/2$). The bars represent $TU_{\text{crust sum}}$ for each sample. The coloured stacks within the bars represent the compound-specific TUs of the top 10 contributors by maximum TU (over all samples), and are sorted by increasing values. Further compounds are aggregated in the black coloured stacks at the bottom of each bar (“X further compounds”). The bold line indicates the risk threshold of 1. The dashed line indicates the risk threshold incl. a dilution factor of 10. Samples from WWTPs with advanced treatment are highlighted in red (suffix “a”). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2,4-dichlorophenol (35/56), N,N-dimethyldodecylamine (21/56) and spiroxamine (21/56).

Some of the 61 TPs found in total were detected as often as, or even more frequently than their parent compounds. For example, acetyl-sulfamethoxazole (52 detects), a TP of sulfamethoxazole (55), metoprolol acid (51), a TP of the beta blocker metoprolol (56) and several carbamazepine TPs, which occurred ubiquitously, were found in similar numbers. The TPs of the legacy pesticides metolachlor (29) and metazachlor (18), metolachlor OA and metazachlor ESA were detected almost twice as often, namely in 52 and 46 samples, respectively. The frequent detection of TPs of pharmaceuticals, pesticides and “other” compounds is in good agreement with previous studies in the U.S. (Mahler et al., 2021), and Germany (Halbach et al., 2021; Kiefer et al., 2019), detecting higher numbers of TPs in higher concentrations compared to parent pesticides. Since biological wastewater treatment may transform parent chemicals to several stable TPs (Petrie et al., 2015; Richardson and Ternes, 2018), the actual number of TPs is probably much higher than covered by the current target screening. It should also be considered that screening approaches necessarily disregard

chemicals that are out of the method domain or can be detected only at MDLs above effect thresholds, including metals, glyphosate, pyrethroids and certain steroids.

4.2. Concentration ranges

Many compounds detected in high concentrations are common wastewater-related contaminants and in agreement with previous studies. The so far most comprehensive monitoring study on European WWTP effluent samples by Loos et al. (2013) showed acesulfame at place one based on median concentration ranking (14.3 $\mu\text{g/L}$), followed by 1H-benzotriazole (2.7 $\mu\text{g/L}$), 5-methyl-1H-benzotriazole (2.1 $\mu\text{g/L}$), sucralose (1.7 $\mu\text{g/L}$) and carbamazepine (0.8 $\mu\text{g/L}$), which in our study showed median concentrations of 1.9 $\mu\text{g/L}$, 3.6 $\mu\text{g/L}$, 1.8 $\mu\text{g/L}$, 15.3 $\mu\text{g/L}$ and 0.5 $\mu\text{g/L}$, respectively. Hence, except for acesulfame and sucralose having switched the position, similar median concentrations were measured (Fig. 2). Moreover, the diuretic hydrochlorothiazide, the diabetes drug metformin, the analgesics diclofenac and tramadol, lamotrigine as another anticonvulsant, as well as different sartan

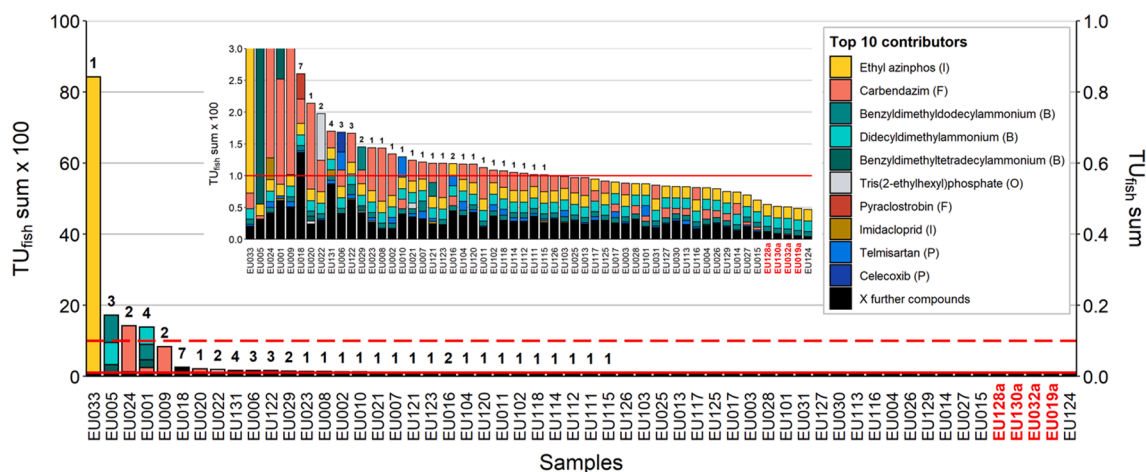


Fig. 13. Risk driving compounds according to $TU_{fishsum}$ (based on dataset B; $NA = MDL/2$). The bars represent $TU_{crustsum}$ for each sample. The coloured stacks within the bars represent the compound-specific TUs of the top 10 contributors by maximum TU (over all samples), and are sorted by increasing values. Further compounds are aggregated in the black coloured stacks at the bottom of each bar (“X further compounds”). The bold line indicates the risk threshold of 1. The dashed line indicates the risk threshold incl. a dilution factor of 10. Samples from WWTPs with advanced treatment are highlighted in red (suffix “a”). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

hypertension drug-derivatives were found in similar concentration ranges as in previous studies (Alygizakis et al., 2020; Gago-Ferrero et al., 2020; Margot et al., 2015). The 30 compounds that have been detected at the highest maximum concentrations in this study can be divided into two groups:

- (1) Point source industrial chemicals that are emitted at very high concentrations from industrial production sites and occur in low or non-detectable concentrations in other effluents, with typical maximum to median differences of more than two orders of magnitude (Fig. S2). This holds true for the compounds HMMM, secobarbital, tetrapropyl ammonium, triethylphosphate, cyclohexylamine, 7-diethylamino-4-methylcoumarin, 2,4-dichlorobenzoic acid, tetraglyme, pentobarbital, bentazone and 7-(ethylamino)-4-methylcoumarin. HMMM is used in the automotive industry for the formulation of lacquers. Secobarbital is not registered for medication in Europe, but is produced for the international market and has some application as an illicit drug. Together with pentobarbital, it has been reported previously in the Mulde and Elbe rivers (Hug et al., 2014; Krauss et al., 2019; Peschka et al., 2006; Schwarzbauer and Ricking, 2010). The high concentrations are discharged with a mixed industrial and municipal effluent from a WWTP, treating among others a production site for these chemicals, complemented with contaminated groundwater that contains high concentrations of the herbicide bentazone peaking in this effluent (LHW Sachsen-Anhalt, 2013). The three peak concentrations of tetrapropyl ammonium, triethylphosphate and cyclohexylamine were belonging to the same effluent. The first compound is a rarely reported chemical, and the second a plasticizer, used in the production of polyvinylchloride, polyester resins and polyurethane foam and reported also in high concentrations in previous studies (Kandie et al., 2020; Wei et al., 2015). Cyclohexylamine is an industrial chemical, but also a known TP of cyclamate (Renwick et al., 2004). 7-Diethylamino-4-methylcoumarin, which is used as fluorescent dye, together with its TP 7-(ethylamino)-4-methylcoumarin were detected previously in the receiving river Holtemme with a concentration of 14 $\mu\text{g/L}$ (Muschkett et al., 2018). 2,4-Dichlorobenzoic acid is a known pesticide TP and production intermediate of the insecticide spirotolifen (Kiefer et al., 2019), which is why it was classified as such. However, due to some very high peak concentrations in different WWTP effluents, it is expected to be linked rather to other unknown sources. Most of these chemicals have

rarely been detected before and highlight the need to complement target screening of known WWTP effluent components from domestic and industrial uses, with suspect screening, based on known sources and non-target screening to cover local point sources with unknown emissions. In general, such prominent maximum concentrations occur in 15 out of 56 WWTP effluents and indicate the great relevance of industrial emissions for surface water quality. Interestingly, also saccharin and caffeine were found in very high maximum to median ranges, however, rather indicating poor treatment efficiencies at individual WWTPs than industrial uses.

- (2) About half of the compounds with prominent maximum concentrations exhibit a relatively small maximum to median ratio, indicating ubiquitous detection at high concentrations and thus diffuse consumption in high volumes (Fig. S2). These compounds include particularly synthetic sweeteners (sucralose, acesulfame) that have been already previously detected in high $\mu\text{g/L}$ and even mg/L ranges (Loos et al., 2013), but also the two corrosion inhibitors (1H-benzotriazole and 5-methyl-1H-benzotriazole) and several pharmaceuticals (4-aminoantipyrine, valsartan, N-formyl-4-aminoantipyrine, atenolol, hydrochlorothiazide, metoprolol acid and metformin) used for the treatment of common diseases. Apart from the two UV-filters phenylbenzimidazole sulfonic acid and benzophenone-4, the high occurrence of the rarely analysed industrial compound 4'-aminoacetanilide also speaks for diffuse sources.

4.3. Fraction of persistent and bioaccumulating compounds

According to the predicted BCF, indicating the lipophilicity-driven bioaccumulation behaviour of organic chemicals, none of the top 30 compounds exhibited a substantial risk to bioaccumulate (Fig. 2). Since detectable water concentrations require sufficient water solubility and thus a rather low hydrophobicity (low $\log K_{OW}$) (Palma 2014), these results are expected. In order to address the risk of compounds that tend to bioaccumulate, other matrices, such as biosolids, should be sampled (which may pose a threat in areas where biosolids are used in agriculture).

High persistence of environmental contaminants, either due to slow biodegradation, low chemical reactivity or poor physical degradation by sun-light, is considered as a key driver for the risk of chemicals to accumulate in the environment until exceeding hazard thresholds (Cousins et al., 2019). Since (consistent) data on persistence of most

water contaminants are widely lacking, estimates typically rely on QSAR models such as OPERA (Mansouri et al., 2018). Applying half-life ranges adapted from recommendations under REACH scoring from 1 (poorly degradable, half-life time greater than 180 days) to 5 (easily degradable, half-life time less than 15 days) was not suitable to rank compounds by persistence (Fig. 2). For example, the WWTPs marker for poor degradability in treated wastewater, carbamazepine (Hai et al., 2018), and the marker for readily degradable substances in untreated wastewater, caffeine (Buerge et al., 2003) share the same score of 5. Experimental persistence information seems to have higher discriminative power and environmental realism, even if the number of compounds covered is limited and a few data are in contradiction to our findings (e.g.; $\mu\text{g/L}$ -range concentrations of acetaminophen in many WWTP effluents despite reported 100% degradability). High concentrations of readily degradable compounds such as saccharin, cyclamate and caffeine in individual effluents indicate poor treatment in the respective WWTP. Unfortunately, consistent experimental half-life times as comparable to modelled values are lacking because of missing standardization and thus highly differing experimental designs.

4.4. Risk assessment

In this study, risk assessment of individual compounds and mixtures detected in WWTP effluents was based on three different risk metrics, namely (i) RQ, (ii) HU and (iii) TU (Table 3). The three approaches rely on different concepts, such as (i) protection against all impacts on all organisms and endpoints, considering uncertainty of toxicity data, (ii) assessment of potential impacts on a whole aquatic community and (iii) separate assessment of potential impacts towards different BQEs represented by sensitive model species, as inspired by the WFD assessment groups to classify the ecological status. Depending on the approach, distinct procedures to consider acute or chronic risks exist; either by defining BQE-specific acute and chronic risk thresholds (Malaj et al., 2014), or by using different toxicity data (NOEC-type endpoints for HU_{ch} as applied in the current study vs. EC_{30-70} for HU_{ac} (Posthuma et al., 2019b)). The RQ approach only considers chronic risk (i.e. AA-EQS). In the following, in accordance with the second objective of this study, the main risk drivers and cumulative risks based on the three different risk metrics were identified.

The risk metric RQsum, often used in the regulatory context, such as compound registration and authorisation, or to identify priority substances in European surface waters, is based on the most conservative assumptions of the three approaches. It yields the highest risk metric ratios for all chemical mixtures found in effluents and thus is in good agreement with the precautionary principle and the use of this approach to identify water samples, which may be considered as 'sufficiently safe'. In the current study, however, none of the effluent RQsum values fell below 1, but all effluents exceed this threshold of concern by a large margin, i.e. 33- to 8400-fold (Fig. 9). The assessment of chemicals according to RQ is based on PNECs which have been selected based on expert judgement, considering the availability of toxicity data to any endpoint and in any organism, also considering uncertainty represented by respective AFs. This approach is considered to be protective for the individual compounds and provides incentives to improve data quality, especially in case of predicted acute toxicity values using an AF of 1000. For 16 out of the 30 top priority compounds ranked by RQ95, no experimental toxicity data for the BQEs are available in the ECOTOX database (Fig. 4). In fact, half of the compounds identified as risk drivers were based on predicted PNECs that involve an AF of 1000, among them the top-ranked compounds vancomycin and HMMM. The latter strongly dominates the cumulative risk metric values in two effluent samples. The other half of the compounds are prioritized despite lower or no AFs in combination with chronic endpoints, which is in agreement with previous findings by Von der Ohe et al. (2011). These compounds include particularly pesticides, such as fipronil, imidacloprid, fenoxycarb, diuron and diazinon. Thus, the prioritization of chemicals

according to the RQ approach requires different actions, which might range from efforts to provide scientifically sound toxicity data with reduced uncertainty to an immediate need for management and regulation. While being highly protective in general, the RQ approach (based on EQS and PNECs) bears the potential of overlooking chemicals with high acute toxic risks. Since decision makers may intuitively focus their management actions on the top n candidates, there is a risk that toxic chemicals that might require immediate management and regulation are ranked lower than chemicals that are highly prioritized due to greater uncertainty (i.e. new chemicals without measured toxicity for which only uncertain PNEC values are available).

Two further approaches on mixture assessment have been explored in this paper. They include the SSD-based HU_{chsum} approach and the BQE-based TUsum approach for individual organism groups. Both approaches agree with the results of the RQsum approach that most of the effluents are not sufficiently safe. Based on HU_{chsum} , 93% of the effluents exceed the threshold of concern, with a maximum exceedance of 42-fold (Fig. 10). This is in line with the percentage of exceedances using the TUsum approach, which indicates 100%, 91% and 54% of the effluents being not sufficiently safe for crustaceans, algae and fish, respectively (Figs. 11-13), considering the chronic toxicity thresholds defined by Malaj et al. (2014), which have been integrated into the TU calculations in this study. Considering HU_{chsum} as a metric inherently integrating over all three organism groups mentioned above, complemented with insects, molluscs and others, the obtained results indicate robustness and coherence of both assessments. While both metrics list a broad variety of chemicals from different use groups, the HU_{ch} approach (Fig. 5) and the TU approach for crustaceans (Fig. 7) and algae (Fig. 6) agree on a clear dominance of pesticides and biocides among the top 30 ranked compounds with insecticides being prioritized for crustaceans and herbicides and biocides for algae, while some fungicides are relevant for both groups. These findings are well in agreement with the biological target the chemicals are designed for. Data-poor chemicals lacking experimental toxicity data have a lower probability to be prioritized. Using HUs, these compounds are even excluded altogether, due to lacking SSDs, while using TUsum they are characterized by predicted toxicity data using baseline toxicity QSAR models that prioritize these individual chemicals only at very high concentrations. Thus, RQ-based approaches are complementary in prioritizing chemicals that require additional efforts to derive sound toxicity data.

Compared to algae and crustaceans, $\text{TUsum}_{\text{fish}}$ exhibits a quite different picture with relatively low risks and a larger number of chemicals without experimental toxicity data for fish (8 out of top 30 chemicals by TU_{95} lack these data, Fig. 8). This is due to the fact that hardly any chemicals are in use that are designed to kill fish and thus exhibit particularly high fish toxicity. Thus, despite the still high percentage of pesticides and biocides among the top 30, data-poor pharmaceuticals and industrial chemicals gain weight. A major gap related to the assessment of risks to fish may be the use of acute toxicity data as a basis for the risk metric calculation, while major adverse effects are possibly due to long term effects caused by endocrine disruption (Kidd et al., 2007), behavioural changes (Martin et al., 2019), inflammation (Naslund et al., 2017), fitness reduction by sublethal effects on metabolism (Bojarski and Witeska, 2020) etc.

4.5. Evaluation of WWTP effluents and impact of advanced treatment

Prioritization of management measures such as an upgrade of WWTPs typically involve mixture risk assessment using the metrics applied in this study. For all assessment methods, mixture risks of WWTP effluents cover ranges of several orders of magnitude, although the nominal treatment technology for 52 out of 56 of them is very similar, using mechanical and biological treatment. Thus, individual contamination obviously strongly depends on i) the type and intensity of pollution sources, and ii) the actual performance of the WWTP. The exploration of individual source-treatment-effluent relationships were

beyond the focus of this study. However, HU_{chsum} , RQ_{sum} and $TU_{algae sum}$ exhibited correlations with r between 0.63 and 0.69 indicating some robustness of the rankings (Fig. 14). Correlations of community-related risk metrics with TUs based on the least (fish) and the most sensitive species (crustaceans) were lower. For the former this may be due to the low contribution of fish toxicity to the community risk. In addition, 4–5 sites with high values for $TU_{fish sum}$ (based on mainly ethyl azinphos and carbendazim, as well as two QACs due to high MDL/2 values) highly impacted to the slopes. The low correlation with crustaceans is assumed to be caused by very toxic individual chemicals, driving risks particularly on crustaceans, such as diazinon, which may be masked in the RQ_{sum} approach by less toxic compounds with high AF and in the HU_{chsum} by considering the whole SSD rather than a particularly sensitive model organism. It should be considered that correlations involving RQ_{sum} were calculated excluding EU009 as an outlier with an RQ strongly driven by a HMMM and thus a compound with high concentration and an AF of 1000, but without reliable toxicity data.

All three applied assessment tools agree that the four WWTP samples with advanced treatment, using either ozonation or AC, were those with by far the lowest risks for aquatic organisms. While using RQ, they still exceeded the threshold of concern, in the assessments using HU_{chsum} and TU_{sum} , these effluents were clearly below toxicity thresholds, except for the chronic risk threshold for invertebrates. Thus, advanced treatment was obviously very successful in reducing mixture risks to an acceptable level. This is also confirmed by a direct comparison of samples of the same WWTP – before and after the ozonation of effluents (Fig. S3) – and is in agreement with previous studies reporting on the elimination of micropollutants by ozonolysis (Hollender et al., 2009).

In all correlations of the absolute values of the different risk metrics, the TU_{sum} of very-low-contamination advanced-treatment effluents deviate from the linear regression line towards higher estimates. This indicates that the TU approach, as used in this paper tends to overestimate risks of these samples by setting a kind of minimum mixture toxicity based on concentrations of MDL/2 for all chemicals below MDL, and using baseline-toxicity predictions for all chemicals lacking experimental data. Taking this into account, threshold exceedance for chronic risks to crustaceans in effluents from WWTPs with advanced treatment should be put into perspective and are not in disagreement with the achievement of the reduced pollution goals using this technology.

4.6. Consensus risk drivers

One of the objectives of the current study was to use the three different risk metrics to prioritize risk drivers in a pragmatic consensus approach. In total, 299 out of 366 chemicals detected have been identified by at least one risk metric as relevant mixture component above the threshold of 1 in at least one effluent (Fig. S6, left & Table S14). Thus, mixture risks are highly relevant for WWTP effluents with many components contributing. In total, 185 compounds are prioritized by the RQ approach, only. Most of these compounds are currently characterized by an AF of 1000, and hence require the revision of the underlying PNEC before taking regulatory management actions. Four compounds are prioritized only by TU and no compound by HU , only. In total 110 compounds have been identified by at least two metrics as consensus mixture components of concern, with 25 compounds prioritized by all metrics. Considering default dilution of the effluents in the receiving water by factor of 10, 32 consensus mixture components of high concern were identified (Fig. S6, right & Table S14). The 32 consensus mixture components of high concern include 21 pesticides and biocides, 5 pharmaceuticals and 6 other compounds (Table 4). Among them are the top risk drivers of TU for algae, i.e. diuron, terbuthryn and clarithromycin, the top risk drivers of TU for crustacean, i.e. diazinon, imidacloprid and fipronil as well as the top risk driver of TU for fish i.e. carbendazim and ethyl azinphos. In addition, diclofenac and azithromycin are among the main risk drivers according to RQ and HU_{ch} . All of them have been reported previously in treated wastewater samples (Alygizakis et al., 2020; Kienle et al., 2019; Loos et al., 2013; Munz et al., 2017; Nickel et al., 2021; Velki et al., 2019), except for ethyl azinphos. Two of these compounds are listed as priority substances according to WFD, while two of them are part of the selection of substances for the 3rd watch list under the WFD (2020).

It remains important to recognise that mixture components of high concern that lack consensus may include substances of high risk even if two approaches fail to identify them. Reasons for a lack of such a consensus have already been given in previous sections of the discussion, such as the lack of SSDs for certain substances or the use of baseline toxicity for specifically acting chemicals with missing toxicity values. Particularly RQ covers endpoints which are not addressed by TU and HU , such as risks to human health or the risk of secondary poisoning, which is exhibited for example by PFAS (Ankley et al., 2021). Moreover, certain EQS used for RQ calculations are based on drinking water limits, which satisfy particularly high levels of precaution.

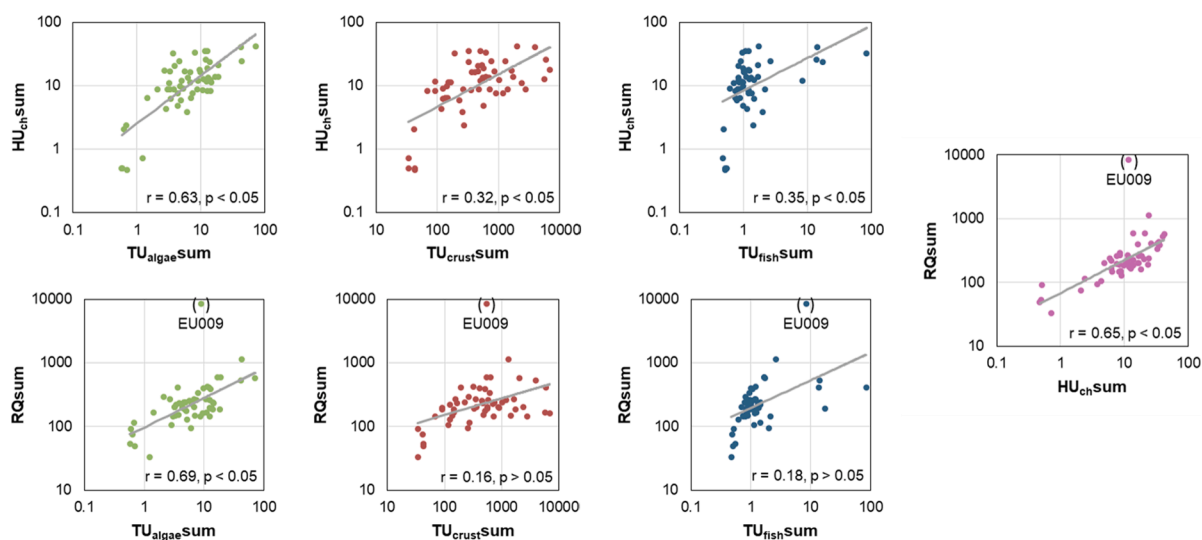


Fig. 14. Pearson's correlation of the three risk metrics (based on dataset B; NA = MDL/2). Correlation coefficient r is based on the entire dataset, excluding EU009 in case of RQ_{sum} .

Table 4

Consensus mixture components of high concern. Identified by at least two risk metric as relevant mixture component above the threshold of 10 (incl. dilution factor) in at least one effluent. *According to the JRC Technical Report “Selection of substances for the 3rd Watch List under the Water Framework Directive” (2020).

Name	Counts	Use Group	RQ	HU	TU	Priority substance or EU watch list candidate*
2,4-Dichlorophenol	35	Industrial	1	0	1	—
7-Diethylamino-4-methylcoumarin	55	Dye	1	0	1	—
Acetamiprid	43	PPP	1	0	1	(1st watch list)
Azithromycin	47	Pharmaceutical	1	1	0	(1st watch list)
Azoxystrobin	49	PPP	1	0	1	3rd watch list
Benzyltrimethyltetradecylammonium	5	Biocide	1	0	1	—
Caffeine	18	Stimulans	1	1	0	—
Carbendazim	52	Biocide	1	0	1	—
Celecoxib	39	Pharmaceutical	1	0	1	—
Clarithromycin	51	Pharmaceutical	1	0	1	(1st watch list)
Clothianidin	22	L-Pesticide (legacy)	1	0	1	(1st watch list)
Diazinon	33	Biocide	1	1	1	—
Diclofenac	56	Pharmaceutical	1	1	1	—
Didecyldimethylammonium	2	Biocide	1	0	1	—
Diflubenzuron	1	L-Pesticide (legacy)	1	0	1	—
Dimethoate	13	L-Pesticide (legacy)	1	0	1	—
Diuron	53	Biocide	1	1	1	PS
Ethyl azinphos	1	L-Pesticide (legacy)	1	1	1	—
Fipronil	54	Biocide	1	0	1	3rd watch list
Fluoxastrobin	1	PPP	1	0	1	—
Hexa(methoxymethyl)melamine	56	Industrial	1	0	1	—
Imidacloprid	54	L-Pesticide (legacy)	1	1	1	(1st watch list)
N,N-Dimethyldodecylamine	21	Surfactant	1	0	1	—
Pirimicarb	25	PPP	1	0	1	—
Pirimiphos-methyl	1	PPP	1	0	1	—
Propiconazole	55	L-Pesticide (legacy)	1	0	1	—
Sertraline	54	Pharmaceutical	1	0	1	—
Spiroxamine	21	PPP	1	0	1	—
Terbuthylazine	46	PPP	1	0	1	—
Terbutryn	53	Biocide	1	1	1	PS
Thiacloprid	17	L-Pesticide (legacy)	1	0	1	(1st watch list)
Tris(2-ethylhexyl)phosphate	6	Plastic additive	1	0	1	—

5. Conclusions

Using wide-scope chemical target screening for 56 European WWTP effluents, a total of 366 chemicals from different use groups could be detected in at least one effluent. These chemicals include many compounds found almost ubiquitously in WWTP effluents and surface waters. However, in 27% of the effluents, high concentrations of site-specific compounds, for example from industrial emissions, have been detected, indicating the need to involve local information on specific commercial usages and industrial processes in order to avoid overlooking these chemicals. Using three different risk metrics (RQ, HU and TU), 299 of these 366 compounds contributed to mixture risks by exceeding the thresholds for chronic effects, highlighting the need for a more comprehensive analysis and assessment of mixture risks, rather than focusing on individual compound risks only. The different risk metrics have their individual strengths and weaknesses, with RQ being the most protective one, using predicted effects and AFs to consider uncertainty, integrating over many toxic endpoints. About 60% of the mixture components of concern were prioritized only by RQ, typically based on high AF. TU and HU assessment metrics were less protective, but probably provide a closer link to the impacts on individual BQEs (TU) or the entire aquatic community (HU) and tend to prioritize chemicals with high known toxicity. Although partly prioritizing different chemicals, effluent mixture risks determined with RQ and HU were well correlated, indicating the robustness of both approaches. Care has to be taken if individual chemicals with high uncertainty and thus high AF drive the RQ, which can result in strong outliers. The TU approach allows a BQE-specific assessment that is not necessarily in agreement with HU and RQ, which address the whole community. Consensus chemicals of very high concern, as identified in this study, include 32 chemicals dominated by pesticides and biocides, indicating the very high relevance of these compounds also in municipal settings and not only in agriculture. Appropriate consideration of risks of

effluents HU and other contamination sources containing endocrine disruptors, immune- and neurotoxins to fish would require the consideration of sublethal effects on reproduction and fitness, going beyond acute toxicity.

While all risk metrics allow for appropriate component-based mixture risk assessment involving a large number of chemicals, further improvements are possible and required. They include a further enhancement of the number of chemicals considered involving both extended target and non-target screening, as well as improved detection limits below effect thresholds for all chemicals potentially contributing to risks. Together with advanced imputation methods beyond using MDL/2 or zero for non-detects, improved MDLs will enhance environmental realism of low mixture risks. A major source of uncertainty is the lack of measured toxicity data for many frequently detected compounds and high uncertainty, if toxicity is predicted by baseline-QSARs or other *in silico* tools. Substantial efforts are required to fill these gaps prioritizing chemicals for effect testing and enhancing QSARs for screening purposes. While in the current paper, a default dilution of 1/10 has been assumed for getting an overview on WWTP-effluent related risks, the assessment and prioritization of individual WWTPs triggering management measures has to take the real dilution into account. Independent of the dilution factor and of the risk metric used, the current study provides clear indications that ozonation and active carbon treatment are able to minimize toxic risks based on known target chemicals, typically below risk thresholds. Due to the formation of possibly harmful TPs that may be missed by target screening and lack reliable toxicity data, these results should be confirmed with effect-based methods (Brack et al., 2019a).

CRediT authorship contribution statement

Saskia Finckh: Conceptualization, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review &

editing, Visualization, Project administration. **Liza-Marie Beckers:** Writing – review & editing, Supervision. **Wibke Busch:** Methodology, Data curation, Writing – review & editing. **Eric Carmona:** Writing – review & editing, Supervision. **Valeria Dulio:** Methodology, Data curation, Writing – review & editing. **Lena Kramer:** Investigation, Data curation, Writing – review & editing. **Martin Krauss:** Methodology, Investigation, Data curation, Writing – review & editing. **Leo Posthuma:** Conceptualization, Methodology, Writing – review & editing. **Tobias Schulze:** Conceptualization, Methodology, Software, Formal analysis, Investigation, Data curation, Writing – review & editing, Funding acquisition. **Jaap Slootweg:** Methodology, Software, Formal analysis, Data curation, Writing – review & editing. **Peter C. Von der Ohe:** Methodology, Data curation, Writing – review & editing. **Werner Brack:** Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition.

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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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2022.107234>.

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